International Conference

Advances in grain legume breeding, cultivation and uses for a more competitive value-chain

BOOK OF ABSTRACTS

LEGATO
LEGumes for the Agriculture of TOmorrow

EUROLEGUME
2014-2017

27-28 SEPTEMBER 2017
NOVI SAD, SERBIA

International Conference

Advances in grain legume breeding, cultivation and uses for a more competitive value-chain

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*This conference marks the final year of two EU FP7-KBBE projects devoted to Grain Legumes, **LEGATO** and **EUROLEGUME**, and thus the complementarity of the scientific and technical outputs developed by more than 100 researchers from both projects. This occasion will disseminate the highlights from the research accomplished, to stakeholders and society in general in a stimulating programme including leading EU projects and invited speakers. The Conference is also open for receiving free communications from the projects´ members and from other teams not belonging to the projects, to be presented as posters/oral communications.*

Invited plenary speakers

Professor Timothy Close (UC Riverside, USA)
Professor Moira Dean (QU Belfast, UK)
Dr. Stephanie Mittermaier (IVV, München, D)
Professor Mark Peoples (CSIRO, Canberra, AU)
Dr. Thomas Nemecek (Agroscope, Zürich, CH)

Sessions

1. Genetics & breeding
2. Biotic stress resistances
3. Enhancing legume quality - novel food & feed
4. Defining ideotypes root phenotyping and microbial interaction
5. Agronomy
6. Climate changes, stress adaptation & mitigation measures

Round-tables

Increasing legume consumption
Increasing legume cultivation

Poster session with flash presentations

Legume Show Cooking event with leading chefs!

Scientific committee

Richard Thompson - LEGATO
Paolo Annicchiarico - LEGATO
Diego Rubiales - LEGATO
Christophe Salon - LEGATO
Carlota Vaz Patto - LEGATO
Erik Steen Jensen - LEGATO
Steve Belcher - LEGATO
Antonio López-Francos – LEGATO
Eduardo Rosa - EUROLEGUME
Boris Rewald - EUROLEGUME
Graça Pereira - EUROLEGUME
Juan Fernandez - EUROLEGUME
Liga Lepse - EUROLEGUME
Ruta Galoburda - EUROLEGUME

Organizing committee

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Ligia Pinto - EUROLEGUME
Caroline Sautot - LEGATO

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Invited speakers

Cowpea: a Warm Season Legume and its Genome

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Cowpea, *Vigna unguiculata* L. Walp, is a diploid warm-season legume (tribe Phaseoleae) with a genome size of ~620 Mb. Cowpea, known as blackeyed pea among other common names, is relevant as a grain legume in the USA and Europe, and as a fresh vegetable in China and elsewhere, but is of major importance as food and fodder in sub-Saharan Africa. An annotated reference genome sequence of an elite African variety, IT97K-499-35, is now available through Phytozome (www.phytozome.net). The v1.0 cowpea pseudomolecules contain 519 Mb of sequence, derived from superscaffold sequences with N50 = 16.4 Mb and L50 = 12. A total of 29,773 gene models were annotated using a combination of ab initio and transcript (RNA-Seq and Sanger EST) evidence, providing a measure of 95.9% plant completeness using BUSCO v2. Synteny between cowpea and other warm-season legumes has been clarified, including common bean (Phaseolus vulgaris L.), which provided the basis of new cowpea chromosome numbering. The genome assembly is based on single molecule real-time sequencing (91x coverage; Pacific Biosciences) together with two optical maps (BioNano Genomics) and ten genetic linkage maps containing a total of 44,003 SNPs. Transition to the use of the reference genome has improved the resolution of QTL mapping in biparental and multiparent populations, and using a minicore of diverse cowpea germplasm. An accumulating list of QTLs and candidate genes for traits now includes flowering time, pod shattering, seed coat patterns and texture, leaf shape, seed size, resistance to several pests and pathogens, yield in several environments, and others. Cowpea and its genome information constitute an important set of resources to understand the biology of this and related species, and to apply such knowledge to agricultural needs and germplasm conservation. This work was conducted mainly under the NSF BREAD project “Advancing the Cowpea Genome for Food Security” with partial support from the Feed the Future Innovation Lab for Climate Resilient Cowpea.

Environmental impacts of the production and use of grain legumes

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Grain legumes have several characteristics with consequences on the environment. During the last years, LCA studies analysed the environmental impacts of the introduction of legumes into cropping systems (see Watson et al., 2017). The main effects found were: i) Reduction of N fertiliser use, due to absence of N fertilisation in the legume crops and reduced fertilisation of the following crop. N fertiliser manufacturing is associated with high consumption of fossil fuels and greenhouse gas (GHG) emissions, mainly N2O and CO2, and needs for transporting and spreading the fertiliser. Furthermore, this means also that N emissions after fertiliser application are reduced, in particular N2O, NH3, NO3 and NOx. ii) N leaching can be increased after legume crops, but not necessarily over several years. iii) Diversification of the crop rotation, which results in breaking pathogen cycles, potentially increased yields and possibly reduced pesticide inputs. It can also increase associated biodiversity. iv) Legume crops produce higher protein yields at the expense of a lower yield of digestible energy. v) Legumes are feeding sources for pollinators and have therefore the potential to maintain or increase their populations. The concrete effects of grain legumes in the cropping system depend on factors like the intensity of the production and the diversity of the rotation. Grain legumes can replace soybean meal in animal feed rations. Soybean production has rapidly expanded during the last decades, often at the expense of former forest areas. Soybean produced on clear-cut land result in very high GHG emissions and is detrimental for biodiversity due to habitat loss. LCA studies investigated the substitution of imported soybean meal by European grain legumes for cattle, pigs and poultry (see Watson et al., 2017). The main effects were reduced transport distances, avoided deforestation impacts and rotational benefits. Since soybean is also a legume, the advantages of symbiotic N fixation are present in both systems. However, replacing soybean meal in a feed ration requires adapting the whole feed ration and the resulting environmental impacts can therefore be lower, similar or higher, depending of the feed components selected. Few LCA studies also investigated the environmental impacts of using grain legumes in human nutrition. Replacing animal protein sources, in particular meat, by grain legumes can generally reduce environmental burdens, since animal protein sources are associated with relatively high impacts. Again the concrete outcome depends on the complete nutritional pattern and the production methods. Grain legumes are indispensable elements of sustainable farming systems and future human nutrition. Reference: Watson C.A., Reckling M., Preissel S., Bachinger J., Bergkvist G., Kuhlman T., Lindström K., Nemecek T., Topp C.F.E., Vanhatalo A., Zander P., Murphy-Bokern D. & Stoddard F.L., 2017. Grain Legume Production and Use in European Agricultural Systems. Advances in Agronomy, 144: 235-303.

Grains and Claims: Consumer Perceptions, Understandings and Intention to Buy

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Health claims on food products, which aim at informing the public about the health benefits of the product, represent a type of nutrition communication; the use of these is regulated by the European Union. This talk will discuss findings from research investigating public perceptions and willingness to use cereal products with health claims. The studies investigated how perceptions and intentions are affected by individual needs and product characteristics. Results show that adding health claims to products does increase their perceived healthiness. Claim structure was found to make a difference to perceptions, but its influence depended on the level of relevance, familiarity and individuals' need for information. Further, the type of health benefit proposed and the base product used also affected perceptions of healthiness. The talk concludes that while healthiness perceptions relating to products with health claims may vary between men and women, old and young and between countries, the main factor influencing perceived healthiness and intention to buy a product with health claim is personal relevance.

Inputs of fixed N and soil mineral N benefits derived from legume cropping

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The nitrogen (N) contributed by legumes is an important component of the N supplied to subsequent cereal crops. However, most Australian grain-growers have little idea about the potential inputs of fixed N provided by their pulse crops, and few routinely monitor soil mineral N before applying N fertiliser to crops grown after a legume. The presentation reports insights into the role of legumes in cereal-dominated cropping sequences gained from 16 dryland (rainfed) experiments conducted in eastern Australia between 1989–2016, and from on-farm measurements of the symbiotic performance of 50 commercial pulse crops undertaken since 2001. The data collated from these studies were examined to identify the main factors regulating inputs of fixed N, and to explore the possibility of developing simple predictive relationships which farmers' could use to benchmark (i) likely inputs of fixed N, (ii) the expected availability of soil mineral N after legumes, and (iii) the relative value of legume N to a following wheat crop.

Food ingredients from lupins

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Plant proteins and dietary fibres are gaining in importance for food industry due to their high nutritional quality and their good functional properties. Furthermore, securing the supply with high quality and sensory attractive proteins for a growing world population is also a main issue nowadays. Thereby, lupin seeds are promising sources for the production of valuable food ingredients due to their high contents of proteins and dietary fibers. Plant based food ingredients are facing a range of requirements, which should be met:
-High nutritional quality
-Highly functional regarding e.g. structure formation, emulsification, and easy to apply
-No or only slight off-flavors
-Meeting consumer expectations like free from genetically modified organisms, environmentally friendly production, regionally available
In general, sweet lupin varities with alkaloid contents below 0.02% are applied for the production of functional ingredients. After successful selection of promising candidate varieties in close cooperation with breeders, the typical flavor compounds associated to lupin proteins and dietary fibres and their possible origins have been identified. Subsequently, strategies for preventing the formation of such volatile compounds and the reduction of already formed odorants were developed. As odorants from different chemical classes like aldehydes, ketones, pyrazines and carboxylic acids were responsible for the typical grassy-green, beany, and cereal-like off-flavor of lupin ingredients, the processing steps comprise de-oiling using supercritical CO2 as well as different aqueous extractions for the reduction of off-flavors and protein isolation. In addition, dietary fibers from lupins having excellent sensory properties (e.g. white in color, smooth mouth feel) are obtained by applying the extraction process. Besides being sensory attractive, the newly developed food ingredients from lupins comprise excellent functional properties, which enable the application of lupin proteins and dietary fibres in a wide range of food products including desserts, ice cream, salad dressings, vegan products as well as bakery products and pasta. The developed processes were implemented into industrial scale by founding Prolupin GmbH as a Fraunhofer IVV spin-off in 2010. Furthermore, several human intervention studies indicate that lupin proteins and dietary fibres can reduce blood serum cholesterol and enhance intestinal health.
Altogether, these developments emphasize the high potential of lupin proteins and dietary fibres for the production of food ingredients. However, several aspects of using lupins as food ingredients remain still unclear and should be investigated in detail. Thereby, the feasibility to use other lupin varieties for the production of food ingredients, the use of a differently processed lupin protein as fat replacement as well as further studies on applying dietary fibres from lupins as functional food ingredients should be in the focus of further research.

1. Genetics & breeding

Evaluation of field grown grain legume genotypes for stress resilience and seed quality

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Legumes are invaluable for sustainable agriculture and protection of the environment providing high protein food and feed, enhancing soil fertility and structure, and suspending soil erosion. The challenges imposed by climate changes highlight the need for concerted research approaches in order to develop crops that are able to cope with environmental stresses, while increasing yield and quality in rainfed cropping systems. Legume crops are subjected to biotic stresses that in association to abiotic stress may diminish crop yield and quality. The study is focused on evaluation of thirty selected grain legume genotypes including winter pea, chickpea, faba bean, lupin and lathyrus. The selected genotypes were evaluated in a randomized field plot design for two consecutive years. The field experiments were rainfed and no fertilizers or chemicals were used as biocontrol agents. Plant growth and crop performance were assessed for a set of agricultural characters including seed germination, development, winter hardiness, yield, 1000 seed weight, seed quality parameters, fungal diseases, viruses and pests tolerance. Seed quality was scored for protein and phenolic content and antioxidant capacity. Statistical analyses of data revealed that increased yield is correlated with seed size for genotypes of the same species and large seed size is correlated with high protein content. Tolerance to biotic stress of faba bean varieties is correlated to small seed size, while in chickpea this correlation is different. Further results are presented and the role of functional markers in breeding is discussed.

Utilization of MAS in pea breeding program

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Peas is a dominant legume species in the Czech Republic, which plays an irreplaceable role in crop rotation. The yielding instability together with the agro-technical requirements are the main limiting factors influencing the greater representation of legumes in crop rotation, which is only 1.8% in the Czech Republic. Peas is a fairly challenging on environment, also on cultivation process and is highly susceptible to both biotic and abiotic stress. Pea breeding has a long tradition, profitable varieties resistant to both fungal and viral pathogens are grown here.The aim of our work was to increase and stabilize the resistance against a broad spectrum of pathogens in afila types of field peas. Declared sources of resistance were tested in inoculation tests. To speed the breeding process the eIF4E genes corresponding with SBM-1 (LG VI) and SBM-2 (LG II) loci, mediating recessive resistance to several viral pathogens of the genus potyvirus including PSbMV were identified. In selected genotypes involving all donors of PSbMV resistance used in practice, alleles of eIF4E gene were identified,. Based on sequence analysis PCR molecular markers were designed, tested and selected, allowing the identification of both the homozygous and heterozygous plants with 100 % reliability. Suitable materials with high levels of biological properties were used in the hybridization process. The result are lines, which will be used for breeding, but also new registered varieties resistant to complex pathogens. Acknowledgements of financial support project project nu.613551 EU FP7 LEGATO.

A new high-density consensus linkage map of white lupin

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White lupin (Lupinus albus L.) is appreciated as a source of seed protein, carbohydrates and oil for food and feed but requires genetic improvement to enhance its agronomic quality. In this study we developed a new high-density consensus linkage map of white lupin carrying sequence-defined landmarks anchored to its transcriptome. We performed mapping of four important white lupin agronomic traits, namely, early flowering, low alkaloid content in seeds, and resistance to two diseases caused by pathogenic fungi, anthracnose and Phomopsis stem blight. We assessed the collinearity of this map with the reference genistoid genome of narrow-leafed lupin (L. angustifolius L.) cv. Tanjil. Polygenic control of vernalization responsiveness and anthracnose resistance, as well as single gene regulating seed alkaloid content were revealed. Conserved inter- and intra-generic synteny was observed in genome regions carrying these quantitative trait loci, however none of these QTLs matched narrow-leafed lupin genes conferring vernalization independence (Ku), anthracnose resistance (Lanr1 and Anman1), low alkaloid profile (iucundus) and Phomopsis stem blight resistance (Phr1 and PhR). Allele-sequenced markers anchored in white lupin QTLs may be implemented for marker-assisted selection in white lupin breeding.

Potential MLO homologous genes in legume crops

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Knock-out mutation in one member of the MLO family lead to durable and broad spectrum resistant against powdery mildew in several crops including barley, tomato and pea. Lack-of-function of this MLO has been associated with higher efficiency of papilla formation impeding cell penetration by a wide range of pathogenic isolates of powdery mildew independently of their races. Despite mlo-based resistance is the unique exploited source of resistance so far to confer powdery mildew resistance in pea in the fields, this resistance mechanism have not been exploited in other legume. As a first step to exploit mlo-based resistance in other legumes, we characterised the MLO gene family in eight legume species from different clades of the Papillionoideae sub-family in the frame of the LEGATO EU-project. This study revealed the multiplication of clade IV and V members that regroups all MLO linked to powdery mildew susceptibility. Thus three candidate MLOs were identified in chickpea, barrel medic and lupin while six were identified in common bean and pigeonpea. Based on these results, candidates to confer mlo-based resistance to powdery mildew were also identified in faba bean, grass pea and lentil. Similarly to chickpea, three potential candidate genes were identified in these species that are being mapped on their respective genetic and physical maps. In addition, allelic variation of these genes is being identified in lentil germplasm collections including wild relatives. These studies confirmed the presence of several candidate MLO genes that should allow exploitation of mlo-based powdery mildew resistance in legume crops.

Yield and protein yield stability in faba beans (*Vicia faba*) under organic conditions in Northern Europe to gain a further insight in the adaptability of gathered genotypes

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Despite large number of faba bean accsessions in gene banks, there is still lack of information on plants performance. Thus, in order to overcome this situation, it is required an extensive multi location evaluation. The field experiment to evaluated yield stability and protein yield in twenty local genotypes and commercial varieties of broad beans (Vicia faba var. major) and field beans (Vicia faba var. minor) was conducted for EUROLEGUME project task 2.2 in four europe countries for two years. A randomized design was used to test each genotype in 4 replications according to field trial layout. The main objective was to evaluate yield and protein stability, as well as maturity and resistance in organic field to gain a further insight in the adaptability of gathered genotypes from EUROLEGUME project task 2.1. Bouth faba bean groups were analysed separately. Indicated traits was average seed weight per plant, 100 seed weight and seed weight per plot. Protein content was measured protein yield was calculated. Between both year was difference between yield, but in protein yield wasn’t major differences, except some cases. Local genotypes showed better yield qualities than commercial varieties. After two year monitoring in field sites, the main difference in genotype yield and protein stability was indicated by gro – climatic conditions between years and sowing time and rainfall during growing period. The data obtained allow to select the most appropriate genotypes for further development of new cultivars as well as to obtain new food and feed products.

Breeding for resistance to chocolate spot in faba bean

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Among the different fungal diseases that affect faba bean, chocolate spot incited by Botrytis fabae is one of the most important, causing in many cases significant yield reductions, being particularly damaging when high relative humidity and mild temperatures concur. Control by fungicides is possible, but it is not environmentally and economically efficient. The employment of resistant varieties appears as the best option. In the last years we have developed a comprehensive breeding program with the main objective of obtaining cultivars combining resistance to chocolate spot and adaptation to Mediterranean environments. This included screening large germplasm collections both in seedlings under controlled conditions and in adult plants in the field over a series of seasons and locations. Identified resistances were introduced in our breeding program and progenies selected for combined resistance and productivity. As a result of this process, now we have available a set of advanced breeding materials. A cultivar has already been submitted for registration and some more are in the offing. In addition to these applied breeding activities we are approaching genetic studies and have developed a recombinant inbred line (RIL) population that has been properly phenotyped over several seasons and genotyped using DArT-Sea markers to identify quantitative traits loci (QTLs) related to resistance. These results will be presented and critically discussed.

Clock resetting during cowpea seed development. Implications on storage protein

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Cowpea (*Vigna unguiculata*) is an important source of protein supply for animal and human nutrition. The current hypothesis is that primary metabolism is to a large extent controlled by the plant circadian clock. We identified cowpea orthologues of the core clock genes, the protein storage genes VuLEG, VuLEGJ, and VuCVC. The core clock genes showed a rhythmic expression profile in leaves with a typical evening/night and morning/midday phased expression in case of VuELF3 and VuLHY, respectively, while VuTOC had a non-typical midday phase. Rhythmicity and phasing apparently faded during early pod development and was regained in ripening pods for VuTOC1 and VuLHY, while VuELF3 showed a different rhythm. VuELF3 and VuTOC were also rhythmic in mature seeds, but VuTOC showed again a modified rhythm. A stable rhythmic expression was observed for all proteins with peak phase 12 hours after dawn in intermediate ripe seeds. Comparing time windows during developmental stages we found that VuCVC and VuLEG were significantly down regulated during the night in mature pods as compared to intermediate ripe pods, while changes in seeds were non-significant due to high variance. The expression of storage protein genes was partially rhythmic, depending on tissue type and ripening stage. Rhythmicity was most prominent in developing seeds, but also pods and mature seeds showed diurnal transcription profiles. During development, the circadian clock is reset between leaves and late pod development. Storage protein deposition may be circadian regulated, this is probably an adaptation to increase energy efficiency.

Seed moisture content of different field pea genotypes during the maturation

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The aim of research was to determine seed moisture content of field pea during the maturation. The research included ten field pea cultivars: NS Junior, Jantar, Timo, Trezor, Javor, Dukat, Jezero, Kristal, Angela and Partner. Seed moisture content of field pea was the highest in the first harvesting period, on average 64.6%. Seed moisture content decreased exponentially until the last harvesting period, amounting to 12.3% on average. The intensity of decrease in seed moisture content was significantly different between harvesting periods. The lowest decrease of 6.3% was between the first and the second, and between the fifth and the sixth harvesting period (8.2%). Average daily loss of seed moisture in these intervals was 1.6%, i.e. 2.0%. Intensity of seed moisture loss between the second and the third harvesting period (10.1%) was similar to the intensity between the fourth and the fifth (10.2%). Daily loss of seed moisture during these intervals amounted to 2.5%. The highest decrease of seed moisture content was 17.5% between the third and the fourth harvesting period with daily loss of 4.4%. In the first harvesting period moisture content varied from 68.6% (NS Junior) to 59.5% (Angela) and two groups of cultivars were distributed according to seed moisture content: 1. Cultivars with indeterminate and determinate stem growth and with conventional leaf type in which interval of variation for seed moisture content varied from 68.6% (NS Junior) to 64.8% (Jantar); 2. Cultivars with determinate stem growth and afila leaf type where the highest seed moisture content had cultivar Jezero (63.2%) and the lowest had cultivar Angela (59.5%).

Peculiarities of development of soybean breeding material in the southern steppe of Ukraine

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The dry environment of the Southern Steppe of Ukraine makes soybean crossing very difficult. To increase the hybrid number, we carried out both artificial and natural hybridization using lines with gene pollen sterility. The investigation was carried out in the experimental field of Plant Breeding and Genetics Institute (the South of Odessa region). . Seeds of female sterile lines with pollen sterility genes (msp, ms4, ms1Urbana or ms1Tonica) mixed with seeds of local high-yielding varieties were planted in 10-rowed plots for natural hybridization. Natural pollination was carried out using lines with pollen sterility genes. This approach increases the hybrid plant number about by 6 times in comparison with manual crossing. Homozygous fertile families are selected from hybrid progeny. Using this approach, we bred variety „Farvater‟, which is listed in the State Register of Plant Varieties of Ukraine. Every year, about 1,000 soybean collection accessions were examined. The main criteria for selection of parents were yield capacity, drought resistance and biochemical parameters. Over more than 30 years of study, we investigated about 6,000 soybean collection accessions and selected the most adapted to our environment forms, which we recommend as starting material for breeding drought-resistant varieties that would be adapted to the steppe zone. Crossing accession VIR 5048 (Kazakhstan) with Ukrainian variety „Medeia‟ generated a series of highly productive breeding lines with protein content of 43-46% and a set of valuable agronomic features .

Creation of chickpea varieties for south Ukraine conditions

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Chickpea till recently did not cultivate in Ukraine except for private gardens and homestead lands. But high prices and the complex of positive agrotechnical descriptions were caused the last years the real "boom" in our country. Farmers began to cultivate it on hundreds of hectares. In most cases his yield makes 1,5-2,5 t/ha. The chickpea breeding in our institute began at the end of the last century. Its basis was made more than 1000 collection genotypes, got from International Crops Research Institute for Semi-Arid Tropics (ICRISAT, India), came from 26 countries. New breeding material was created by intervarietal hybridization. At taking of pairs for crossing followed a ecology-geographical principle. Crossing conducted with and without castration, taking into account that pestle of flower is ready to the pollination from one to two days to ripening of own pollen. The most effective terms for artificial hybridization take place in morning. Paternal components were picked up taking into account a presence for them of marker morphological traits. Maternal forms carry recessive, and paternal dominant traits. Quite good results are got also during hybridization of chickpea in phytotron conditions. The use of artificial climate allows short term to get the sufficient amount of breeding material. As a result of intensive breeding work more than 10 chickpea varieties were created, among that 3 types of desi, other behave to the kabuli group. The most wide distribution in a production were varieties Rozanna, Pamyat, Triumph, Budzhak. Them yield in the production conditions reached 2,8-3,0 t/ha.

Biochemical markers in the soybean breeding of food direction

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Glycinin (11S globulin) and β-conglycinin (7S globulin) are important seed storage proteins in the soybean for food production. The goal of research was to study a character of changes of content and component composition of 11S and 7S globulins, their ratio in the protein of varieties, hybrids and hybrid lines of soybean seed of different genetic origin in the connection with breeding of food direction. 7S and 11S globulins were separated by methods, which were developed in the Laboratory of Plant Biochemistry (Patent # 42181, Patent #107671). The pictures of 11S and 7S globulins were taken with SDS-PAGE electrophoresis. It was shown that the studied soybean genotypes differ widely in the total protein content and 7S and 11S globulin fractions content. The features of 7S and 11S globulins content, its ratio and component composition of genotypes of different phylogenesis origin, F3 - F8 hybrids of soybean and their paternal forms were identified. The polymorphism between varieties on the electrophoresis spectrums of component composition of 7S and 11S globulins of soybean was established. It was shown that soybean varieties of different genetic origin are characterized by polimorphism of α, α1, β, A3, A5 subunits and components in the 7S and 11S globulins component composition. These subunits and components have influence on the human health. The got results open fundamentally new approach of estimation of soybean seed on a quality of protein and can be used for authentication of soybean varieties of the food direction.

Cowpea genetic resources: their use in plant breeding and for new uses

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The genetic heterogeneity present in crop landraces allows their response to various abiotic and biotic stresses, as well as to the different consumer preferences. In a Global Climate Change scenario, this genetic diversity is crucial. The characterization and evaluation of local populations genetic diversity using morphological and agronomical traits is essential for their exploitation in breeding programmes and for their use as novel food. Although cowpea is typically grown for its dry grains, its immature pods and fresh seeds are also consumed in different parts of the world, including some areas of South Europe. A collection of cowpea landraces from South Europe revealed a great diversity in morphological and agronomical traits, reflecting the agro-climatic crop growth conditions and consumer preferences. The most promising landraces of the collection were included in multi local environments (3 countries and 4 locations) aiming the study of genotype x environment interaction during a two-year period. Several traits, such as first pod height, data of maturation, dry seed production and protein content were recorded. A significant interaction among genotypes, locations and years was observed. Regarding fresh pods evaluation, a considerable phenotypic variability but no significant differences regarding the countries of origin, was observed among landraces. Acknowledgements The research work was funded by the European Union’s Seventh Framework Programme for Research, Technological Development and Demonstration under grant agreement n◦ 613781, project EUROLEGUME

Generation and use in MAS of linked markers for low vicine-convicine (lvc) trait

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Vicine/Convicine are compounds which have anti-nutritional effect on human individuals and some domesticated animals therefore the consumption of these faba bean varieties are limited in these groups. Fortunately, new faba bean lines suitable for food and feed have been developed which do not contain or have very low anti-nutritional level for vicine/convicine (lvc). Breeding for lvc have begun promptly, similarly, developing linked markers to aid breeding by MAS has started. Published linked markers provided sequence information in order to generate DNA based linked markers for parental lines provided by LEGATO partners. Based on genetic map and marker information we have developed markers by designing primer pairs for PCR based genotyping of the parental lines. Primers were developed in such a way to be able to get DNA polymorphism after restriction enzyme digestion (Cleaved Amplified Polymorphic Sequences, CAPS method). Some primers pairs were generated in order to prevent primer dimer formation. The primer pairs which were used in the second period were VfVc11 and VfVc13. In the third period we were using new primer pairs which are more closely linked to the lvc locus. PCR amplification is performed using parental DNA and primer pairs. The Lvc and lvc parents could be distinguished unambiguously by markers VfVc13 and VfVc18 after cutting the PCR product by TasI and TaqI restriction endonucleases, respectively. The result of the genotyping will be presented at the final meeting of LEGATO in Novi Sad.

Illumina Cowpea iSelect Consortium Array used to evaluate the genetic diversity and population structure of Iberian Peninsula cowpeas

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Cowpea (Vigna unguiculata L. Walp) is an important legume crop due to its high protein content, adaptation to heat and drought and capacity to fix nitrogen. Characterization of cowpea's genetic diversity is important for properly preserve and utilize germplasm in crop improvement. The aim of this study was to characterize genetic diversity and structure of a set of Iberian Peninsula cowpea accessions in comparison to worldwide cowpea material. A total of 96 cowpea accessions from two Vigna unguiculata subspecies (unguiculata and sesquipedalis), including 43 landraces and cultivars from Iberian Peninsula and 53 landraces collected worldwide, were genotyped using the Illumina Cowpea iSelect Consortium Array containing 51,128 SNPs. Using 44,056 polymorphic SNPs among samples, STRUCTURE identified four subpopulations. All accessions of subspecies sesquipedalis belonged to one subpopulation, while subspecies unguiculata accessions were distributed among the other three subpopulations, which were mainly differentiated by geographical origin. Most Iberian Peninsula accessions clustered together with those from other southern European and northern African countries and had low genetic diversity. However, there were two Iberian Peninsula accessions having admixed ancestry and one that had ancestry from a different subpopulation. These three accessions could be used as donors to incorporate new diversity into Iberian Peninsula breeding programs without compromising local adaptation. Results obtained in this study also brought some insights into possible dispersion routes of cultivated cowpea. Acknowledgements: The research work was funded by the European Union's Seventh Framework Programme for Research, Technological Development and Demonstration under grant agreement n◦ 613781, project EUROLEGUME

Grass pea association analysis of bioactive compounds for quality precision breeding tools development

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Consumers worldwide are increasingly concerned with food quality and environmental sustainability. Grass pea (*Lathyrus sativus* L.) is a robust grain legume with high nutritional value. This environmentally friendly legume may be an excellent response to consumers' concerns. However, a reduced breeding investment has hampered its use in Europe. To increase grass pea consumption and cultivation, breeding objectives and consumer preferences should be aligned. However, breeding for improved end-user´s quality is a complex task in legumes due to high trait interaction. Particular grass pea metabolites might act as health-promoting agents as well as antinutrients, influencing both taste and consumers' acceptability, and interfering with plant resistance to different stresses. Under the scope of LEGATO EU project, we studied the genetic basis of several of these dual-action bioactive compounds as a first step to develop molecular tools (functional markers) for redirecting breeding towards more attractive grass pea varieties. For attaining this, seed metabolites contents (such as phenolic compounds or β-ODAP) of a worldwide collection of 116 grass pea accessions were analyzed by HPLC and HPLC-MS/MS (liquid chromatography tandem - mass spectrometry). A genome-wide association study using the phenotypic information from one to two years of field experiments, combined with the genotypic information from a DArTseq based-SNP screening is currently ongoing. Relevant sources of these bioactive compounds will be detected and marker-trait associations tested using a mixed linear model that accounts for population structure and familial relatedness. Results obtained from this analysis will be reported.

Genetic diversity of a worldwide collection of *Lathyrus sativus* assessed by cross-species amplifiable microsatellite molecular markers

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Grass pea (*Lathyrus sativus*) is an annual legume crop with great potential among grain legumes, presenting high protein content, extraordinary adaptability to unfavorable environments, and resistance to severe diseases. However, little is known about the genetic mechanism underlying these biotic and abiotic stress resistances. The identification of molecular markers associated with these interesting traits would ease their transfer into more attractive modern varieties. Under the scope of the EU LEGATO project we gathered a collection of 137 different accessions from Europe, Africa and Asia representing the worldwide grass pea diversity. As a first step towards the use of this collection on association studies, and with the objective of identifying the most genetically contrasting grass pea accessions we performed a preliminary analysis of the genetic diversity of this L. sativus collection. An average number of 10 individuals per accession were genotyped using four Expressed Sequence Tag-SSR markers (EST-SSR). Several genetic diversity parameters and the genetic relationships among accessions were estimated. The implications of the observed genetic variability on the worldwide grass pea collection on future association studies and grass pea breeding will be discussed.

Genetic diversity in a world collection of white lupin

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White lupin genetic resources are limited to the primary gene pool. The rescue of white lupin as a major grain legume is hindered by limited breeding work that suffers, inter alia, from lack of information on the structure of crop genetic diversity. The aims of this study were: (i) investigating the structure of landrace genetic diversity across three diversity layers relative to variation among nine major historical cropping regions (Italy, Egypt, Spain, Portugal, Turkey, Maghreb, Madeira-Canaries, Near East, East Africa), landraces within cropping region (6-14 sampled landraces per region), and genotypes within landrace (3-4 sampled genotypes per landrace); (ii) assessing patterns of genetic variation for 86 landrace accessions and 15 varieties or breeding lines (where the latter encompassed spring-type, winter-type and intermediate phenological types). Overall, 323 genotypes were characterized by a genotyping-by-sequencing approach that provided about 6,500 polymorphic SNP markers per genotype. An analysis of molecular variance indicated the following ranking of variance components for landrace germplasm: genotype variation within landrace > landrace variation within region > variation between historical cropping regions. This result, not expected for this predominantly self-pollinated crop, highlights the importance of exploiting also within-landrace diversity. Landrace multi-dimensional scaling ordination displayed mainly a latitudinal gradient, along with outstanding variation among landraces from Near East that agrees with the hypothesis of crop origin in this region. Variety germplasm varied depending on its phenological type but tended to be fairly distinct from landrace material, suggesting that just a minor portion of the available genetic diversity has been exploited by breeders.

Evaluation of pea accessions for seed yield in Southern Portugal

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Sixteen pea accessions were selected from the genepool collection provided by the EUROLEGUME partners according to the results obtained from the primary screening performed for earliness and productivity. These accessions (4 from Latvia, 1 from Portugal, 9 from Estonia, and 2 from Sweden) were installed in the experimental field of INIAV, Elvas (38°53’N, 7°09’W), during two years in order to evaluate the yield stability. Thirteen accessions have white flowers and three have violet flowers. The evaluation was performed using eight traits: days to flowering, duration of flowering, plant height, resistance to lodging, days to maturity, seed yield, 100 seed dry weight and diseases incidence. Data were analysed by ANOVA followed by Tukey’s test using the IBM Statistics 20 program. The analysis of variance revealed significant differences between accessions for all the parameters evaluated. The accessions with higher seed yield are associated with a large flowering period and earlier maturation. The accession Eesti Kollane söögihernes has the smallest seeds (9.76 g/100 seeds) and the Portuguese variety Grisel (22.61 g/100 seeds) has the highest value for 100 seed dry weight. The most promising accessions in INIAV-Elvas were Seko, Kirke, Clara, Mehis, K-6973, Capella and Grisel.

Yield potential of faba bean accessions grown in Portugal

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Faba bean (*Vicia faba* L.) is an important legume crop well adapted to most climatic areas of Europe and widely used for feed and food. In Portugal, faba bean is a winter growing crop, sown in autumn and harvest in late spring/early summer. In INIAV-Portugal, the major objectives of faba bean breeding programme are to develop new varieties with high yield potential, early maturity and tolerant to the major diseases (*Botrytis fabae* and *Uromyces fabae*). In this work, twelve accessions (one from Estonia, eight from Latvia and three from Portugal) selected from the genepool collection provided by the EUROLEGUME partners were evaluated by eight parameters: days to flowering, duration of flowering, days to maturity, plant height, number of seeds per pod, seed yield, 100 seed dry weight and diseases incidence. The trial was conducted in the experimental field of INIAV-Portugal (38°53’N, 7°09’W), in a randomized complete block design with four replications. Data were analysed by to one-way ANOVA followed by Tukey’s test using the IBM Statistics 20 program. In general, the most productive genotypes are the Portuguese accessions since they are better adapted to our environmental conditions.

Evaluation of pea (*Pisum sativum*) genetic resources under Norwegian field conditions

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Genetic resources of pea (*Pisum sativum*) may represent valuable traits for inclusion in breeding programs, but may also be valuable directly due to good adaptation to local agro-climatic conditions, or have market potential as heirloom foods. Grain legume production in Norway is limited compared to many other European countries, but increased acreage is desired and expected for several reasons. A short growing season and the specific agro-climatic conditions makes choice of genotypes important for reliable grain and protein yields. During three years, several evaluation field trials with pea genetic resources were carried out in Southern Norway, assessing morphological and phenological traits, seed yields and protein levels. The trials included pea accessions from Norway(1), Sweden(6), Finland(1), Latvia(8), Estonia(9) and Portugal(1), as well as commercial cultivars(6). The genotypes differed widely in grain yield, time and duration of flowering, maturity, plant height, and resistance to lodging. Seed protein levels ranged from 24.0 to 32.7%. Protein levels for these pea genotypes under Norwegian field conditions appear to be higher than in comparable trials in countries at lower latitudes. The research was supported by the EU FP7 project EurolegumeGA 613781 and NordGen (Nordic Genetic Resource Center).

Polymorphism of Ukrainian soybean varieties by Gy5 gene marker

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Soybean (*Glycine max* L.) is important agriculture crop which is used due to the high content of glycinin and ß–conglycinin storage protein complexes. Glycinin is hexamer, subunits are derived into groups: group I (G1, G2, G3 or A1aB2, A2B1a, A1bB1b) encoded by genes Gy1, Gy2, Gy3; group IIa (G4 or A5A4B3), encoded by Gy4 gene; group IIb (G5 or A3B4), encoded by Gy5 gene. G5 glycinin has the major part in contributing to tofu firmness with any coagulant. Indels in gene sequences determine absence of some subunits. Different combinations of subunits cause different properties of glycinin. Some molecular markers were designed for different regions of Gy5 gene. Thus, molecular marker A3(Gy5) is dominant-recessive (Jegadeesan et. al., 2012) and produces 864 bp amplification fragment or null allele by polymerase chain reaction method . Soybean varieties with 864 bp are used for tofu producing but ones with null allele have a negative effect on tofu quality. We have tested 14 Ukrainian soybean varieties by A3(Gy5) marker of Gy5 gene: Anatoliivka, Antares, Apolon, Berehynia, Chernivets'ka 9, Farvater, Kyivs'ka 98, Mel'pomena, Odes'ka 150A, Proteyinka, Ustia, Valyuta, Vasyl'kivs'ka, Yuh 30. As control soybean variety Harovinton was used that has all glycinin subunits and must generated 864 bp product by A3(Gy5) marker. In result, all varieties including Harovinton were identified and had 864 bp product of amplification. This indicates that overall Ukrainian soybean breeding is currently showing positive effect on tofu quality.

The genomic and phenotypic evaluation of chromosome segment substitution lines of wild pea (*Pisum fulvum*) to widen genetic diversity of pea crop

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Plant evolution under domestication has altered numerous traits, introducing domestication bottleneck resulting in high degree of relatedness, leading to narrower genetic base of cultivated germplasm, prone to pests and diseases. We report development of the chromosome segment substitution lines (CSSL) of wild pea (Pisum fulvum WL2140) in the cultivated pea (P. sativum subsp. sativum cv. Terno) genetic background. The 145 lines were genotyped by genome wide DARTseq technology. All together 1,880 sequence based marker could be placed into respective linkage groups using synteny to Medicago and 50 lines were also genotyped by 13.2k Pea Illumina SNP assay. Once pea genome is available it is expected that larger number could be positioned. Fifty lines were selected for field trials, recording morphological traits and agronomic parameters (14 traits). There was substantial transgression in most of the traits, including seed weight, flowering time and maturity compare to cv. Terno control. Harvested seeds were analyzed for total protein, soluble proanthocyanidins, raffinose-verbascose-stachyose, raffinose family oligosaccharides, total starch, phytate-total phosphorus and galactomannan contents. Establishment of such permanent introgression library will allow phenotypic characterization of unlimited number of target traits, which will provide means for QTL and gene identification and subsequent incorporation into desired commercial genotypes. Acknowledgements: This work received funding from the European Community's Seventh Framework Programme (FP7/ 2007-2013) under the grant agreement n°FP7-613551, LEGATO project.

Identifying positional candidate genes controlling low vicine-convicine in faba bean (*Vicia faba* L.)

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Vicine and convicine (v-c) are faba bean compounds with anti-nutritional effects in monogastric animals and potential toxicity in humans. A single gene, vc-, responsible for a 10-20 fold reduction (1, 2) was identified and several attempts to identify candidate genes (3, 4) have been pursued. The approach is limited by its reliance on the priori knowledge about the physiological function of candidates. In case of v-c, the lack of knowledge of the v-c synthetic pathway, the large faba bean genome size (~13 Gbp) and the lack of a reference genome, result in a significant bottleneck for the application of this approach. Although the task is challenging, significant progress has been achieved within LEGATO and several closer markers have been identified. To determine which enzymes or transcriptional factors could be encoded by the vc- gene, we have used comparative genomic approaches (5), KASPar SNP genotyping assays (6) and high-throughput genome profiling DarTSeq (7) in a F2 from the cross Vf6 x vc-, to generated a map with more than 4000 markers. The target region was confined between Medtr2g008210 and Medtr2g010180, containing 136 genes, but the causative gene remains unknown. Recently, 21 new candidates have been genotyped in the population, using the Medicago and the faba bean transcriptome sequences (8). Fourteen should be discarded due to nonspecific/lack of amplification or to the absence of SNPs in the target sequence. The remaining seven could be mapped, thus providing potential candidate genes and offering a step towards breeding lines free of these compounds.

Evaluation of *Pisum sativum* subsp. *elatius* and *Medicago truncatula* seed dormancy as adaptation to environment

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The timing of transition from seed to seedling is important developmental transition determining the survival of individual as well as population and species. Thus seed germination is vital for plant adaptation and acts in the context of natural selection. Little is known about clues which release the physical dormancy. It is hypothetized that temperature and soil moisture oscilations are the major players. Wild pea (Pisum sativum subsp. elatius) and Medicago truncatula offer excellent model to study seed dormancy in ecological context, due to wide range of species geographical distribution and existing variation in dormancy levels. We ask following questions: Is there an association between seed dormancy variation and geography along wild pea (Pisum sativum subsp. elatius) distributional range? If so, which ecological factors (climate, soil conditions, local habitat conditions) as adaptation drivers are correlated with geographical distribution of dormancy? 55 geographically different wild pea and 142 Medicago lines were scored at fluctuating temperatures of 35/15°C, 25/15°C and constant 15°C and 7°C regimes. Variation in dormancy was recorded and significant differences were found. Selected bioclimatic, topographic and humidity conditions were extracted based on geographical coordinates and subjected to Principal Component analysis. Main gradient is correlated with altitude and is related to the temperature variation (BIO 2, 4, 7) and mean temperature (BIO 1, 6, 11). The second most important gradient is related to the latitude. The results of seed dormancy behaviour will be discussed in light of adaptation. Acknowledgements: This is supported by the Grant Agency of the Czech Republic, 16-21053S project.

Genomic selection for production traits in white lupin and pea

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Genomic selection (GS) aims to predict complex, polygenic traits by a statistical model constructed from genome-wide marker information. Genotyping-by-sequencing (GBS), which can provide many genome-wide SNP markers at a lower cost than array platforms, may facilitate the cost-efficient application of GS. This work aimed to explore the value of GBS-based GS for production traits of white lupin and pea. 1) White lupin. A world collection including 83 landraces from 11 historical germplasm pools and eight French varieties was subjected to GBS by genotyping two to four genotypes per landrace and one genotype per variety, and was phenotyped for several agronomic traits. GS using a Ridge-regression BLUP model applied to allele frequencies of 6,578 SNP markers exhibited high predictive ability (as correlation between true and predicted phenotypes in a cross-validation procedure > 0.50) for grain yield in a sub-continental climate site (Lodi), grain yield in a Mediterranean site (Sanluri), and several other traits (winter survival; onset of flowering; etc.). 2) Pea. A GS model with high ability to predict grain yield under severe terminal drought ($r = 0.69$) was established on the basis of joint data from a rainout sheltered environment in Lodi and an agricultural environment in Morocco. Results are provided for recent experiment work aimed at assessing the value of this GS model in terms of actual yield gains obtained for novel germplasm evaluated in a managed-stress environment.

Grain and biomass yield of white lupin germplasm under contrasting moisture availability

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White lupin breeding can rely on agronomically outstanding landrace genetic resources. However, information is scant on the genetic variation for drought tolerance. Two contrasting moisture conditions were imposed in an experiment under a field rainout shelter. One condition implied severe stress, with available water (field capacity minus wilting point soil water content) in the range 0-20% from the late vegetative period onwards; the other was favourable, with available water in the range 60-80% throughout the crop cycle. We evaluated 21 landraces from 10 countries along with one variety and two breeding lines. We found wide variation between moisture conditions and among genotypes for grain yield, dry biomass yield, and harvest index. A few landraces displayed good grain yield in both conditions, and a few others were consistently low-yielding. Several genotypes showed genotype × treatment interaction of cross-over type between favourable and stressed conditions, and specific adaptation to either condition. A breeding line selected in Morocco exhibited greatest specific adaptation to stressful conditions as expressed by highest value of the grain yield ratio between stressed and favourable conditions. A breeding programme specifically tailored to drought tolerance is advisable for severely drought-prone regions, whereas selection for wide adaptation could be searched for when targeting less unfavourable or climatically-variable regions.

Increased production of pods and seeds in pea through mutation in the MFN genes

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Annual legume plants, such as pea, go through a vegetative phase in which it the shoot apex produces nodes with lateral vegetative branches. After floral transition the shoot apex becomes a primary inflorescence that, while it is active, produces flowering nodes, with lateral secondary inflorescences with flowers. The production of pods and seeds depends on the number of flowering nodes formed by the plant. We have identified two genes, which we have named MORE FLOWERING NODES (MFN), encoding homologous transcription factors that control the period of time in which the primary inflorescence apex is active and, therefore, the number of flowering nodes. Homozygous plants with null mutations in MFN1 or MFN2 produce up to twice the number of pods and seeds than their corresponding wild-type parental line, without affecting flowering time, both under greenhouse conditions and in field trials. Evaluation by NIRS indicates that the basic nutritional quality of the seed is not affected by the mfn mutations. To asses whther the MFN genes can be used to increase the production of seeds in pea, we are analysing in detail the phenotype of the mfn mutants. In addition, to analyse their performance in commercial varieties, we are currently introgressing the mfn mutant alleles into several pea elite cultivars. We expect that MFN genes can be used as a useful tool to increase yield in grain legumes.

Unravelling autofertility in faba bean (*Vicia faba* L.)

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Autofertility is the ability of plants to set seeds without disturbance of the flowers. In faba bean autofertility seems to be related to the rupture of the stigmatic cuticle, which occurs two days prior to anthesis in autofertile lines, whereas it remains mostly intact along the anthesis in autosterile lines [1]. For a successful pollination it is necessary a synchronization between the availability of viable pollen and the stigma receptivity. Our main aim in this study is to identify those genes related to autofertility in the segregating population from the cross Vf6 (autosterile) x Vf27 (autofertile). Previous field assays in this RIL population, scoring undisturbed and tripped flowers, allowed the identification of QTLs for traits underlying autofertility (flowers/node, pods/node and pods/flower), but their confidence intervals are still too wide for a meaningful candidate-gene analysis. In order to dissect the QTLs operating in the trait we are performing a refined analysis in parental and RILs displaying contrasted autofertility levels by: (1) analysing the viability of the pollen, (2) measuring floral architectural traits (flower and ovary length and stigma-ovary angle), (3) counting pollen deposition and pollen tube growth in the stigma of undisturbed flowers using fluorescent microscope at different developmental stages and (4) examining stigmas under scanning electron microscope. The final aim is to achieve a detailed understanding of the genetic and molecular basis of the autofertility, a highly complex quantitative trait influencing the level and the stability of yield in this crop.

Selecting appropriate field pea varieties for breeding under nordic environmental conditions

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Pea as important human food crop is grown on over 6.7 million hectares worldwide. Plant phenotyping has become the bottleneck to provide the physiological and genetic basis of plant growth and performance for its translation into crop improvements. Therefore the aim of experiments was to provide suitable field pea varieties for further breeding on the basis of higher productivity and plant growth best performance under Nordic environmental conditions. A field experiment was carried out at the Estonian Crop Research Institute in 2015 and 2016. The field pea varieties were: Bruno, Capella, Clara, Eesti Kollane Söödahernes, Hele, Kiir, Kirke, Leili, Looming, Mehis, Rahel, Seko, Zaiga. The highest and more stable yield (t/ha) come from accessions Hele, Kiir, Kirke, Leili and Zaiga. 100 seed weight (g) was highest in accessions Clara and Kiir. As best flowering period lasts from 10 - 20 days, then the best varieties are Capella, Clara, Kirke and Seko. Accessions with shortest growing period were Capella, Clara, Kirke, Leili, Rahel and Seko. Susceptibility to pod spot was not statistically different both years. Susceptibility to downey mildew was highest in accession Leili. Most resistant accessions to lodging were Capella, Clara, Leili and Rahel. Conclusions: Out of these yield and phenotype characteristics results it can be concluded that accessions Kirke, Mehis and Rahel are most suitable varieties for breeding in Nordic conditions. Some other varieties might be also good, when looking at concrete characteristic. This investigation was supported by EU FP7 project EUROLEGUME No. 613781.

Molecular-genetic research of legumes in Ukraine

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In Ukraine legumes (soybean, pea, chickpea, bean, lentil, lupin etc) are grown throughout the territory and play important role in our country economy. Realization of legumes significant genetic potential depends on using in breeding process of modern marker technologies based on latest achievements of genomics, to create varieties with higher yields and resistance to different stress factors. The main directions of our research are: soybean and chickpea genetic diversity studying by different molecular markers, molecular identification of varieties, bioinformatic and molecular genetic analysis of genes / loci of agronomical important traits, development and validation of markers for marker-assisted selection (MAS). We developed the original approach for soybean and chickpea genotypes identifying and varieties registering as genetic formula with the microsatellite loci allelic state information. Bioinformatics and molecular genetic studies of Gy and Cgy genes of soybean storage proteins - β-conglycinine and glycinine are conducted to create system of molecular markers for development of varieties with certain seed globulin component composition to avoid negative effects on human health, in particular allergic reactions. Because of photoperiod impact on growth duration and productivity of different soybean varieties, we are analyzing soybean genotypes on E1-E8 genes that control flowering and ripening phases. Detection of E-genes will allow involving certain genes in breeding material for high-performance soybean varieties by MAS. Studies of Foc genes / loci of resistance to fusarium wilting (pathogen - Fusarium oxysporum f. sp. ciceris) are carrying out. Markers creation is necessary for pyramiding of effective resistance genes into elite chickpea genotypes.

2. Biotic stress resistance

Prospects for durability of available resistances to fungal diseases and parasitic weeds in pea and faba bean

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Peas and faba beans, as any other crops, can be damaged by a number of diseases. Breeders have explored a range of strategies to exploit available resistances, that unfortunately use to be scarce, incomplete and of limited heritability. As a result, only cultivars with moderate levels of resistance are available to farmers in most instances. This is further complicated by the fact that several clearly distinct species (i.e. *Orobanche crenata* and *O. foetida*; *Uromyces viciae-fabae* and *U. pisi*; *Erysiphe pisi* and *E. trifolii*) can infect the same legume crop. Here we will revise current status of resistance in pea and faba bean to a number of fungal diseases and parasitic weeds discussing historic and recent achievements, with a special focus on potential durability.

Characterization of pea defense mechanisms against *Fusarium oxysporum*

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Fusarium oxysporum f. sp. pisi (Fop) is a ubiquitous soil pathogen reducing field pea (*Pisum sativum*) yield worldwide. Monogenic resistance have been already described and successfully used in breeding. However, monogenic resistance is at risk of breakdown by the constant evolution of the pathogen. To improve the durability of resistance, the genetic base of resistance should be broadened and a better knowledge on the defense mechanisms efficient against Fop should be gathered. To this aim, we identified several Fop-resistant Pisum spp. accessions and characterized the underlying defense mechanisms. A first study on pre-penetration mechanism revealed that the root exudate of three accessions inhibited Fop germination. Further analyses indicated that this pre-penetration mechanism was mainly mediated through the constitutive secretion of pisatin, the main pterocarpan pea phytoalexin. A second study focused on post-penetration defense mechanism at the cellular level, showing that involvement of three main mechanisms, namely papilla formation, cell walls strengthening and accumulation of phenols and carbohydrates. These studies highlight that pea accessions combined several strategies to delay and efficiently prevent disease development which offer interesting opportunities for breeding.

Comparative analysis of proteome changes induced by *Botrytis fabae* in faba bean (*Vicia faba*)

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Chocolate spot, caused by the necrotrophic fungus Botrytis fabae is an important disease affecting faba bean in many regions of the world where it is cultivated. Although chemical control is an option, it presents serious drawbacks in terms of economic and environmental costs, being the use of resistant varieties a better and more efficient alternative. Varying levels of resistance have been previously identified. In this study we used a proteomic approach (2-DE coupled to MALDI-TOF/TOF analysis) to compare the leaf proteome of three faba bean genotypes showing different resistance response to B. fabae. Multivariate statistical analysis identified 270 differential protein spots when genotypes and treatments (non-inoculated and inoculated) were compared, with a total of 89 proteins identified using a combination of peptide mass fingerprinting (PMF) and MSMS fragmentation. Interestingly a high proportion of identified proteins (13%) were proteases, suggesting that protein degradation might play an important role during pathogen infection. Results will be presented and critically discussed in terms of differences between genotypes in response to B. fabae.

Potential application of fungal and plant metabolites in rust and powdery mildew disease management

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Rusts and powdery mildews are major limiting factors worldwide for many crops, including legumes. Despite of continued efforts to develop cultivars with high level of genetic diseases resistance, fungicides are, currently, the mainstay control method and a wide range of synthetic compounds are widely used. However, a renewed interest in the discovery of natural products as alternatives to synthetic fungicides application is recently emerged. Considering that spore germination and fungal penetration are key phases for the initial development of biotrophic plant pathogens, a further approach proposed for their management could be the use of natural metabolites produced by plants and fungi as pathogen inhibitors in these pre-penetration stages. With this aim we studied potential application for disease management of eight fungal and plant metabolites. Their inhibitory effect on early stages infection was studied on 4 rusts and 2 powdery mildew species. Results will be presented and critically discussed ACKNOWLEDGEMENTS This research was financially supported by AGL2014-52871 projects and Programme STAR financially supported by UniNa and Compagnia di San Paolo, Italy.

Construction of a high-density integrated DArTseq SNP-based genetic map and identification of genomic regions controlling rust resistance in *Pisum fulvum*

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The fungus *Uromyces pisi*, causing pea rust, is one of the major limiting factors in pea production worldwide to which limited resistance is available. We searched in previous years for resistance in a large germplasm Pisum spp. collection and failed to identify complete resistance, being the higher levels of incomplete resistance identified in P. fulvum. To unravel the genetic control underlying resistance to U. pisi in P. fulvum we generated a recombinant inbred line (RIL) population from a cross between resistant and susceptible P. fulvum accessions, which was genotyped on the Diversity Arrays Technology Pty Ltd platform. A total of 9,569 high-quality DArT-Seq and 8,514 SNPs markers were generated that assembled in seven linkage groups. The newly constructed integrated genetic linkage map of P. fulvum and the QTLs associated with rust resistance both in seedlings and adult plants will be presented. ACKNOWLEDGEMENTS This research is funded by AGL2014-52871-R project.

Identification and validation of positional candidate genes for partial resistance to *Didymella pinodes* and *Aphanomyces euteiches* in pea

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Ascochyta blight and Aphanomyces root rot are the most damaging diseases of pea worldwide. Both linkage and association mapping studies identified numerous quantitative trait loci (QTL) controlling partial resistance to either of the main pathogens responsible for these diseases, i.e. *Didymella pinodes* and *Aphanomyces euteiches*. The recent development of new molecular marker SNP resources recently made publicly available (Tayeh et al, 2015 ; Boutet et al, 2016) allowed identifying candidate genes, within refined QTL confidence intervals, likely to be involved in partial resistance expression. Differential expression of some of the genes lying within the most important QTLs confidence intervals is upon study using RTPCR on inoculated/non inoculated leaves or roots of susceptible and partially resistant pea genotypes.
This work is carried on with the support of the Legato project.

Tayeh et al, 2015
Boutet et al, 2016

Screening faba bean (*Vicia faba*) for resistance to aphids (*Aphis fabae*)

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Black bean aphid (*Aphis fabae* Scopoli) is considered the most important and damaging insect pest of faba beans worldwide, causing damage by direct feeding on the phloem and acting as vectors of viruses. In attempt to identify sources of resistance, a germplasm collection (180 accessions) was screened under field conditions at Córdoba (Spain) during two consecutive growing seasons (2014-2015 and 2015-2016), in assays with 2 replications. The susceptible cvs. Baraca and Alameda were sown each five lines, both as spreaders to ensure the aphids distribution attack and to be used as checks. Heavily infested pots of cv. Alameda were randomly distributed along the field trials by the end of March, usual time of first observations in the field. By mid-May the aphids coverage was quantified and at the end of the growing season the plants harvested and the yield was measured. The 80 most resistant and productive accessions were sown a third seasons (2016-2017) in a 3 replications assay. Several rows of cv. Alameda were uniformly distributed and sown along the trial. At mid-March artificial aphids inoculation was carried out. During spring, infestations levels and aphids damage were recorded several times. The 35 most resistant accessions, selected from field trials, were also studied under controlled conditions to confirm the resistance, in a trial with two repetitions. Seedlings were grown in a growth chamber at 22ºC and inoculated with adult aphids; the aphids coverage was periodically recorded. Several accessions seem to be moderate to highly resistant to the faba bean aphids development.

Influence of climatic conditions on the adaptive capacity of different morfotipe cultivars of peas

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Experiments were conducted in 2006-2016 years using common technologist's cultivation of peas for the zone South of the steppe Ukraine.To characterize the regional climatic conditions during vegetation period 2006–2016 pea years used indicator of hydrothermal factor (HTF). Over years of research of modern domestic and foreign varieties of mustachioed type demonstrate good productivity both in favorable years and years with a deficit of moisture. Heteromorphy yields the forms generally depends more on moisture conditions. Due to their high plasticity (Ri = 0.83–1.09) in the most favorable rainfall years they form large yield (1.81 t/ha) than in the acute drought (1.24 t/ha). Among heteromorphy varieties of the most stand out Spartak, Az 1420 and Az 390, which yields the most close to the baleen and leafless classes.All the studied varieties have a linear response to fluctuations in weather conditions. Their productivity increases or decreases proportionately to the change in ambient conditions, but there are also differences in the severity of the reaction of sorts. So sorts Kamerton, Modus, Glians, Combine 1, Berkut, Madonna, Blagodatniy give high genotypic effects (Eì) and have favorable adaptive capacity, so the form higher and stable yield relatively standard and other varieties. With the deterioration of the conditions for growth and development benefits of these varieties are amplified. Spectacular varieties, Chekbek, Sweet, Gotievskiy, Topaz 2, Kharkovchanin, Intensive 92, Spartac, Orel, Az 1420 when explicit plasticity (Ri) have a higher average level of productivity for a number of years.

Antixenosis and antibiosis to pea aphid in *Pisum* spp. germplasm

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Acyrthosiphum pisum is a polyphagous aphid of major importance on pea crop to which little resistant cultivars are available. In the frame of LEGATO project we screened a germplasm collection of Pisum spp. under field conditions at Córdoba, Spain over two seasons. A number of accessions displayed significantly lower levels of infestation than the check Messire in both seasons. Selected accessions were further studied in pot assays under controlled conditions corroborating field data. Susceptible check cv. Messire showed the highest levels of aphid infestation and the highest damage as shown by plant wilting. On the opposite side, accession P40 showed the lowest infestation and the lowest level of plant damage at all observation times. Accession P29 showed similar values of aphid infestation at all time points than Messire, but suffered less damage both in terms of chlorosis and wilting. Accession P665 showed moderate infestation but high chlorosis. Multiple choice assay showed that P40, P29 and P665 were less preferred than Messire.

Identification and characterisation of resistance to *Bruchus pisorum* in *Pisum* germplasm

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Pea weevil (*Bruchus pisorum*) is a damaging insect pest affecting pea production worldwide. In the frame of LEGATO project we searched for resistance in a germplasm collection in two locations in Spain during 3 consecutive growing seasons assessing seed infestation (SI) and larval development (LD). Accessions P665, P669 and P656 showed a low and stable SI in the different assessed environments. Accessions P1 and P314 showed stable reduction of larval development. Further studies were performed under controlled conditions in choice and no choice bioassays to discern possible effects on oviposition. No choice assays showed a significant genotypic effect on oviposition, being reduced to half on pods of particular pea accessions and negligible on pods on other legumes. Dual choice assays also showed significant differences in oviposition preference.

Identification and characterization of the MLO family in legume genomes

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Powdery mildew is one of the most widespread and damaging fungal pathogen of many crops including legumes. One of the most efficient and durable powdery mildew resistance mechanisms is conferred by homozygous recessive alleles at the mlo and er1 loci in barley and pea respectively. These efficient papilla-based penetration resistances are due to loss-of-function mutations in one MLO protein. This protein belongs to a highly conserved plant specific family of proteins that possess seven trans-membrane domains. Although the exact function of MLO is still not known, phylogenetic analysis classified MLO proteins in seven clades of which clades IV and V contained all MLO linked to powdery mildew susceptibility in monocots and dicots respectively. As a first step to exploit mlo-based resistance in other legume crops, we identified and characterised the Mlo gene family in eight legume species from different clades of the Papillionoideae sub-family in the frame of the LEGATO EU-project. This analysis revealed several evolutionary differences between tropical and temperate legume crops. Interestingly, legume genomes were shown to encode several clade IV and V MLO members that are potential candidates to confer resistance to powdery mildew in these species. In addition, several clade-specific motifs were identified in the MLO protein sequences as hallmark of each MLO clade. These motifs could be useful to identify additional clade V MLO members in non-sequenced legumes and to search for novel papilla-based penetration resistance sources in crops.

A targeted proteomic approach to identify protein markers for *Didymella pinodes* resistance in pea

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Ascochyta blight, caused by *Didymella pinodes*, is one of major diseases of pea worldwide. Only low levels of resistance are available in pea cultivars. Quantitative Trait Loci associated with resistance to this disease have been identified, but the resistant genes underlying these QTLs are unknown what limits their practical application in Marker Assisted Selection. These QTLs are currently being saturated with additional markers and candidate gene identification is in progress what will certainly improve their use in MAS. In a further attempt to exploit other types of markers we identified protein markers and explored the feasibility for their use in selection breeding. Shotgun proteomic experiments revealed several stress-related proteins differentially expressed in resistant reactions to D. pinodes in pea, 35 of them matching with previous Microarray and SAGE gene expression analysis. By using a targeted proteomic strategy these proteins were searched and measured at constitutive level in a RIL population segregating for resistance. Results still in progress, suggest that at least 18 of these proteins are mostly represented only in the resistant lines and could be used as markers for resistance. Applicability of this strategy in MAS will be presented and discussed.

Search for resistance gene orthologs in *Vicia faba* (LEGATO T2.3.1)

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Orthologues MLO (Mildew Resistance Locus O) genes have been characterization in non-legume and legume species (1, 2). Only members of a specific phylogenetic clade (IV and V) play a role in powdery mildew susceptibility/resistance (3). In Medicago truncatula, the genes MtMLO8 (IV), MtMLO1 and MtMLO3 (V) could confer broad-spectrum powdery mildew resistance (2). To identify sequences of MLO orthologues in faba bean, we have done a search against a recent faba bean transcriptoma (4). Only three homologues sequences for MtMLO1 (MTR_6g033330) were found which cover over 1443 pb. The lack of faba bean lines segregating for this resistance prevented to map or to search for likely loss-of-function variants. On the other hand, four pea candidate genes for ascochyta blight resistance (DRR49a and RGA1.1 (5); JERF and BLEC4) were analyzed in faba bean lines differing for this resistance (29H and Vf136). Searching of orthologues sequences through the faba bean transcriptome databases (4) identified contig4188 (Ethylene-responsive transcription factor RAP2-12) as a JERF orthologue (89% identity) and contig18749 (Non-seed lectin) as a BLEC4 orthologue (84% identity). The other two of putative orthologs were not found. Several primers pairs were designed for each of the previous mentioned genes and the amplified fragment sequenced by Sanger. Unfortunately the analysis revealed no SNPs in any of the gene sequences, preventing mapping or other further analysis.

QTLs and eQTLs detection for rust resistance in *Lathyrus cicera*

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Lathyrus cicera L. (chickling pea) is a cool season legume crop used mainly as animal feed. Chickling pea presents tolerance to abiotic stresses and resistance to some severe diseases, such as rust (*Uromyces pisi*) and powdery mildew (*Erysiphe pisi*). However, *L. cicera*'s resistance mechanisms to these pathogens are still poorly understood. As part of the LEGATO European project, we are interested in unveiling the genetic basis and the molecular strategies of resistance against rust in Lathyrus spp.. To accomplish this, genomic and transcriptomic approaches have been integrated on a L. cicera recombinant inbred line (RIL) population, segregating for resistance to this disease. Previously, Quantitative Trait Loci (QTLs) for resistance to rust were mapped in a first version of a L. cicera RILs population linkage map. In the current work, the resolution of this map was improved by adding new EST-SSRs and DArTseq based SNPs. The revised L. cicera genetic linkage map was used to confirm and refine the location of the QTLs previously detected. In addition, the expression levels of five allelic variants (SNPs), within candidate genes for rust resistance previously identified through RNAseq, were quantified by RT-qPCR using in the same RIL population inoculated with U. pisi. These expression data are being used to detect expression QTLs (eQTLs) for rust resistance. The co-localization of QTLs and eQTLs will be discussed. This study will allow a better understanding of the molecular basis of L. cicera's rust resistance extending considerably the available genomic resources in the Lathyrus genus.

Identification of constitutive metabolites in pea responsible for antixenosis and antibiosis against *Bruchus pisorum*

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Pea weevils (*Bruchus pisorum*) is a pest of the field pea (*Pisum sativum*) of great economic importance. Recently, pea accessions have been reported to reduce seed infestation, and/or larval development. Pods and seeds from these accessions were collected to check constitutive metabolites able to block the egg lying on pods (seed infestation) while pea seeds to check metabolites hampering larval development. Pea pods and seeds were lyophilized in order to evaluate metabolites that may be involved in pea weevil infestation. Standard method for metabolomics analysis was carried out on lipophilic and hydrophilic metabolites extracted, then compared to find a biomarker of resistance in pea. In this communication the studies aimed to identify metabolites responsible for antixenosis and/or antibiosis in pea resistant accessions to weevils will be discussed.

Testing of *Vicia faba* accessions on resistance to bruchids (*Bruchus rufimanus*)

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During 2014 – 2016 Vicia faba accessions provided by Czech genetic bank (Prague, Czech Republic) and CSIC/IAS (Córdoba, Spain) were tested on resistance to Bruchus rufimanus. In 2014 301 accessions were exposed to natural infestation under field conditions (Šumperk, Czech Republic). The accessions with levels of infestation below 5 % were chosen for other testing in glass house: Fioletovy Czysowskich, Chorostovskij, Maris Bead, Afganistan, Fereales, Uran, Ostojski, Stredan, Outlook, Karna, Erfano, M6-9019, BG – 752, BG – 1106 and BG – 1643. In 2015-16 these 15 accessions together with BG – 1265, BG – 1613, BG – 1030 and BG 1017 (infestation below 10 %), two commercial varieties (standards: Merkur and Merlin) and two accessions which showed high levels of infestation in 2014 (controls: BG – 427 resp. BG – 403; infestation above 75 %) were tested. Significantly lower attractiveness of BG-1106, Chorostovskij, Afganistan, Outlook and Erfano for bruchid females proved in more-choice tests. But in one-choice tests females laid eggs on these accessions, too. More than 40 % of individuals died at the stage of egg or during the pod cover penetration in Chorostovskij, Afganistan, Outlook and in Merkur. The seed coat was the most complicated obstacle for larvae in Fioletovy Czysowskich, Maris Bead, Erfano, M6-9019, BG – 1106, BG – 1643 and BG – 1265 (mortality above 30 %). The levels of larval mortalities in cotyledons were not significantly different among the accessions. The overall highest mortalities of B. rufimanus were in Chorostovskij, Afganistan, Outlook, Erfano and BG – 1106 (80 – 100 % of larvae died there during their development).

Mechanism of resistance in faba bean (*Vicia faba*) against broomrape species

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Broomrapes are weedy root parasitic plants that severely constraint faba bean (*Vicia faba*) production in Mediterranean basin. The most widely distributed species affecting faba bean is *Orobanche crenata*, although *O. foetida* and *Phelipanche aegyptiaca* are of local importance. Extensive and long efforts have been made by breeders, but unfortunately only moderately resistant cultivars are available to farmers. Here we summarize a series of experiments intending to characterize the resistance mechanisms involved in accessions with varying levels of resistance earlier reported. In a first rhyzotron experiment we studied the responses of six faba bean accession against *O. crenata*, *O. foetida* and *P. aegyptiaca*. A proportion of broomrape radicles successfully contacting faba bean roots might later become necrotic and fail the tubercle development against a Spanish *O. crenata* population. Formed broomrape tubercles might later become necrotic, mainly in the case of some of the resistant faba bean accessions to the Spanish O. crenata and to P. aegyptiaca but not to a Syrian *O. crenata* or to *O. foetida*. There was little effect on induction of seed germination. In an attempt to go deeper in the germination step a separate series of experiments was performed with other accessions allowing the identification of accessions inducing low germination of *O. crenata*, *O. foetida* and *P. aegyptiaca*, a mechanism that we consider most promising and we are combining with the other ones in our breeding program.

Characterization and expression analysis of MLO gene family members in cultivated and wild species of lentil

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The Mlo gene family has been included into the plant defence-related genes since it is associated with powdery mildew resistance in monocots (barley) and dicots (*Arabidopsis thaliana*, tomato, and pea). We identified 14 Mlo genes in the lentil genome using the available genes of pea and soybean in order to carry out sequence mining based on protein similarity. The phylogenetic analysis of the gene sequences grouped two genes (LcMLO_01 and LcMLO_02) in the same clade that pea PsMLO1. Loss-of-function alleles of PsMLO1 are responsible of durable powdery mildew resistance in pea. Another lentil Mlo gene (LcMLO_10) was included in the clade of the barley resistance genes. Specific primers were designed to amplify the complete LcMLO_01 and LcMLO_02 genomic sequences from interesting lentil (Lens culinaris culinaris) accessions and wild relative species (L. c. orientalis, L. tomentosus, L. odemensis, L. lamotei, L. nigricans, and L. ervoides). The analysis of the sequences revealed a high conservation. Most of the nucleotide differences were located at intronic regions, although a few aminoacid changes were also identified for both genes. Conserved transmembrane motives are present in the deduced protein sequences and no loss-of-function alleles have been detected in the sequences of both genes from the analysed accessions. RNAseq analysis showed significant differences in the expression levels of LcMLO_01 and LcMLO_02 when cultivated susceptible lentil and wild moderately resistant L. odemensis were compared. Such difference was also observed for LcMLO_11. No differences in expression levels were observed for other seven Mlo genes. Expression of four genes (including LcMLO_10) was not detected in either species.

Identification of candidate genes for resistance to ascochyta blight in pea by combining transcriptomics and high-throughput mapping of differentially expressed genes

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Peyronellaea pinodes (Syn. *Didymella pinodes*), causing ascochyta blight, is the most destructive foliar pathogen of dry peas. Resistance to this disease is quantitative and polygenic. Quantitative Trait Loci (QTLs) associated with resistance have been identified, but the genes underlying these QTLs are unknown. The objective of this study is to identify candidate genes for resistance to ascochyta blight in pea by selecting genes differentially expressed in resistant reactions and located into QTLs associated with resistance. With this aim, in the frame of “LEGATO” project, a detailed transcriptomic study was carried out, using MACE technique, to identify genes differentially expressed in the resistant accession P665 compared to the susceptible one cv. Messire. The resulting MACE sequences were used to identify SNPs between P665 and cv. Messire for these differentially expressed genes. These SNPs were further genotyped in the RIL population P665 x Messire in a high-throughput way by sequencing MACE libraries from 94 RIL families. The mapping of these differentially expressed genes in the P665 x Messire genetic map and the study of their possible co-localization with the QTLs associated with resistance to P. pinodes is in progress.

Screening a *Vicia faba* L. collection for Sitona lineatus resistance

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A screening in field condition was carried out in 2014 of a collection of 250 accessions of Vicia faba for intensity of sitona adult damages on leaves and for N2 fixing activity. Extensive damage on leaves was seen in, with no differences between genotypes. 16 most contrasted genotypes for N2 fixation activity were selected for further screening of sitona susceptibility trial at INRA Dijon in spring 2016 and spring 2017. On the 2016 trial again extensive damage on leaves was seen, with no differences between genotypes. We sampled root systems in the field and recorded nodule damage induced by sitona larvae (% of nodules with larval attack). 2 genotypes were highly damaged (56 and 76% of nodules damaged), other genotypes displayed medium attack (11 to 40%) and no completely resistant nodulation system was found. These differences were not significant. However they were negatively correlated with N2 fixation measurements of 2014. This tendency is under reevaluation in spring 2017 in order to establish the significance of genotypic differences.

3. Enhancing legume quality - novel food & feed

Nutritional valorization of cowpea stovers in animal feeding

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The expected increase in legume consumption by humans will generate large amounts of fibrous residues that have the potential to be incorporated in animal feeding. For this purpose, nutritional valorization of cowpea stover was promoted through biological treatments that included its ensiling with discarded apple and its valorization through solid state fermentation with white-rot fungi. Silages containing mixtures of cowpea stover up to 85% showed higher nutritive value and longer aerobic stability compared to the ones with 70% of inclusion, indicating that mixtures of cowpea stover and discarded apples can be conserved by the ensiling process. White-rot fungi treatments showed that Pleurotus citrinopileatus strain was the most effective in the delignification process resulting in higher in vitro organic matter digestibility. This strain was selected to treat sufficient amount of cowpea stover in order to allow its inclusion, in a commercial rabbit growth compound feed, at 10% level. No differences were observed for the rabbits' performance (daily weight gain, daily feed intake and feed conversion rate) when comparing feed containing treated cowpea stover and a normal commercial diet. Furthermore, no differences were obtained for meat quality, carcass traits characteristics, digestive tract histology, as well as for blood haematology and serum biochemistry. Data obtained so far points out the potential of using cowpea stover in animal feeding. However, animal trials should be conducted in order to evaluate the inclusion of the silages in ruminant feeding and, in the case of rabbits, higher levels of inclusion should be tested. Acknowledgements: The research work was funded by the European Union's Seventh Framework Programme for Research, Technological Development and Demonstration under grant agreement n◦ 613781, project EUROLEGUME.

Cowpea fresh pods, a new legume for the market. Assessing the quality and dietary characteristics of 37 cowpea landraces grown in southern Europe.

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Cowpea is traditionally cultivated in some regions of southern Europe mainly for dry seeds; however there is scarce information on the quality and dietary characteristics of fresh pods. In an experiment conducted within the framework of the EUROLEGUME research project at the Agricultural University of Athens, several quality, and dietary characteristics of pods from 37 accessions originated from southern Europe (Greece, Portugal and Spain) were assessed, in an attempt to provide information on pod quality and nutritional properties and identify relationships between quality traits and origin of the accession. Pods from the sesquipedalis subspecies were heavier and larger, and reached commercial maturity two days later than those from the unguiculata subspecies. There were large differences among the accessions in each quality and dietary characteristic. The pods of most accessions were rich in proteins, chlorophylls, carotenoids and phenolics, exhibited high antioxidant activity and low concentrations of nitrates and raffinose-family oligosaccharides. Cluster analysis based on quality, dietary or anti-nutritional traits could not reveal any apparent grouping of accessions. All quality characteristics were independent of the origin of the accessions or the subspecies they belong. Most of the accessions tested produced cowpea fresh pods of acceptable quality and high dietary value, which indicates that they can be introduced to the market and constitute a valuable genetic material for the development of new improved varieties

Economic gains from the consumption of legumes in broiler chicken and dairy cows diets

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Economic assessment of the new products constitutes an essential evaluation to accomplish the general aim of identifying the economic advantages of using domestically grown peas and beans used as ingredients in dairy cow and broiler diets, suitable to replace the currently used soybeans and thus, enhancing the sustainability and competitiveness of the agro-food sector. With this objective, the following tasks have been performed: identification of the effects of different ratios of domestic peas and beans added to diets on feed costs and productivity of broilers and dairy cows. Based on the experimental data, one can conclude that feed rations may comprise peas and beans grown in Latvia, thereby reducing the consumption of imported soybeans. Live weight of broilers and the production efficiency factor increased by 5.5 and 5.2 %, when peas were included up to 20 % in the broilers' diet. Cows feeding trials showed that the feed cost per kg milk produced for dairy cows in the group fed with 10-12% peas and 10-12% beans was 2.3-4.4% lower than for the control group. The average milk yield in the 3rd group (22.0–24.0 % faba beans, var. 'Lielplatone' in feed) was significantly higher than in the control group. This gives an opportunity to decrease the amount of imported soya in the diet, as well as to lower feed costs and raise the growth of peas in the country and the income for farmers.

Bean Blue - a faba bean based blue mould cheese

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There are few vegan and vegetarian alternatives to cheese beside products based on soya beans. The consumer group interested in non-dairy based products is predicted to grow in the near future and thus there is a need to develop foods that mimicry eg ice cream, cheese, milk and cream but are based on plant origin. There is generally interest in food products based on legumes that either more or less imitate standard animal-based food (soya milk instead of cow milk), or are new expressions of food (ready-to –eat bean products). Soya beans have for a long time been used for production of tofu and is suitable to replace milk in eg ice cream. However, in the Eurolegume project we have been able to develop a "cheese" bases entirely on faba beans with a creamy texture and taste of blue mould culture. The production method is partly confidential, but basically, faba beans are soaked in water and milled to a milky slurry. The dry matter is removed by pressing the slurry and the remaining milk is then boiled. Curding agents and commercial blue mould culture is added after boiling. During the chilling process, starch is decanted and the curding process is finalized. By placing the curd in cheese forms we obtain a product that after maturation in about three weeks is ready for consumption. The prototype "cheese" will in 2017-2018 be further developed and commercialised. Consumer release in late 2018 or early 2019.

Minimal processing of fresh legume seeds

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Regular consumption of fruits and vegetables is associated to essential benefits for human healthiness. Within vegetables, legumes are an important source of protein, carbohydrates, vitamins and minerals. Nowadays lifestyle, with little time left to prepare balanced meals, combined to an increased interest in healthy food, have led consumers to demand natural, fresh, and ready-to-eat products. These are represented by fresh cut or minimally fresh processed (MFP) fruits and vegetables, which are typically packaged in modified atmospheres (MAP). Consumption of legumes is sometimes reduced due to difficulties for its preparation. So, the development of MFP pea, faba and cowpea immature seeds could be seen as a good option to avoid shelling. Few reviews deal with the physiological answer of these species to fresh processing. In the studies presented here, respiration rate, compositional changes, deterioration, mostly due to browning, dehydration and microbial contamination of these immature seeds were analyzed. Several sanitizers and antibrowning agents (chlorine, UV-C light, edible coatings, acidified sodium chlorite) were tested as well as the optimal atmosphere composition within packages for each product. Sensory attributes (overall quality, taste, aroma, visual appearance, texture and loss of lightness) were also determined. Obtained products were stored at different temperatures (1 and 4ºC). Anti-nutritional factors, that can limit legumes consumption, were also analyzed. Results suggest that typical quality loss of immature seeds due to processing can be reduced by using the proposed technology. Low temperature storage combined with MAP allowed to obtain high quality products with a shelf of up to 14 d.

Effect of processing on nutritive value of peas and field beans for ruminants

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There has been two research activities within EUROLEGUME at NMBU. Improving feed value of field peas and field beans for ruminants through processing has been the main target in both activities. The processes examined have been steam pelleting, expander pelleting, extruder processing and roasting. The temperatures and treatment times used were selected to reflect a normal range in processing conditions. Steam pelleting is the most commonly used method in feed processing for ruminants, but the temperature rarely exceed 90ºC. Expander pelleting is used to some extent in ruminant feed production and the temperature may exceed 130ºC. Extruder processing is not commonly used, but the method is flexible and allow for a wide range of processing conditions. Roasting is an alternative heat processing method using heat from an external heater (like propane burner), allowing for thermal processing at lower cost and at farm level. The effect of the various processing methods have been examined through analyses of main nutrients (protein, starch, neutral detergent fibre (NDF), ash and fat), analyses of amino acids and rumen nylon bag incubations (in sacco) measuring rumen digestion of protein and starch. The conclusion is that nutritive value of field peas and field beans for ruminants can be improved by processing, but the effect is depending on processing conditions. Compared to an untreated control, processing at mild condition increased rumen digestion of protein. Although with variation, processing at higher intensity and higher temperatures reduced rumen digestion of protein, implying that heat processing can be used to increase protein value of field beans and field peas for ruminants. With respect to starch, heat processing increased rumen digestion. Thus, since there is a positive correlation between rumen digestion and small intestine digestion of starch in ruminants, heat processing can be used to improve energy value of field beans and field peas for ruminants, unless the rumen microbial environment is harmed. Roasting at high temperature in combination with extended treatment time, increased analysed concentration of NDF. At the same time, analysed concentration of total amino acids and in particular lysine was reduced. This indicate overtreatment of protein and care should be taken to avoid that when roasting. No indications for overtreatment of protein was observed for steam pelleting, expander pelleting or extruder processing.

Quality changes of fresh and then microwaved minimally processed faba seeds

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Faba beans (*Vicia faba*) are one of the most common grain legumes, rich in proteins, carbohydrates, antioxidants, fiber, vitamins and minerals. Therefore, the production of minimally processed immature faba seeds would be an alternative to promote its consumption. In addition, that product can be then microwaved, if packaged in an appropriate package. In this study, the effects of sanitation with NaOCl (150 ppm, pH 6.5) or alternatively UV-C light (3 kJ m-2) during storage (10 d, 4º C) on fresh and then microwaved (700 W, 1 min) faba seeds were analyzed. Here, the evolution of sensory attributes (overall quality, taste, aroma, visual appearance, texture, browning, dehydration, and loss of brightness) and tannins, a group of antinutritional compounds that might adversely affect the consumption of legumes, are presented. The microbiological quality of the fresh seeds was also evaluated. Seeds treated with UV-C maintained their sensory attributes above the limit of acceptability after 10 days, while those treated with NaOCl were acceptable until day 7. After 10 days reductions of 1 log CFU for mesophilic aerobes, 0.5 log CFU for psicrophilic and 1.5 log CFU for enterobacteria were achieved for the UV-C treatment respect to NaOCl. Tannins decreased during storage, regardless of the type of treatment. They also decreased after microwaved, with a reduction of around 35% in relation to the fresh product at the beginning of storage. More research is needed to study the effect of these treatments on other quality parameters.

The effect of processing and different thermal treatment on the antioxidant capacity of peas puree

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Legume purees development with eco-innovative technologies is an interesting market, since consumers demand appetizing ready to eat products with healthy properties. For processing, pea seeds were treated with steam (STEAM, 5 min, 80°C) or continuous microwave (MW) flow (9 kW, 40s) and then blended in a food processor (3 min, 1.450 g-1) to obtain a puree. Then, they were packaged under vacuum. Control pea puree (CTRL) consisted on fresh peas blended in a food processor (3 min, 1.450 g-1) at room temperature and vacuum packaged. The effect of different treatments (CTRL, MW and STEAM) on fresh pea and on pea purees on total antioxidant capacity (TEAC) was evaluated by two different assays (DPPH and FRAP). The TEAC evaluated by DPPH in fresh seeds was around 515.1±5.8 mg TEAC kg-1 fw, however the TEAC value decrease around 49% in CTRL puree samples. Additionally, the antioxidant activity evaluated by FRAP in fresh seeds were 354.7±50.9, 454.8±43.4 and 519.3±21.1 mg TEAC kg-1 fw in CRTL, MW and STEAM, respectively, and in pea puree samples were 188.7±10.4, 492.5±30.4 and 343.4±10.4 mg TEAC kg-1 fw in CRTL, MW and STEAM, respectively. STEAM seeds and MW seeds of pea puree samples showed the highest FRAP values, however not significant differences between treatments were observed in DPPH values. The lowest antioxidant capacity was observed in CTRL puree samples for both FRAP and DPPH. MW treatment improved the quality of pea puree, because of the inactivation of enzymes involved in redox status of peas.

Use of *Pisum sativum* (L.) seeds and straw as alternative in feeding of dairy goats

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Grain legumes are considered an alternative vegetable protein resource in animal nutrition. The aim of this study was to assess the effect of the inclusion of pea straw and peas in the diet of Murciano-Granadina dairy goats. The trial was divided into two periods. In the first period pea straw replaced barley straw and in the second period peas partly replaced soybean, corn and wheat in the diet. In both periods of the assay, gross composition and hygienic quality of the milk were not affected by the diet. 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 ($P<0.08$) to 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. 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 for consumer health.

Improvement of maize bread formulation and fortification with legumes based on consumer liking

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The fortification of maize bread with legume flour has been explored in order to increase the total protein content of traditional bread (broa) within the scope of the LEGATO project. Broa is a Portuguese ethnic bread comprised of more than 50% maize flour mixed with either wheat or rye flours. This bread is very well-known in the Country and eaten separately during meals. The quality of maize flour assumes primary importance, determining consumer perception. The optimization of legume formulations considering the influence of maize flours variability was studied. A set of baking tests were performed with white and yellow, regional and industrial maize flours blended with flour from pea, chickpea, faba bean and lentil species. A panel of 60 naïve tasters evaluated under controlled conditions and at different sessions, sets of 5 breads with varying maize flours and each set with a different legume flour. Tasters evaluated overall liking, using a 9-point scale, from 1- "dislike extremely" to 9- "like extremely". A total of twenty composite breads were also analysed for crude protein. The protein content increased in broa with incorporation of 10% legume flours and the highest value was obtained with faba bean (from 8.9 to 12.2 %). There were no major differences of overall acceptability, with the control samples exhibiting the highest liking scores, followed by the broa with yellow industrial maize flour and faba bean or lentils flour. The least liked broas were the ones with the incorporation of chickpea flour, with an average liking of 6.5±1.5.

Consumers' preferences for maize breads fortified with legume flour: an experimental auction in Portugal

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Within the work program of the LEGATO project an experimental procedure with maize breads fortified with legume flour, based on sensory and non-sensory information, was applied to a sample of Portuguese consumers. The main objectives of the experimental auction were: i) to determine the consumers acceptance for different types of fortified breads based on economic valuation of the products – consumers' willingness to pay values; ii) to obtain the preferences hierarchy of the fortified breads, according to these monetary values ("reservation prices"); iii) to assess the impact of information (sensorial, compositional and nutritional) on consumers' willingness to pay. To obtain the consumers economic valuations, participants were put in different information situations where they needed to balance the influences of the sensory (visual and taste) and non-sensory aspects (knowledge of composition and nutrition). The experimental auction was attended by 100 regular consumers of maize bread ("Broa") from the district of Porto. The products under evaluation were four breads fortified with faba bean, chickpea, lentils or peas flour, and also one "conventional" maize bread. The incentive compatible elicitation mechanism used was the original Surplus Comparison Mechanism (SCM) that takes into account the influence of the diversity of products actually available on the market and their comparison. The results revealed that the sensory attributes were essential in the confirmation of the monetary valuations of the consumers; but the non-sensorial information, namely the protein content, was important in strengthening the position of each fortified bread in the hierarchy of consumer preferences.

“By the book” - Development of a communication strategy to increase pulses consumption in developed economy communities

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The need to promote pulses’ consumption in all kinds of communities across the world, regardless their economic status, has been recently highlighted and the importance of environmental, agricultural and health benefits of pulses was already recognized. Promoting pulses consumption in developed economy communities may be challenging when considering the availability of food choices and the fact that they are not dependent on pulses for sustenance. Additionally, south European countries, where pulses used to have an important role in the Mediterranean diets, have undergone a “westernization” on their food habits, with pulses having a marked decline. Taking the food trends that are influencing food habits of developed economy communities to identify its needs, a culinary book on pulses is being prepared as a tool to contribute to increase pulses consumption in a regular basis. The material is being designed in a way to be both a convenient pulses recipe guide and a source of scientific information referring to the genetic diversity, production status, environmental and health benefits and adequacy to specific diets (gluten free, vegetarian, flexitarian). Recipes are being collected, selected and tested based on the simpler, healthier and more convenient trend criteria, but also taking in consideration the gastronomic experience involved with the meal. This last criterion which is a growing trend, is being supervised by author Modesto, considered to be one of the most trusted food authorities in Portugal, and who pioneered cooking television in the country and authored many iconic best-selling cookbooks.

Genetic diversity of basic and carotenoid composition in a germplasm collection of pulses exploited by European breeders

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Although the UN declaration of the 2016 International Year of Pulses expresses the potential of pulses, like peas, chickpeas, lentils, or faba beans in nutrition context, the benefits that pulse offered were not systematically characterized. The objective of this research was to examine the difference of basic composition and concentration of carotenoids in 106 pea, 93 faba bean, 79 chickpea and 48 lentil accessions from a representative collection of the germplasm used by European breeders. The accessions were grouped according to different seed traits (size, shape, color, surface, genotype types). The results showed that protein content falls between 15 and 30 g.100 g-1 and mean values for pea, faba bean, chickpea and lentil accessions were 22.3, 24.0, 19.0 and 25.5 g.100 g-1 respectively. The crude fiber ranged from 3.4 to 11.9 and faba bean exhibit the greatest mean content (9.3 g.100 g-1). The chickpea accessions revealed the maximum mean fat content (5.6 g 100 g-1) of the collection. The two carotenoids identified were lutein and zeaxanthin. Pea accessions had the higher lutein mean (10.8 µg.g-1 flour), chickpea revealed the higher zeaxanthin mean (4.1 µg.g-1 flour) and faba beans accessions zeaxanthin content were negligible. The overall germplasm variability, within and between classes of morphological seed traits (size, shape, color, surface) was discussed. This research identified pea, chickpea, lentils and faba beans outstanding accessions that can respond to European breeders needs for the development of high-yielding varieties with specific grain quality required for markets world-wide. The research leading to these results has received funding from grant agreement n°FP7-13551, LEGATO project.

Innovative and sustainable exploitation of plant protein in future foods

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FoodProFuture aims to develop a knowledge platform for optimal production and utilization of Norwegian plant resources into tasty, healthy and attractive plant-based food products with high protein content. This will be an important stepping-stone to achieving a more sustainable diet with a reduced environmental footprint and improved health benefits for large population groups. Knowledge from this platform will enable the Norwegian food system to produce sustainable plant-based products of high quality, giving opportunities for economic gain both in the agricultural sector and in the food industry. Key bio-resources will be grain legumes. The project aims to produce a range of protein-rich model products created through the combination of improved crop production adapted to the Norwegian climate, and innovative technologies based on dry fractionation combined with other processing technologies. This also includes characterization of functional and sensory properties of the selected model products. The project will further provide in-depth knowledge on health and safety aspects of plant-based products including effects on nutrient bioavailability and possible immune responses. The environmental impact of the entire food system will be assessed using LCA and social analyses. Finally, the project will provide insight about consumer demands for plant-based products and their markets, and uncover current barriers in the food system (agricultural, consumer, industry) blocking the shift towards a more sustainable diet. Increased understanding of the Norwegian consumers and industry's perceptions related to their demands for high protein plant-based food products is crucial knowledge to succeed in a future shift towards a more sustainable diet.

Focused approaches for strengthening seed production system to enhance pulses productivity in India

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Pulses are the staple source of protein, support sustainability of prevalent cropping system, reduce usage of chemical fertilizers and improve soil fertility. The productivity of pulses in the country is still low due to cultivation on marginal lands/rainfed areas and low adoption of improved agronomic practices particularly use of farm saved seeds. The crop development programs like NFSM, BGREI and RKVY are being implemented across the country to increase production and productivity of pulses. Under NFSM, more than 50% of allocation is earmarked for various production interventions of pulses covering 638 districts of country. From 2016-17, an amount of 0.24 million US dollar for each seed hub (150 seed hubs) was allocated to produce 1000 quintals of quality seed. About 3.17 million US dollar was provided to 12 ICAR institutes /SAUs for strengthening infrastructures to produce quality breeder seeds. About 31 thousand ha. of pulses demonstrations conducted in 2016-17 to create seed availability of newer varieties and fast spread of improved technology. The seed minikits of newer cultivars distributed free of cost to pulses growers. The production oriented interventions will be continued in next five years. The pulses production attained a record of 22.40 million tonnes in 2016-17 as result of effective implementation of crop development programs, active involvement of development stakeholders coupled with substantial increase in MSP, procurement through government agencies.

Towards the identification of the interactive partner of the Bs5 protein responsible for the entry of the effector protein molecules in plant – pathogene interaction

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The mutant Bs5 gene, bs5 confers resistance against Xanthomonas bacteria in pepper. These groups of bacteria are also pathogenic in legumes like X. medicaginis and X. axonopodis. The bs5 gene was identified and sequenced by map based cloning. There is a two amino acids (AAs) deletion in the transmembrane domain (TM) of the protein. In the mutant plant the effector protein entry from the bacterium is severely reduced, consequently pepper is resistant after infection. We suppose that the deletion of these two leucines will confer resistance in legumes too. The N-terminal cytoplasmic part of Bs5 contains AAs which are built up in repeats of 5-6 residues having disordered structure with unknown function. The TM domain is cysteine rich therefore it is denoted CYSTM. GFP-Bs5 protein fusions allowed us to demonstrate that the Bs5 protein is located in the plasma membrane. In this period we used specific dye to test whether or not the CYSTM protein is going through endocytosis. It is plausible to suppose that the CYSTM proteins interact with plasma membrane proteins responsible for effector translocations. Having high percent of identity between the human and plant CYSTM domains we argued that high degree of identity should exists between the TM domain(s) of the interacting partners. Since TM domains are identified in human and plants, we started bioinformatic analysis in order to identify homologous human-Arabidopsis TM segments to identify protein candidates interacting with CYSTM domains both in human and Arabidopsis. The results of these experiments will be presented.

Innovative legumes based food products

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Legume products are an alternative to traditional animal-derived products, having all nutritious components of legumes: quality protein, complex carbohydrates, dietary fibre, minerals, and vitamins. The grains of legumes are an important source of vegetable protein in the diet of the world population, being especially important for vegetarians and meat-avoiders and meat reducers. Pulse carbohydrates are slowly digested, which allows some of the lowest glycaemic index (GI) among carbohydrate-containing foods. The aim of the study was to develop innovative legume based products. After screening of available pulses, dry seeds of maple peas (Pisum sativum var. arvense L. cv. Bruno), faba beans (cv Barteks) and cowpeas (*Vigna unguiculata* (L.) Walp. cv. Fradel) were selected. Three types of new products and their production technologies were developed – extruded snacks; pulse spreads, and protein-rich snack bars. In all product groups variety of ingredient combinations were tested. The new product qualities: physical parameters, chemical composition, nutritional value, sensory attributes, and microbial parameters were evaluated and as a result the best products were selected for further studies. The quality of new products during storage in various packaging materials (for extruded snacks and snack bars) and the capacity of alternative processing methods such as sous vide and high pressure processing (for pulse spreads) were studied to provide extended shelf-life on new products. Used extrusion parameters, packaging materials, and technology proved to ensure stable microbiological quality f snacks during 24 month storage. Sous vide and high pressure processing maintained the microbiological quality of pulse spreads during 62-day refrigerated storage.

Characterization of phenolic content and antioxidant activity of legume accessions from five different species

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Legumes are important nutritional food sources, particularly due to their high content in fibre and protein. They are also rich in slowly digestible starch, minerals, vitamins, and phenolic compounds, which have been reported to have multiple biological effects, including antioxidant activity (AA). Dietary intake of phytochemicals through frequent legume consumption may provide health benefits, protecting against several diseases. Within the scope of LEGATO project, in order to characterize and compare the total soluble-free phenolic composition (TPC) and AA of a collection of accessions representing the fava bean, pea, chickpea, grass pea and lentil germplasm diversity in use by the European breeders (93 Vicia faba, 119 Pisum sativum, 87 Cicer arietinum, 116 Lathyrus sativus and 94 Lens culinaris different accessions), phenolic compounds were extracted with 50:50 ethanol:water. The TPC was determined by Folin-Ciocalteu spectrophotometric method and HPLC (high performance liquid chromatography). AA was determined by oxygen radical absorbance capacity (ORAC) assay. TPC and AA of the different legume species were compared. Fava beans showed the highest TPC (1701-452mg gallic acid equivalents/100g of sample), followed by lentils. Peas, chickpeas and grass peas presented similar TPC and AA values. Similarly, fava beans showed the highest AA (4663-14119µM trolox equivalents/100g of sample), followed by lentils, peas, grass peas and chickpeas. A principal component analysis (PCA) was performed to assess the relationship among the accessions based on the TPC and AA data. Results are discussed considering the differences observed among the different species and within accessions of each legume species studied.

Prediction of grass pea (*Lathyrus sativus*) phenolic composition and antioxidant activity by FTIR-ATR

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Lathyrus sativus (grass pea) has been cultivated since ancient times. It is a good source of calories and proteins and it has a relatively high phenolic content, comprising phenolic acids and flavonoids. However, the extraction procedure and the methods of phenolic analysis are time-consuming hampering their use on routinely plant breeding evaluations for quality improvement. Therefore, developing rapid analytical methods, such as FTIR (Fourier transform infrared spectroscopy), for predicting the concentration of nutraceutical components in plant raw materials is a topic of growing interest. Under the scope of LEGATO EU project,116 grass pea varieties were characterized for soluble-free phenolic composition and antioxidant activity (AA). The total phenolic content (TPC) of the sample extracts was measured by (1) Folin-Ciocalteau assay and (2) total peak areas of the chromatograms at 280nm obtained by HPLC (high performance liquid chromatography). Phenolic compounds were identified by HPLC and LC-MS (liquid chromatography tandem - mass spectrometry) and quantified by HPLC. In order to determine the AA, ORAC (oxygen radical absorbance capacity) assay was performed. TPC of grass peas ranged from 41 to 129mg GAE/100g of sample, and AA ranged from 467 to 3719µM trolox equivalents/100g of sample. Grass pea samples were simultaneously analysed using a Thermo Scientific FTIR-ATR spectrometer. PLS (partial least squares) regression was used to analyse data from FTIR spectra, in order to predict grass pea phenolic content, phenolic composition and antioxidant capacity. A multivariate analysis was performed using the Unscrambler X software to compare all samples. FTIR-ATR may be used to get rough estimates for phenolic content and antioxidant capacities.

New legume foods: innovative legume-based foods and drinks for enhanced resource use efficiency in food systems

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The modern food system is a main driver of many environmental problems. A large portion of the human-edible plant products, such as cereals and grain legumes, is fed to animals instead of being used as food. This causes inefficient resource use and larger climate impact than direct use of plant biomass in food products. The New Legume Foods project (Jan 2017-Dec 2020) is a Swedish multidisciplinary and trans-sectorial project aimed at developing climate-smart and protein rich food products based on domestic legumes. The overall goal is to contribute to increased food system sustainability through the development of novel, attractive, health-promoting and environment-friendly foods. Here we present results from the project's initial phase, reviewing current knowledge and recent developments in using legumes as foods in a Swedish context. The presentation covers health benefits of legumes, ways legumes are processed, marketed, sold and consumed in the Nordic countries, experiences of growing legumes in a Nordic climate and environmental impacts of producing legume-based foods. Great potential for increased legume cultivation in Sweden was identified from a market and cropping system perspective as well as potential to explore novel food uses of grain legumes that are currently mainly used as animal feed. The environmental impact of legume based products was found to be lower than corresponding meat based alternatives. Challenges include uncertainties among farmers about choice of legume species for different soil types and cropping systems, and lack of knowledge about crop management in areas with limited experience from grain legume cultivation.

The nutritional value of legume based extruded products

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Legumes are food that has good nutritional qualities. It’s rich in protein and have low fat content, so they should be used more in daily diet. But usually their cooking process can be rather long and time consuming. So in order to make legumes more available extrusion-cooking was used and legume based products were developed. For such products nutritional value should be studied, and such parameters as protein, starch and fat content should be determinated. Thus the aim of this study was to determine nutritional value of extruded legume based products. The study was cried out in Latvia University of Agriculture, Faculty of Food technology and Biotechnology scientific laboratory. Peas (Pisum sativum L.), faba beans (Vicia faba var. majora) and cow peas (Vigna unguiculata (L.) Walp.) were extruded by twin screw extruder. For each type of legume, two types of flour were extruded, one containing only legume flour and another one with 45% wheat flour addition. Protein content in samples with added wheat flour to their extrusion mixture, was significantly ($p<0.05$) lower, that in samples without addition, contrary to starch content – adding wheat flour to extrusion mixture significantly increased starch content in extruded samples. No significant differences ($p>0.05$) were found in fat content of extruded samples and it didn’t exceed 1 g/100g. Total nutritional value for legume based samples didn’t exceed 300kcal, except for cow pea/wheat extruded products – 333 kcal. Lowest nutritional value – 272 kcal was for faba bean/wheat products, but for pea based legume products nutritional value was 296 kcal.

Changes in sensory quality of sous vide and high pressure processed pulse spreads during storage

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Sensory parameters of food are closely related to consumer demand; therefore, sensory quality research is particularly important during the development of innovative products. The aim of the research was to assess sensory quality changes of sous vide (80°C/15 min) and high pressure (700 MPa/10 min/20°C) processed cowpea (*Vigna unguiculata* (L.) Walp. cv. Fradel) and maple pea (*Pisum sativum* var. arvense L. cv. Bruno) spreads in PA/PE and PET/ALU/PA/PP flexible vacuum packaging during 62-day storage at 5±1 °C temperature. Sensory quality was evaluated using descriptive methods (ISO 8586:2012) and the assessment of such sensory parameters as the overall appearance, aroma, mouthfeel and taste of pulse spreads was conducted by consensus of all selected experts. Each sensory parameter was assessed within a 5-point scale; a quality number (QN) was calculated for each sample. Sensory quality of pulse spreads was evaluated by six experts on days 0, 15, 29, 42, 50, 57 and 62. The quality of sous vide treated pulse spreads was very good (QN=4.96–4.75) up to day 29 regardless of packaging film material. Spreads without bruschetta seasoning were of good quality at day 62, whereas spreads with bruschetta maintained good quality up to day 57 and average quality – after day 57. High pressure processed spreads maintained very good quality (QI=4.99–4.79) up to day 57 and good quality after day 57. Both processing methods were able to maintain the quality of pulse spreads during 2-month storage, however, high pressure processing could ensure better quality.

Total phenolics and antiradical activity of sous vide and high pressure processed pulse spreads

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Legumes are principal sources of antioxidant activity in food, based on the abundant phenolic content. The present study was undertaken to assess the effect of sous vide treatment (80°C/15 min) and high pressure processing (700 MPa/10 min/20°C) on the content of total phenolics and antioxidant activity in cowpea (*Vigna unguiculata* (L.) Walp. cv. Fradel) and maple pea (*Pisum sativum* var. arvense L. cv. Bruno) spreads during 62-day storage. Antioxidant compounds were extracted using 70% ethanol and acetone blend (1:1). Total phenolics were determined spectrophotometrically by the Folin-Ciocalteu assay using gallic acid as standard. Free radical scavenging activity of samples was measured using ABTS·+ assay and 2.2-diphenil-1-picrylhydrazyl (DPPH·) free radical and expressed as Trolox equivalent (µmoL) per g of sample. Higher total phenolic content was found in sous vide treated ($p = 0.010$) and high pressure processed ($p = 0.023$) maple pea spreads compared to cowpea spreads. Maple pea spread with bruschetta seasoning had the highest concentration of phenolic compounds – 12.14 mg GAE g-1, whereas the lowest amount was found for cowpea spread without seasoning – 7.49 mg GAE g-1. Overall, high pressure processing showed higher values for total phenolics and antiradical activity. A strong, positive correlation was found for total phenolics and antiradical activity of sous vide treated and high pressure processed pulse spreads using both ABTS·+ ($r > 0.96$) and DPPH· ($r > 0.96$) assays. Based on the high correlation coefficients, both methods are suitable to evaluate the association between total phenolics and antiradical activity in pulse spreads.

Effect of sous vide treatment and high pressure processing on colour difference of pulse spreads

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Colour difference of sous vide treated (80°C/15 min) and high pressure processed (700 MPa/10 min/20°C) cowpea (*Vigna unguiculata* (L.) Walp. cv. Fradel) and maple pea (*Pisum sativum* var. arvense L. cv. Bruno) spreads in PA/PE and PET/ALU/PA/PP flexible vacuum packaging after processing and during 62-day storage at 5±1 °C under daylight luminescence were assessed. Colour was measured with Colour Tec PCM/PSM colorimeter according to the CIE L*a*b* colour system and the total colour difference (ΔE*) was calculated. Colour parameters of pulse spreads were less affected by high pressure processing than sous vide treatment. Significant differences were found ($p < 0.001$) between processing methods and spread samples in different packaging materials, with a significantly lower L* value for sous vide treated samples packed in PA/PE. After processing ΔE* value of sous vide treated samples was significantly higher than that of high pressure treatments ($p < 0.001$). During storage ΔE* value of sous vide treated and high pressure processed samples packed in PA/PE increased to 2.13 and 2.01, respectively, on day 62, however, the colour difference threshold (ΔE* 2.8) for untrained panellists and consumers was not exceeded for any of the samples. Pulse spreads packed in PA/PE had a significantly higher total colour difference compared to samples packed in PET/ALU/PA/PP ($p < 0.001$) after 62-day storage. Light proof packaging film was able to preserve colour of processed pulse spreads better than transparent film under daylight luminescence. If the products are sold in secondary packaging with light proof properties, it is possible to use either of the investigated packaging materials without the loss of quality.

Demonstration and knowledge transfer network for expanding and improving cultivation and utilisation of field peas and field beans in Germany [DemoNetErBo]

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Starting in March 2016, we have set up a demonstration and knowledge transfer network consisting of 75 conventional and organic farmers with longstanding experience of growing field peas (*Pisum sativum*) and/or field beans (*Vicia faba*) across ten federal states of Germany. Furthermore, distributors, processors, advisors, and scientists are integrated in this network. Due to several reasons cultivation of peas and beans has been on a rather low level in Germany in the past years. DemoNetErBo is now supporting and enhancing the cultivation as well as the usage of peas and beans. Based on the experiences of the network participants, we show how to grow, process and feed legumes. We also collect data of the practitioners to question why some of them achieve an additional value from legumes and why some of them do not. The participants of the network are demonstrating diverse best practice examples for cultivation, processing, and utilisation of legumes. The network implements expertise in the extension service by organising exchange and transfer of specific knowledge through field days, conferences, newsletters and press and webpage articles. We connect stakeholders along the whole legume value chain from the feed and food sector. The overall aim is to point out the benefits of using grain legumes at every stage of the value chain. Our efforts can contribute to the substitution of imported soy with regionally produced non-GMO protein crops. The project is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) of Germany within the Federal Protein Crop Strategy (EPS).

Characterization of the biosynthesis of saponins during seed development in peas (*Pisum sativum*) and faba beans (*Vicia faba*)

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The use of pulses as ingredients for the production of food products rich in plant proteins is increasing. However, protein fractions prepared from pea or faba beans contain significant amounts of saponins, glycosylated triterpenes which can impart a bitter taste to the final food product. In addition, saponins have also been described to be involved in plant responses to biotic and abiotic stresses(1). In this study, we identified and characterized the genes involved in saponin biosynthesis during pea seed development(2), and optimized a saponin extraction protocol to follow the biosynthesis of these compounds during the development of pea and faba bean seeds. The identification of mutants affecting the function of key genes of the saponin biosynthetic pathway is currently underway in pea(3). Acknowledgement: This study is funded under the LEG'UP FUI (Unique Interministerial Fund) project (AAP No. 18) References [1] Faizal, A. and D. Geelen (2013). "Saponins and their role in biological processes in plants." Phytochemistry Reviews 12(4): 877-893. [2] Morita, M., M. Shibuya, et al. (2000). "Molecular cloning and functional expression of triterpene synthases from pea (Pisum sativum) - New alpha-amyrin-producing enzyme is a multifunctional triterpene synthase." European Journal of Biochemistry 267(12): 3453-3460. [3] Dalmais, M., J. Schmidt, et al. (2008). "UTILLdb, a Pisum sativum in silico forward and reverse genetics tool." Genome Biology 9(2).

4. Defining ideotypes root phenotyping and microbial interaction

Indigenous nitrogen-fixing rhizobial strains isolated from cowpea in Greece: Phylogeny and genetic diversity

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Cowpea (*Vigna unguiculata*) is a promiscuous grain legume, capable of forming nitrogen-fixing root nodules with diverse symbiotic bacteria, mainly slow-growing rhizobial species belonging to the genus Bradyrhizobium. The aim of this study was to study the genetic diversity of indigenous rhizobia isolated from field-grown cowpea nodules in five geographically different regions in Greece. Cowpea-nodulating rhizobia isolated from alkaline soils were exclusively fast-growing rhizobia belonging to the genus Ensifer/Sinorhizobium, while those from acid to neutral soils were slow-growing rhizobia belonging to the genus Bradyrhizobium. Based on MLSA analysis of housekeeping genes and symbiotic phylogenies of nodC and nifH genes, the Bradyrhizobium isolates showed close relatedness with the type species B. pachyrhizi PAC48T and B. liaoningense USDA 3622T and confirmed that the B. liaoningense-related isolate may constitute a putative novel species and symbiovar within Bradyrhizobium. The B. pachyrhizi-related isolates belonged to symbiovar vignae, which was recently identified in Bradyrhizobium strains isolated from Spain. The Ensifer isolates were classified within the same species with E. fredii USDA257, while their symbiotic gene content was divergent to those from all known E. fredii strains suggesting that they may constitute a new symbiovar for which the name sv. aegeanense was proposed. The Ensifer strains were able to nodulate and fix nitrogen in cowpea but not in soybean and common bean. Our results constitute the first phylogenetic analysis of indigenous cowpea-nodulating rhizobia in Greece and further confirm the promiscuity of cowpea and extend our knowledge regarding the diversity, distribution and evolution of cowpea-nodulating rhizobia in European soils.

Multilocus sequence analysis for the assessment of phylogenetic diversity of rhizobia associated with cowpea (*Vigna unguiculata* (L.) Walp) in Portugal

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Cowpea (*Vigna unguiculata* L. Walp) is known to establish a symbiotic relationship with slow-growing rhizobia known as “cowpea miscellany”, now classified in the genus Bradyrhizobium. Cowpea bradyrhizobial species from Africa, Asia and South America were identified as *Bradyrhizobium elkanii*, *B. japonicum*, *B. liaoningense*, and novel *Bradyrhizobium lineages*. Fast-growing rhizobia have also been isolated from cowpea’s nodules classified in the genera Rhizobium, Mesorhizobium and Sinorhizobium. However, the efficiency of native rhizobial strains is very variable. Several studies reported that the yield of cowpea was not improved by inoculation which could be due to the competitive ability of native cowpea rhizobia and the promiscuity of cowpea to rhizobia species. Thus, the knowledge of naturally occurring populations is fundamental for the development of elite inoculant strains. The aim of this study was to study the diversity of cowpea-nodulating bacteria, collected in different geographic regions in Portugal for selection of effective strains adapted to drought. After phenotypic characterization of the isolates in differential media and evaluation of the nodulation ability in vitro, phylogenetic analysis of the isolates was evaluated using the 16S rRNA gene, 7 housekeeping genes (recA, gyrB, glnII, SMc00019, truA, thrA and atpD) and 2 nodulation genes (nodA and nodC) as well as their concatenated sequences. It was found that nuclear protein-coding genes were more polymorphic compared to the symbiotic genes (nodA and nodC). Furthermore, conventional markers (16S rDNA and recA) were not as informative as the recently newly described markers (SMc00019).

Impact of biostimulator application on the morpometrical parameters of winter pea seedlings (*Pisum sativum* L.)

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In the cooler part of Europe the success of winter pea cultivation highly depends on appropriate plant development before winter. Previous research suggests that plants have to develop short internodes and at least their first two leaves. However, obstacles may come about by sowing which takes place too late due to unpredictable weather conditions or the delayed harvest of a previous crop. An effective solution may be the application of plant growth regulators before the sowing of the seeds. The aim of the study was to assess the pea seedling parameters depending on temperature conditions and growth regulator application. The experiment was conducted in control conditions. Seven different cultivars were compared with regards to the temperature and growth regulators application. The seeds were sterilized and subsequently soaked in growth regulator liquid. The seeds were germinated in a growth chamber for 21 days. The growth development was observed and biometrical parameters of the seedlings were measured (the length and weight of shoots, and roots). We have found that as low temperature slow down seedling growth, because radicles were observed after 10 days of growing. Among the compared cultivars, the best seedling parameters at 5oC gained cv. Enduro. The efficiency of biostimaultors was low, however slightly better results were found compared to those of the control group. Different results were observed in high temperature conditions. The growth and development of the seedlings were visible after just 3 days. The best results were observed in the E.F.B.33 cultivar after the Kelpak application.

Influence of Rhizobium and AMF inoculation on Faba bean yield and protein content

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Symbiotic properties of faba bean with Rhizobium sp. bacteria and arbuscular mycorrhizal fungi (AMF) are well known and reported by others. Stimulating influence of symbionts on faba beans yield and protein content was investigated in the trial performed in Pūre Horticultural Research Centre in the period of 2014-2016. Two strains of Rhizobium (No 23 and No 407) from collection of Latvia University of Agriculture were included as inoculants in the trials of all three seasons. AMF supplied by “Simbion” Ltd. were tested only in 2016 as pure inoculants and in mixture with Rhizobium strains. Inoculated variants were compared with two controls: 1) mineral nitrogen fertilizers applied and 2) without any mineral fertilization. Yield of dry beans was harvested and expressed in tons ha-1. Protein determined analytically by Kjeldahl method, and by technique of near-infrared spectroscopy. Results indicate positive influence of Rhizobium on the yield of faba beans. Especially notable stimulus of Rhizobia was observed in 2015, extremely dry vegetation season. Tendency of positive influence of Rhizobia on the yield was observed in 2014 and 2016. There was found different cultivar response to inoculants – VF-001 was more responsive to Rhizobia strain No 23, but VF-002 to No 407. It leads to speculation that mixes of Rhizobia strains are more efficient for broader spectrum of genotypes. This should be investigated in further trials. AMF had stimulating influence together with Rhizobia treatment, but without statistically significant difference. There was not found influence of inoculants on the protein content in the beans.

Does the rhizobium partner may modulate the capacities of symbiotic pea to compensate a partial water stress?

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Soil is an heterogeneous and fluctuating environment. Water limitation in agronomic soils is often partial but lead locally to the suppression of symbiotic activity and therefore to reduction of N acquisition by the plant. A split-root system was developed in pea to evaluate the capacities of Pea-Rhizobium leguminosarum bv viciae (Rlv) symbiotic associations to compensate a local reduction of water availability. Root systems are split in two parts watered separately. Soil water content was monitored. Water limitation was exerted by stopping watering of a half root system. Arrest of nitrogen fixation is very rapidly observed in root directly exposed to water stress. This local and partial water limitation of the plant triggered a systemic signaling on well-watered roots of the same plant. The compensatory response occurs by increasing nodule biomass (and therefore nitrogen fixation capacities) of these roots not directly exposed to the water limitation. In preliminary experiments pea plants were inoculated with a reference Rlv strain. Response of and root and nodule development that compensate the local water limitation was confirmed. Interestingly, the compensatory response varies according to the Rlv strain as another Rlv strain, displaying similar nitrogen fixation efficiency in watered conditions, was not able to promote equivalent compensatory response. This experimental system has been used as a screen to select Rlv strains able to promote rapid compensatory responses to water stress. This work has received funding from the European Community's Seventh Framework Program (WP3 Task 3.1) under the grant agreement n°FP7-613551, LEGATO project.

Model based prediction of mature pea root systems from seedling root phenotyping information

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Seedling root phenotyping is a high throughput approach to compare cultivars for distinct root system characteristics. However the restricted time of observation makes the inference on mature plant root systems questionable. In this study 16 pea genotypes were phenotyped for root traits in (i) a high throughput system at seedling stage and (ii) a low throughput column based system at flowering stage. A root architecture model was parameterized using the seedling root data to predict the fully developed root systems of the different pea genotypes at flowering. Regression analysis did not show any empirical function that would allow direct prediction of the fully developed root system traits from seedling root data. However, using a mechanistic root architecture model to bridge the ontological gap between the two phenotyping platforms allowed prediction of the mature root systems (root length, root distribution, root branching) of pea genotypes with high accuracy. It was concluded that root models provide an important tool to improve the breeding relevance of information obtained from short term seedling root phenotyping platforms.

Yield formation and protein and nitrogen content of faba bean (*Vicia faba* L.) depending on inoculation

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Rhizobia bacteria and arbuscular mycorrhizal fungi (AMF) are considered to be two of the most important plant symbionts which are beneficial for mineral nutrition and plant growth. A field experiment was conducted in 2016 at Estonian Crop Research Institute to investigate the effect of Rhizobium and AMF inoculation and and their mixture with nitrogen fertilizer application (15 kg ha-1) on the protein and nitrogen content and yield of faba bean. Rhizobium leguminosarum was isolated from Estonian soils and chosen in field experiment based on previous laboratory testing. AMF was obtained from Symbiom Ltd. Czech Republic. Rhizobium and AMF coinoculation with N application proved to be the most effective treatment – 5011 kg ha-1 seed yield was obtained (513 kg ha-1 extra yield). Protein and nitrogen content were significantly higher in in treatment with Rhizobium, AMF and nitrogen together and also in single inoculation with Rhizobium. This work was funded by project EUROLEGUME, grant no 613781

Rhizobia and mycorrhiza fungi inoculum evaluation for the pea and bean growth promotion

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Legume seed inoculation with rhizobia is a common practice in agriculture, for the nitrogen fixation ability of the plant, induced by rhizobia. While rhizobia ensure plant with the necessary nitrogen, mycorrhiza fungi inoculum enhances nutrient and water supply, increasing overall plant fitness. A combined rhizobia and mycorrhiza inoculum has been suggested, as this consortium is thought to improve the growth of whole plant. Three year long, extensive study has been carried out in Latvia with the aim to select the most efficient rhizobia strains for beans and peas, as well as to test the effect of double inoculation with rhizobia and mycorrhiza fungi. From the large amount of data obtained, only a part of data show that these inoculations have significant effect on bean and pea growth. The efficiency of these inoculants is known to be particularly important in poor environmental conditions. Lack of high performance shown by both rhizobia and mycorrhiza in our trial experiments could indicate that the plants were exposed to adequate growing conditions, leading to plant growth close to an optimal; therefore, inoculated plants could not reach significantly higher results than control plants. Nevertheless, plant growth promoting results could be observed in many cases. The efficiency of these inoculants was affected by several factors, such as soil type, temperature, rainfall, plant developmental stage and cultivar. Based on this study, a combination of rhizobia strains with addition of mycorrhiza fungi is suggested to be the most efficient for bean and pea inoculation, to obtain the optimal yield.

Investigating photoassimilate allocation and development of legume root system, nodules and pods with non-invasive methods

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Growth and development of the root system are dependent on carbon supply from source leaves, in a similar manner as other important sinks such as pods and seeds. In nodulated legumes the nodules also present an additional important sink for photoassimilates. Balancing between these sinks is essential for plant survival, performance and yield. However the dynamics of carbon partitioning and the resulting growth and development in the plant are difficult to quantify on different timescales. Therefore we employ three different non-invasive techniques: 1) Positron Emission Tomography (PET) with the short-lived radiotracer 11C is used to monitor the partitioning of photoassimilates and its dynamics among roots and nodules directly at the scale of hours to days with the potential for investigating pods at the same timeframe. 2) Magnetic Resonance Imaging (MRI) is used to study the structural development of roots and nodules in pots over the course of several weeks. 3) Low field nuclear magnetic resonance relaxometry with portable devices (pNMR) is used to study dry matter and water content in pods over periods of several weeks. We will show results from all three techniques for monitoring development of roots, nodules and pods as well as treatment responses of roots and nodules photoassimilate partitioning and discuss their potential for deep phenotyping legumes, nodules and their responses to stress.

Interaction of rhizobia, arbuscular mycorrhizal fungi and beans (*Vicia faba* L.) in different soils of Latvia

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Legumes are important part of a sustainable agriculture. The agronomic benefits of legumes are not any due to 'nitrogen effect' as result of symbiotic nitrogen fixation, but also as important part of crop rotation. Therefore Rhizobium and their host plant relationship studies are renewed not only in Latvia but also in whole Europe. To improve symbiotic system productivity, legume plants are inoculated with mycorrhizae fungi too. Experiments were established to evaluate the effectivity of Rhizobium leguminosarum strains and mycorrhizae fungi on legumes growth and productivity in the different soil types of Latvia. The bean seeds were treated with rhizobia by soaking in bacteria suspension for 30 minutes directly before sowing. Mycorrhiza fungi preparation was obtained from company Symbiom Ltd, contain a mixture of at least three species of mycorrhiza fungi and was added in soil under seeds before sowing. Plant fresh and dry weight, protein content and the weight of nodules were measured at the beginning of bean flowering. Bean yield and its parameters (1000 seed weight, protein content) were measured after harvesting. Bean yield significantly depended on variety, growing conditions during vegetation period. Unambiguous effect of tripartite symbiosis was not approved. Better results were obtained with the combination of rhizobia strain RV407 with mycorrhiza preparation. Experiments were done in the frame of EU 7th Research Framework Programme of the European Union project 613781, EUROLEGUME (Enhancing of legumes growing in Europe through sustainable cropping for protein supply for food and feed).

5. Agronomy

Design, assessment and feasibility of legume-based cropping systems according to stakeholders in three regions of Europe

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European agricultural land used for legume cultivation needs to increase to meet environmental and food challenges. Agronomic and environmental benefits of legume-based cropping systems (CS) – including a large range of legume species, with various practices in the fields (sole or cover crop, intercrop, etc.) – should be understood and valued by stakeholders. Our aim was to evaluate the sustainability and feasibility of legume-based locally-adapted CS, in three European regions (Scania, Sweden; Paris Basin, France; Andalusia, Spain). First, innovative CS were designed in three steps: (i) an initial diagnosis in each region, i.e., description of the most frequent CS and their advantages and limits, and definition of local improvement targets; (ii) the design of innovative CS including legumes, during a common 2-day workshop involving mainly scientists (iii) multicriteria assessment of the CS with the MASC® tool. Second, stakeholder meetings were organized in each region, to (i) present and discuss innovative CS including legumes and their assessment results compared to the reference CS, (ii) discuss the feasibility of the innovative CS with stakeholders, to see if some of them could be implemented in farmers' fields, and (iii) gather the preferences of stakeholders on ranking of performance criteria (weight sets) of MASC®, reflecting their point of view on sustainability. Weight sets were synthesized into four MASC® trees and each innovative CS was re-assessed according to those trees. The results showed that it was possible to identify feasible CS with grain legumes for each region, that were assessed sustainable according to the four MASC® trees.

Use of intercropping in faba bean crops for weed control

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Weed competition hampers the growth of crops and may bring about important yield reductions. Chemical control with herbicides is the most used solution although it entails several problems, such as the appearance of weed resistances to herbicide molecules, environmental damages or high economic costs for farmers. Intercropping may be an efficient alternative to herbicides for weed control. The introduction of another crop in the field increases biodiversity and may result in reduced levels of weed infestation. In the frame of LEGATO project we tested weed reduction in faba bean intercropped with different crops during three consecutive field seasons at Córdoba. The crops introduced were wheat, barley and pea, employing an alternate-row system. Three different experiments were carried out: in one of them replacement intercropping (where rows of the second crop replace rows of the main crop) was chosen; in another one, addition cropping (with rows of the second crop being sown in between the rows of the main crop) was employed; and in a third one, both methods, addition and replacement intercropping, were studied. Results have shown that addition intercropping achieves important reductions of weed coverage and biomass compared to faba bean sole-crop, being barley the most effective companion crop for weed reduction, followed by wheat and pea in decreasing order. On the contrary, no weed reduction was observed in replacement intercropping.

Faba bean rust and pea powdery mildew management by intercropping with cereals and by cultivar mixtures

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Limited biodiversity is a problem of modern agriculture. The generalization of intensive monocrops has entailed a loss of biological richness in agricultural ecosystems, which has originated several problems, such as reduced soil fertility or a higher incidence of pests and diseases. Two strategies to introduce diversification in our agricultural systems are intercropping, by mixing different crops in the same field, or varietal mixtures, i.e., growing together different varieties of the same crop. In the frame of LEGATO project we have tested during three field seasons both strategies to control two important diseases of grain legumes: rust in faba bean and powdery mildew in pea. In the case of intercropping, the main crop (faba bean or pea) was mixed with either one of two cereals (wheat and barley) or another legume (pea or faba bean). As for varietal mixtures, the experimental design consisted of mixing different proportions of a resistant monogenic variety with a susceptible one Alternate-row mixing was the system employed in both experiments. Results point to a significant reduction of the incidence of both diseases in the intercropped treatments compared to that of the monocrop. Both in the case of rust in faba bean and powdery mildew in pea the best results are obtained when the legume is combined with barley. Varietal mixtures also achieve an important reduction in the levels of both diseases in the susceptible variety. A proportion of 50/50 (resistant/susceptible) appears to be the most efficient combination, balancing adequately disease suppression and increase of genetic variability.

Exploring the potential of cover crop integration for soil health in vining peas

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Intensive production of vining peas for the frozen food market has impacted on soil health leading to yield decline. Efficient, long-term crop production relies on healthy soils with healthy microbial communities and good soil structure. Cover crops can be employed to improve the condition of soils by reducing compaction and improving soil structure, helping root penetration and drainage. The incorporation of green leaf material can increase soil organic matter, conserve nutrients, improve earth worm activity and reduce soil erosion. Isothiocyanates released by Brassica plants such as mustards and radish may also act as biofumigants, suppressing some weeds and soil borne pathogens. PGRO, The Green Pea Company and Birds Eye, with funding from the European Innovation Partnership for Agricultural Productivity and Sustainability (EIP-Agri), are undertaking a project investigating soil health in vining pea rotations. The aim of the project is to investigate whether cover crops can improve pea yields and contribute to the economic and environmental sustainability of the pea industry by improving soil health. Field trials will be established each year throughout the project and a variety of cover crop mixtures including Brassica species, Phacelia, cereals and legumes will be planted each autumn before vining pea crops. The project is in its first year and cover crops were established autumn 2016. In 2017, vining peas were sown after incorporation of the green material or direct drilled after cover crop destruction. Parameters indicative of healthy soils will be monitored, including soil organic matter content, soil structure and foot rot pathogen levels.

Impact of rhizobia inoculation on cowpea performance under nitrogen stress

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In soilless culture, inoculation of legumes with rhizobia can considerably reduce the input of inorganic nitrogen to the nutrient solutions. However, the practical application of this approach encounters some difficulties. On the one hand, the supply of plant available nitrogen is important at the early growth stage when the rhizobia are still not functional in terms of N2-fixation. On the other hand, inorganic nitrogen and especially nitrate N inhibits rhizobia colonization. To cope with these two contrasting issues, a fine tuning of the N supply is needed, when legumes grown in soilless culture are inoculated with relevant rhizobial strains. In the present study, two different indigenous rhizobial strains, Bradyrhizobium spp. and Ensifer spp., and a mixture of them were used to inoculate cowpea plants () grown in perlite and supplied with either full-N (total-N 11.2 mM) or 60% of full-N until the flowering stage. Afterwards, the N supply was either reduced to 30% or fully starved until the end of the cultivation period. BNF was quantified at the early pod filling stage using the 15N natural abundance method. Nodules number and weight were also estimated. The results showed that reduction of nitrogen to 60% up to the vegetative stage and 0% up to the flowering stage lead to increased nodule weight while the number of nodules was reduced. Similarly nodulation of cowpea with Ensifer spp., either alone or in a mixture with Bradyrhizobium spp. enhanced cowpea nodulation by increasing nodule weight and number while nodulation with Bradyrhizobium spp. alone was less efficient.

Does mutual legume intercropping improve microbiological activity and nitrogen content?

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In a temperate region, intercropping benefits of better use of light, nutrients, and soil resources are usually exploited in legume/cereal mixture. Cereal provides stability for annual legumes while legume is nitrogen source for cereal due to symbiosis with Rhizobium. The principle of intercropping was applied in growing sainfoin (*Onobrychis viciifolia* Scop.) and field pea (*Pisum sativum* L.), where annual legume helps the better establishment of the perennial one. Apart from other evaluated parameters, we have tried to identify does two legumes act stimulated on rhizosphere activity and nitrogen content. This research aimed to evaluate microbiological activity, with emphasize on Rhizobium and Azotobacter, and nitrogen dynamics in a mutual legume intercropping system. The research included growing of sainfoin with two morphologically different field pea varieties sown in three seeding rates. The results were compared with pure sainfoin stand. It was shown that a two-legume intercropping effect a higher number of microorganisms. In the rhizosphere of both intercrops higher microbiological activity was in the mixture of sainfoin and normal-leaf pea variety, with lower dry matter productivity in the first cut. However, in subsequent cuts dry matter yield of sainfoin and field pea varieties was similar. As it was expected, nitrogen content changed between the cuts and it was higher in the legumes intercropping. This practice seems very promising as an ecological service and the economically justified system via obtained yield and nitrogen increase.

Soil N availability and crop performance in a 3-year rotation of pea, faba bean and cabbage

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To quantitatively assess the benefits of cabbage from rotation with legumes, a field experiment was carried out at the facilities of AUA, located in Copaida during 2014-2017. In this report, only results from the second year are presented, in which cabbage was grown either after pea, or after faba bean, or after cabbage, by applying either conventional or organic farming practices. The soil NO3--N concentration was higher in plots with pea or faba bean as preceding crop (170.1 mgkg-1 and 151.18 mgkg-1, respectively) than in those with cabbage as preceding crop in samples collected before establishing the cabbage crop in the 2nd year. Pea as preceding crop increased significantly the yield of cabbage (68.8 tha-1) compared with faba bean (63,4 tha-1), while cabbage after cabbage resulted in the lowest yield (up to 54,4 tha-1) when grown according to organic farming practices. However, in the conventional cropping system, the highest yield of cabbage was obtained when the preceding crop was also cabbage. The results of the present study showed that cabbage can get benefits only in organic cropping systems when grown in rotation with peas and faba bean, while in conventional cropping systems the reverse is true. The quantification of biological N2 fixation by measuring the natural abundance of 15N in the tested legume species and reference plants at anthesis revealed that peas were capable of fixing from 74 to 188 kg Nha-1, while faba beans fixed from 196 to 593 kg N ha-1, respectively, during the first cropping year.

Organic weed control on Faba bean (*Vicia faba* L.) and Wheat (*Triticum esitivum* L.)

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Weeds are the most important factors in Egypt agriculture and all the world, weed chemical control consumption many billion euros, and bad effects on environment and climate, this work aim to weed control with eco-friendly method without chemical for weed control in two big crops in Egypt faba bean and wheat. the idea for weed control as organic to use allelopathic crops in cropping systems in agriculture, during summer season 2016 sowing two alleopathic crops forage sorghum and sunflower at the vegetative growth stage cutting crop plants and mixed in soil. at winter season 2017 sowing faba bean and wheat in the same soil with sorghum and sunflower residues and another soil . applied 3 weed control treatments hand pulling, chemical control, weedy check. results indicated that allelopathic crop plants (organic weed control) reduced number of weed and fresh weight of weeds grass weeds and broadleafed in wheat in soil sorghum and sunflower, soil with sorghum residues reduced grass weeds only but sunflower reduced grass and broadleafed compared to chemical weed control.

The role of integrated organic carbon sources and phosphorus for improving productivity of legume crops in arid and semiarid climates

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Low water and high temperature stresses had negative impact on soil health, crop productivity and growers income under arid and semiarid climates. Integrated nutrients management and intercropping of legumes in the cereal based cropping system in arid and semiarid climates could increase soil fertility and health, increase crop productivity and growers income. Integrated nutrients management refers to the maintenance of soil fertility and improvement in crop productivity with application of plant nutrients through combined application of organic manures, inorganic/chemical fertilizers and bio-fertilizers. Our recent field experiments on legumes crops (chickpea, mungbean, mashbean, common bean, pigeonpea, faba bean and soybean) confirmed a significant increase in yield per unit area with integrated nutrients management under moisture stress condition. The combined application of plant nutrients especially major nutrients (nitrogen, phosphorus and potash) along with different organic carbon sources (farmyard manure, animal manures & plant residues, composts, biochar) into the soil had significantly improved legumes growth and increased crop productivity and growers income. Under arid and semiarid climates the application of beneficial microbes (Biofertilizers) was also found beneficial in terms of higher nutrients use efficiencies, yield and net returns. Legumes intercropping in winter and summer cereals crops improved the grain yield and fodder quality under cereal based cropping system in arid and semiarid climates.

The effect of crop structure on the abundances and distributions of bruchids (*Bruchus pisorum*) in field peas (*Pisum sativum*)

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During 2014 – 2017 distributions and abundances of B. pisorum eggs and infested seeds were analysed in field pea crops with different structure: pea monocrop (v. Protecta), pea monocrop founded as a combination of two varieties (Protecta with delayed start of flowering + Cisterski with early start of flowering – the second one sown in 6 m wide strips located along edges with Protecta), pea monocrop founded as a combination of the same two varieties – Cisterski sown in 3 m wide strips located along the plot edges and among the 16 m wide strips with Protecta and field pea/barley (Protecta/Sirael – 60/40) intercrop. In general the levels of seed infestation were not significantly lower in pea/barley intercrops than in Protecta monocrops. In plots where the two field pea varieties were combined, strong tendency for aggregation of eggs into the zones with Cisterski was recorded. This tendency was more pronounced in the plots where Cisterski was sown in 6 m wide strips along the plot edges. The seeds of Protecta harvested from the plots containing strips of Cisterski were significantly less infested than the seeds from Protecta monoculture even the seeds harvested from pea/barley intercrop in 2014 – 2016. When margins with the early variety show width of 6 m and the delay in the start of flowering of the main variety (the variety which occupies the inner part of field) is at least 10 days, the majority (90 % or more) of B. pisorum females lay their eggs on plants located at the margins.

The effect of plant diversity on the spatio-temporal distributions of pea aphids (*Acyrthosiphon pisum*) and syrphids (*Diptera*: *Syrphidae*) in field pea crops

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During 2014 – 2017 distribution patterns of pea aphids and larvae of syrphids (aphid predators) and the spatio-temporal associations between the two groups of insects were analysed in large plots of field pea monocrops vs. pea/barley intercrops. The objectives were to find out if there are some differences in the patterns of the pest and its predator distributions in the crops and if the spatio-temporal associations between the pest and its predator are influenced by the presence of cereal accompany. The spatial patterns of insect counts were analysed using SADIE. To compare two distributions (aphids vs. syrphids) in the crops, SADIE - Quick Association was used. In intercrops pea aphids and, in part, syrphids showed a strong tendency towards aggregation on dates when the highest abundances of aphids were recorded. In monocrops these tendencies were not recorded. There was a tendency towards somewhat higher spatio-temporal association between aphids and syrphids in intercrops. In intercrops the distributions of aphids measured at the time of their highest abundance were also more spatially related to the subsequent (measured later) distributions of their predator. This indicates more effective localizing of the host by its predator in intercrops. Significant spatial dissociations between syrphids and subsequent (measured later) distribution of aphids were more frequent in intercrops, too. The spatial dissociation should indicate effective predation induced by syrphids in intercrops. Stronger and more spatially and temporally linked relations between the pest and its predator may explain earlier decline of aphid populations usually recorded in legumes intercropped with cereals.

Evaluation of strawberry - legume intercropping

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During last decade more and more attention in organic and integrated growing approach is paid to plant-environment interactions. Therefore diverse growing systems are applied - intercropping, catch crops, green manure, etc. In order to match sustainable and optimal land use in strawberry plantation and to preserve biological diversity there was established trial in Pūre Horticultural Research Centre in Latvia in the period from 2014 till 2016. Three species of legumes (Faba bean, pea and alsike clover) were sown as intercrop in strawberry field to evaluate strawberry – legume intercropping. Intercropping influence on the soil plantation was determined by detecting soil respiration rate, activity of dehydrogenase and cellulase. Strawberry yield and quality was evaluated in order to detect legume influence on the strawberry productivity. Results indicate significant difference between treatments in soil respiration rate, dehydrogenase activity and cellulase activity. As the last two years – 2015 and 2016 respectively were uncommonly dry during vegetation season, it strongly influenced microbial activity. Especially it was observed in treatments with legumes especially with beans where soil was significantly dryer than in other treatments. Beans and peas had no significant influence on disease spreading, but clover had significantly negative influence on strawberry sanitary state. During first yielding in 2015 there were no significant differences in yield quality and quantity between treatments with legumes and control treatment with nitrogen application. In the second yielding year treatments with legumes showed significant decrease in strawberry yield. It could be caused by significantly lower soil moisture in treatments with legumes.

Why does not Portugal produce more chickpeas?

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The chickpea is one of the oldest legume crops and offers a range of health benefits, like helping to increase people's satisfaction, boost digestion, keep blood sugar levels stable, increase protection against disease and much more. Chickpeas are characterized for high protein content and a strong vitamin and mineral composition, which is why they are often included in many healing diets. In Portugal the production of chickpeas represents 17% of national production of grain legumes. Even though, in Portugal the consumption of chickpeas/capita/ year is only 1kg and the domestic quantity supply is not enough (10%), and quantity of imports is up to 16 ton per year. Portugal has good conditions to produce grain. But why does not produce more chickpeas? There are some causes among which: • Traditional spring sowing • Low level of cultural intensification • High production costs • General reduction of agricultural activity • Absence of an organized commercial chain leading to the importation of these species • The use of traditional varieties susceptible In INIAV (Elvas, Portugal), the Food Legumes Improvement Program has already registered on Catalogues of Varieties of Agricultural Plant and Vegetable Species, 6 varieties of Chickpeas and 3 advanced lines are in the registration phase. These new varieties contribute with protein content between 20 and 23% for food. With this new genetic material (adapted to the autumn sowing; high tolerance to Ascochyta rabiei and Fusarium oxysporum; adapted to the abiotic stress and with great size of seeds), we are sensitizing more farmers to introducechickpea in the Portuguese agricultural systems.

Effects of cover cropping and tillage system on weed infestation, yield formation and N accumulation of organic pea

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Oral presentation: To develop organic systems that are less dependent on tillage some aspects of usage non-legume cover crops were tested in a three year field experiment. This study estimated the influence of some non-legume cover crops: oat, bristle oat, niger and millet, including also the sowing date of the cover crops on weed suppresion, yield formation and N accumulation of organic peas. Pea plants were sown after reduced tillage and by no-tillage system by the use of cross slot no-tillage seeder. Seeding date of cover crops, cover crop species and the tested years showed clear effects on pea growth, pea N accumulation and weed suppresion. Early sown niger and millet led to high shoot biomass accumulation of the cover crop and low weed growth in reduced tillage as well as in no-tillage pea plants. What is more important, after specific cover crops like niger and millet the grain yield of organic pea plants were higher, up to 4.6 t ha-1 by the use of reduced tillage and 3.8 t ha-1 in no-tillage system. It is necessary to underline that none of mechanical weeding treatments were applied during pea growth. To sum up, the conducted study clearly showed the importance of non-legume cover cropping for weed management and improved crop growth in organic pea production.

The effect of plant diversity on the spatio-temporal distributions of pea aphids (*Acyrthosiphon pisum*) and carabids (*Coleoptera*: *Carabidae*) in field pea crops

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During 2016–2017 distribution patterns of pea aphids (Acyrthosiphon pisum) and predatory ground beetles (Carabidae) and the spatio-temporal associations between the two groups of insects were analysed in field pea monocrops and in field pea / barley intercrops. The objectives of the large plot trials were to find out if there are some differences in patterns of spatio-temporal distributions of the aphids and the carabids in the compared crops and if the spatio-temporal associations between the pest and the predator distributions can be influenced by the plant diversity in crops. The spatial patterns of insect counts (aphids, carabids – assessed totally and separately for the individual species) were analysed using SADIE. The tendency towards significant spatio-temporal association ($p<0.025$) between distribution of aphids and the four important species of carabids were recorded in intercrops even monocrops in some dates. It indicates that these species should play important role in diminishing of aphid populations during their development in crops because they were actively looking for the zones with higher abundances of aphids (at these places the probability that aphids fall down is higher). At the same time in some cases decreases in aphid populations were recorded at the zones in crops where previously higher abundances of carabids were recorded (significant spatial dissociation in their distributions; $p>0.975$). That shows some species (especially Poecilus cupreus, Pterostichus melanarius, Pseudoophonus rufipes and P. griseus) should play important role in diminishing of aphid populations during their development in crops and can serve as effective biological agents.

Inoculation requirement of pea and faba bean and selection of Rlv strains

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Legumes have the unique capacity to form N2-fixing symbiotic nodules with compatible rhizobia. However the level of their bacterial partner in the soil, their ability to form nodules in mixture with other bacteria, as well as their nitrogen fixation efficiency in the symbiotic organ may vary and therefore limit the benefit of symbiosis. Our objective is to assess the sufficiency and suitability of native field populations of rhizobia for biological nitrogen fixation of pea and fababean, and to predict the potential interest of inoculation to maximize nitrogen acquisition and performance of these crops. The Rhizobium leguminosarum bv viciae (Rlv) populations of 5 European experimental stations (INRA Toulouse, IFVCNS Novi Sad, SLU, Agritec, University of Cordoba) have been characterized. Soils from 9-13 plots at each site, representative of diverse cropping systems with or without pea and/or fababean, were used for (1) Rlv trapping experiments with pea and fababean and (2) quantification of Rlv diversity in soil using an NGS-metabarcoding method. Efficiency of isolates to fix nitrogen associated with their plant host has been estimated. One to three best Rlv isolates from each site have been used in mixtures for inoculation trails at the five experimental sites in 2017. Effects of the cropping system on abundance of the various classes of Rlv isolate in soils are investigated. The potential of NGS-metabarcoding method to predict the effectiveness of inoculation strategies is evaluated. This work has received funding from the European Community's Seventh Framework Program under the grant agreement n°FP7-613551, LEGATO project.

Lupin-cereal intercropping to improve weed control in lupin: interactions dynamics and performances

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There is a need to design alternative cropping practices for grain legumes in order to secure their productivity and improve weed control while reducing herbicide use. Intercropping can be a way to decrease yield variability and to increase competitive ability against weeds. A non-fixing crop can be seen as a companion crop providing services to a legume in an intercrop while contributing to grain production. This approach is illustrated with winter white lupin (*Lupinus albus* L.), a crop producing protein-rich seeds that could reduce EU dependency on soybean importation. Lupin is known for its high yield variability and low competitive ability against weeds. Lupin-cereal intercrops were compared with lupin sole crops both in controlled conditions and field experiments in Western France. We show the benefits of intercropping lupin with a cereal crop to control weeds and maintain or increase overall productivity compared to lupin sole crop. The mechanisms explaining the benefits are presented, with a focus on the dynamics of growth, Nitrogen and light sharing between lupin, cereal and weeds. During the first growth stages, the contrast between lupin and a cereal in early growth strategies allows for low competition for N within the mixed stand and a higher soil Nitrogen acquisition than lupin pure stands. We also demonstrate the impact of plant traits on interactions throughout the crop cycle using contrasted combinations of species and cultivars. This work shows the relevance of using trait complementarities when designing intercrops.

LegValue, a new EU funded project under H2020

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LegValue The goal of LegValue is to pave the road to develop sustainable and competitive legume-based farming systems and agri-feed and food chains in the EU. To this end, the project will assess both the economic and environmental benefits for the EU agro industry to widely produce and use legumes in a sustainable manner. The feature of LegValue is an approach to research that takes stakeholder-driven objectives. The gap between research and practice will be overcome with close collaboration between non-academic actors and scientists. LegValue will result in the first decision tool for farmers to choose the optimal legume species with their adapted crop management and to assess the economic and environmental benefits of legumes in the cropping and grassland systems. LegValue will contribute to identifying the supply chains that are the most competitive to foster legumes crops. By removing current market opacity and the design of transition pathways, LegValue will provide scientific support for EU and national policy-makers directed at increasing legume production, support technological innovation and organisational innovation in supply chains, meeting the EU Parliament 2011 motion on increasing self-sufficiency on protein rich plant materials. Started the 1st of June, 2017, LegValue is funded under H2020 (Project ID: 727672)

Does a mixture of pea varieties with different leaf morphology improve crop performance?

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Crop diversification in space and time is a key component in the design of resource-efficient cropping systems with reduced dependency on synthetic inputs. A well-known example of crop diversification is the practice of intercropping, i.e. growing two or more crops together in the same field. Intercropping grain legumes and cereals in low-input systems makes use of complementary acquisition of light, water and nutrients to enhance the crop's resource use efficiency and competitiveness against weeds. Within-species diversification in variety mixtures may also increase yield stability, via e.g. complementary tolerance to abiotic or biotic stress. As part of the European LEGATO project, our study has investigated whether a mixture of pea (Pisum sativum L.) varieties with two types of leaf morphology can increase the legume crop's standing ability and competitiveness against weeds. The pea varieties Dukat (normal-leafed) and Partner (semi-leafless) were grown as single varieties and variety mixtures with and without a wheat intercrop in field experiments at four locations in Europe: Serbia (Novi Sad), Sweden (Alnarp) and France in central (close to Paris) and southern (Toulouse) regions. Lodging, weed biomass and crop/intercrop grain yields were recorded during two growing seasons per location. The results showed that a variety mixture of pea can combine the beneficial traits of the single varieties with maintained legume grain yield relative to the highest-yielding variety. However, the benefits in terms of weed reduction and standing ability were not as strong in variety mixtures as in pea-wheat intercrops, which remained the most efficient mixtures.

Combined inoculation of AM fungi and nitrogen fixation bacteria affects dry matter and yield parameters of pea (*Pisum sativum* L) plants

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The study aimed to investigate the combined effects of two different AM fungi and a nitrogen fixation bacteria on growth and yield parameters of garden pea plants. Control and inoculated (Rh. irregularis; Cl. claroideum; Rh. leguminosarum and Rh. irregularis+Cl. claroideum+Rh. leguminosarum) pea plants were subjected to two different levels of NaCl salinity (0 and 50mM NaCl) at the irrigation water. At the pod maturity stage, randomly selected plants were removed from the substrate and individually analyzed. The lengths of main root and the height of the main shoot were measured and after that the dry matter of roots and shoots were individually weighted. Simultaneously, the number of pods and seeds per plant were measured and the fresh harvested seeds of plants were individually weighted. Saline irrigation water strongly diminished the growth and strongly reduced the yield parameters of pea plants. The AM fungi demonstrated different effects regarding growth and yield parameters of pea plants under saline irrigation conditions; a strong growth promoting effect and higher yield was found due to Rh. irregularis presence, but no positive effect was seen due to Cl. claroideum inoculation. Non significant positive effects were seen in case of lonely application of Rh. leguminosarum, but strong synergetic effects of its combined inoculation with AM fungi were found under fresh (0 mM NaCl and saline (50 mM) irrigation water. Therefore, the potential application of AM fungi as an alternative for salinity alleviation in pea plants should be done only after salinity resistant AM strains are carefully selected.

Results of an analysis of the conventional cultivation of grain legumes in farms

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The shape of the current CAP reform and country-specific agri-environmental measures have resulted in an increased interest in faba beans, peas and lupins in agriculture, although lack of economic attractiveness is often cited as a reason against the cultivation of these crops. Between 2012 – 2014 in the research project "LeguAN - Innovative and holistic value-added concepts for functional food and feed from domestic grain legumes from cultivation to use" it could be analysed why conventional farms are currently growing grain legumes. In addition, the economic viability of faba beans, field peas and lupines in conventional farms was examined on the basis of accounting data. In comparative terms, the legumes were each presented with an economic alternative culture. A total of 97 data sets from the regions of North, East and Central Germany could be evaluated in these three years. The high preceding crop effect, labor-economic advantages and the feeding of the grain legumes on the company's own farms were important reasons for the interviewed farmers. On average, positive direct-cost and labor-free benefits (DLB) were generated for grain legumes. Taking into account the preceding crop effect and using a calculation of the fertilizer costs via nutrient substitution, 64-100 % of farms with faba beans, 38-75% of farms with field peas and 25-33 % of farms with lupines achieved a higher DLB than with comparative cultures.

Wheat/faba bean intercrop response to tillage in rotation with sunflower in a Mediterranean Vertisol

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A 3-yr study was conducted to determine the effects of tillage system (conventional tillage and no-tillage) on wheat/faba bean intercrop and sole crops performance within a dryland rotation with sunflower. Wheat was intercropped with faba bean in rows of 2:1 arrangement. Duplicate sets of plots were established within each block to allow the presence of all phases of the rotation each year. The field experiment was designed in a split plot randomized block, and each block was replicated four times. Grain yield, yield components, land equivalent ratio, nodules (number and weight), bioavalability of nitrogen by a SPAD meter, broomrape infestation, and sitona weevil damage were measured.

Comprehensive evaluation of faba bean (*Vicia faba* L.) lines within the framework of the FP7 project LEGATO

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The overall aim of the project "LEGumes for the Agriculture of TOmorrow" (LEGATO) is to contribute to the increased sustainable reintroduction of grain legumes in European cropping systems. The project focuses on identification and testing of novel legume breeding lines of the major grain legumes cultivated in Europe possessing valuable characteristics such as disease and pest resistance and quality for human consumption. A broad based testing of new varieties gives important information for both breeders and growers. Eleven faba bean varieties from five European countries were tested in field trials in Estonia in 2015 and 2016. Evaluation of morphological and economical characteristics, resistance for biotic and abiotic stresses and quality was carried out. Average seed yield was 4.1 t ha-1 in 2015, 3.4 t ha-1 in 2016, the highest yield was in both years 5,1 t ha-1 (the variety Taifun in 2015, the variety Fuego in 2016). Average protein content in seeds was 29.93% in DM in 2015 (the variety Julia - 32,44% in DM), 30.48% in DM in 2016 (the variety Fury - 32,14% in DM). The most imortant plant disease was faba bean rust (Uromyces viciae-fabae) in both testing years: the damage was 6.5 points in 2015 (1-9 scale, 1 - maximum damage, 9 - no damage), 5.6 points in 2016. Acknowledgements: The project LEGATO, Grant no 613551

Legume cropping systems diversification and intensification for soil fertility improvement and family nutritional benefits

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A study was carried out in 2011/2012 growing season in Kandeu and Manjawira Extension Planning Areas, Central Malawi to determine biomass yield and subsequent effects on soil fertility of the crop residues from various legume cropping systems. The experiments were laid out in a randomised complete block design with farms serving as replicates. In Kandeu, there was significant difference ($p<0.001$) in the dry weights of groundnut haulms in the sole and that intercropped with pigeonpea. There was significant difference ($p=0.007$) in the dry weights of tops for soyabean in the sole and intercrop with pigeonpea. The crop residues of pigeonpea in the sole crop, intercrop with groundnut and intercrop with cowpea were significantly different ($p<0.001$) from each other. All the LER values were greater than 1 which means that intercropping of soyabean, groundnut, pigeonpea and cowpea were efficient in utilising resources, resulting to higher grain yield compared to the sole cropping systems of the same legumes. In both EPAs, LERs for pigeonpea intercropped with groundnut was higher, indicating yield advantage of the pigeonpea intercropped with groundnut. Crop residues from various legumes cropping systems have profound impact on soil fertility, as such, stakeholders should help intensify and scale out legume growing and incorporation of crop residues from various legume cropping systems (eg groundnuts and cowpea) by farmers, supplementing with low levels of inorganic fertiliser (24 kg N ha-1) in order to help them harvest increased maize grain yield.

Integrated nutrients management and intercropping is key to improve legumes crops productivity in drylands

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Low water and high temperature stresses had negative impact on soil health, crop productivity and growers income under arid and semiarid climates. Integrated nutrients management and intercropping of legumes in the cereal based cropping system in arid and semiarid climates could increase soil fertility and health, increase crop productivity and growers income. Integrated nutrients management refers to the maintenance of soil fertility and improvement in crop productivity with application of plant nutrients through combined application of organic manures, inorganic/chemical fertilizers and bio-fertilizers. Our recent field experiments on legumes crops (chickpea, mungbean, mashbean, common bean, pigeonpea, faba bean and soybean) confirmed a significant increase in yield per unit area with integrated nutrients management under moisture stress condition. The combined application of plant nutrients especially major nutrients (nitrogen, phosphorus and potash) along with different organic carbon sources (farmyard manure, animal manures & plant residues) into the soil had significantly improved crop growth and increased legumes crop productivity and growers income. Under semiarid condition the application of beneficial microbes (Biofertilizers) was also found beneficial in terms of higher nutrients use efficiencies, yield and net returns. Legumes intercropping in winter and summer cereals crops improved the grain yield and fodder quality under cereal based cropping system in arid and semiarid climates.

Effect of intra-annual rotations with legume species on horticultural crop production and soil quality

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In this study we assessed the effect of preceding legumes (cowpea: *Vigna unguiculata*) on crop production and soil quality in subsequent non-legumes crops (broccoli: *Brassica oleracea*) during three crop years. We also assessed if cowpea cultivars (Feijão frade de fio preto and Feijão frade de fio claro) and management practices (conventional and organic) significantly influenced crop and soil properties. The study was randomly designed in blocks with four replications, in plots of 10 m2. Soil samples (0-30 cm) were collected from each plot at the end of the crop cycle to measure biochemical and chemical parameters in soil. Crop production and quality were determined at the end of the cycle. In cowpea, after three crop years and considering cultivar and management practice, we observed a significant increase in nitrogen and phosphorus content, together with a significant decrease in sodium and potassium content. Nitrogen was positively correlated with carbon ($R > 0.65$, $P < 0.01$) and phosphorus content ($R > 0.79$, $P < 0.01$), while this was negatively correlated with dehydrogenase activity ($R > 0.74$, $P < 0.01$). Soil parameters in broccoli crop did not follow a clear pattern. There were no significant differences in crop production and quality for the subsequent broccoli crop compared to broccoli in monocrop. These results demonstrated that the inclusion of cowpea in rotation significantly affected the amount of nitrogen and phosphorus, however, this grain legume did not lead to increased crop production and quality in the broccoli crop.

6. Climate changes, stress adaptation & mitigation measures

Effect of humic acids and calcium sulfate application on yield and plant developent of soybean

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Global warming created an opportunity to cultivate soybean in Central Europe. In 2011 the area of its cultivation in Poland amounted to 300 ha. In 2016, it was already 300.000 ha. The aim of the study was to evaluate weather humic acids and calcium sulfate application improve the quantity of yield of three soybean cultivars and influence plants development. Humic acids are known to have a positive effect on plant biomass, and improve growth of roots as well as of shoots. Since the majority of arable soils in Poland are acidic, it is important that calcium sulfate reduces the soil acidity and neutralize the Al toxicity. Moreover, calcium is the basic element needed for good transportation of nutrients. The experiment was conducted in 2016 in Experimental Station of Agricultural University in Krakow (Poland) in chernozem from loess, slightly degraded soil. The experiment consisted of two factors – cultivars (Atlanta, Augusta and Merlin) and soil treatments (humic acids (400 kg ha-1), calcium sulfate (30 kg ha-1), humic acids + calcium sulfate and control plots) – and was carried out with complete randomized design. Conducted research shown that cultivars, as well as used treatments didn’t influence the yield significantly. However, the plant development varied significantly between cultivars. Especially the cultivar of very early maturity – Merlin developed the biggest number of pods and seeds on the plant and gained the biggest seed mass. The application of humic acids increased the number of pods and seeds on the plant, and the weight of seeds, stripped pods and shoots, but not statistically significantly.

Effects of drought stress in cowpea: a gene expression analysis during seed development

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Drought is one the most severe environmental stresses with major impact on plant development and productivity. Cowpea [*Vigna unguiculata* (L.) Walp.] is pointed as one of the most tolerant grain legume crops to drought being considered as an ideal model to study the molecular mechanisms of drought tolerance. The main objective of this study was to understand the effects of drought stress during seed development. In a greenhouse, two Portuguese cowpea genotypes (Cp5051 and Vg50) were submitted to two watering regimens (0% and 100% of field capacity, FC), after flowering during seven days. Two genotypes, Bambey21 (highly susceptible) and IT93K-503-1 (highly tolerant), were used as controls. Leaves, pods and seeds in different stages of development were collected and RNA was extracted. The gene expression levels of eighteen genes related to drought, oxidative stress and important storage proteins were evaluated by semi-quantitative PCR. Two housekeeping genes were used as control. Differences between genotypes and water regimes were observed. A total of six genes were choose to perform a real-time quantitative PCR and consequently to help the understanding the effects of drought stress in reproductive stage.

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Agronomic performance of a worldwide grass pea collection under mediterranean conditions

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Grass pea (*Lathyrus sativus* L.) is a promising source of calories and protein for dryland farming systems. Nowadays underused in Europe, grass pea was in the past widespread in the Mediterranean region. The superior atmospheric nitrogen fixation rate, allied to the ability to tolerate salinity, drought and flood, make this species highly potential for sustainable agriculture. Despite its tolerance to some important diseases, its resistance is however limited to some legume pests such as weevils, causing severe quality losses. Under the scope of the EU LEGATO project, we evaluated a worldwide collection of 150 different grass pea accessions under Mediterranean field conditions. Our objective was to identify sources of resistance to the main biotic stresses, associated with relevant agronomic performance. The collection was tested during two growing seasons in an α-lattice design, with two replications, in double-row plots. The establishment and evaluation of the field trials were done in a participatory approach in a traditional Portuguese grass pea production area – Alvaiázere. Seven quantitative traits (plant growth rate – stage II, plant height, plant yield, 100 seeds weight, number of seeds, number of pods and percentage of weevils infestation) and nine qualitative traits (flower and seed colour, seed shape, growth habit, plant type, stem colour, leaflet shape, leaf tendrils and leaf senescence) were measured. Analysis of variance and multivariate principal component analysis allowed us to perform a comparative assessment of the accessions performance in this region and discriminate outstanding pre-breeding materials to be incorporated in future quality-oriented grass pea breeding programs.

Grain and biomass yield of white lupin germplasm under contrasting moisture availability

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White lupin breeding can rely on agronomically outstanding landrace genetic resources. However, information is scant on the genetic variation for drought resistance. We aimed to investigate such variation, by a managed-environment experiment that imposed two contrasting moisture conditions under a field rainout shelter. One condition implied severe stress, with available water (field capacity minus wilting point soil water content) in the range 0-20% from the late vegetative period onwards; the other was favourable, with available water in the range 60-80% throughout the crop cycle. We evaluated 21 landraces from 10 geographic origins along with one variety and two breeding lines. We found wide variation between moisture conditions and among genotypes for grain yield, dry biomass yield, and harvest index. A few landraces displayed good grain yield in both conditions, and a few others were consistently low-yielding. Several genotypes showed genotype × treatment interaction of cross-over type between favourable and stressed conditions, and specific adaptation to either condition. One landrace from Maghreb and one from Jordan displayed outstanding grain yield under drought. A breeding line selected in Morocco exhibited greatest specific adaptation to stressful conditions (as expressed by highest value of the grain yield ratio between stressed and favourable conditions). Stress escape via early onset of flowering contributed to drought resistance, but mechanisms of intrinsic drought tolerance were important as well. A breeding programme specifically tailored to drought tolerance is advisable for severely drought-prone regions, whereas selection for wide adaptation could be searched for when targeting less unfavourable and/or climatically-variable regions.

Dissection of Pea responses to drought during seed filling and the interplay with sulfur metabolism

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Drought is a major environmental factor limiting crop productivity. In pea drought stress occurring during the reproductive phase can greatly affect seed yield and quality. We investigated the response of pea plants (var. Caméor) subjected to water stress during the seed filling period, a phase associated with massive remobilization of nutrients from the vegetative organs to sustain seed high-nitrogen demand. Transcriptomic profiling of leaf response to water stress revealed metabolic and regulatory pathways affected by drought and enabled the selection of candidate genes for drought resistance. One of these genes, named RAMOSUS1, encodes a carotenoid cleavage dioxygenase involved in strigolactone biosynthesis. Interestingly, preliminary phenotyping of the corresponding mutant showed increased sensitivity to drought compared to the wild-type. Because sulfur nutrition has been suggested to play a role in stress tolerance, we next investigated the interplay between drought and sulfate deficiency. Sulfate-deprived pea plants were subjected to a water-stress during the early reproductive phase. The combined stresses strongly affected yield components and analysis of seed protein composition revealed differences in the accumulation of sulfur-rich (11S) and sulfur-poor (7S) globulins in response to individual or combined stresses. To elucidate the metabolic and regulatory networks connecting sulfur nutrition and drought response, leaf and seed tissues were subjected to proteomics, transcriptomics and metabolomics. A network is under construction that will be enriched with phenotyping and physiological data.

Effects of Rhizobium inoculation on growth, N-fixation efficiency and protein levels in six field-grown faba bean (*Vicia faba*) genotypes

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Matching high performing varieties of legumes with effective symbiotic N-fixing bacteria can potentially enhance production volumes and economic returns when cultivating grain legumes. We investigated whether field inoculation with local or introduced Rhizobia to six different varieties of faba bean improved growth, nitrogen (N) fixation and protein content in a field experiment in Southern Norway. In 2016, a full factorial experiment featuring three inoculation treatments (a mixture of four morphotypes of Rhizobia isolated from locally grown faba bean, a mix of two efficient and well documented Rhizobium strains from Latvia, and a non-inoculated control treatment) and six faba bean (Vicia faba) genotypes (Agua Dulce, Bauska, Jõgeva, Gloria, Julia, Lielplatones) was set up in an experimental field with sandy loam soil with no recent legume culture history (>10 years). At late flowering/early pod formation stage we quantified N fixation of the crop using the N-15 natural abundance method, using weeds from the same plots as reference plants. We also assessed morphological and phenological characters, seed yields and protein levels at plant maturity. Clear differences were observed, and detailed results from this study will be presented at the conference (analyses are still pending). This research is a part of the EU FP7 project Eurolegume.

Combined inoculation of AM fungi and nitrogen fixation bacteria affects root morphology and enhances the nutrient acquisition capabilities of pea (*Pissum sativum* L) plants

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The study aimed to investigate the effects of commercially available AMF inoculate (*Glomus* sp. mixture), a nitrogen fixation bacteria (*Rhizobium leguminosarum*) and of their combined effects on the growth and the nutrient acquisition of garden pea. Control and inoculated (AMF, RhL and AMF+RhL) pea plants were subjected to three levels of NaCl salinity (0, 20 and 50mM NaCl). In randomly selected plants, the morphology of root system was analyzed and the dry matter of roots and the aboveground biomass were individually measured. Furthermore, plant tissue samples were analyzed regarding N, P, K and Na concentration and the total uptake between DAS 90 and DAS 60 were calculated. In addition, RUR were estimated regarding N, P, K and Na, and SAR of N, P and K per unit of root length were calculated. Saline irrigation water strongly diminished the growth of root system and entire pea plants and strongly reduced the nutrient absorption capacity of their root system. The inoculation of AM fungi or Rh. leguminosarum either alone, or combined, improves RUR as well as increases SAR of main nutrient elements (N, P, K). Synergetic effects of combined inoculation of AM fungi and Rh. leguminosarum were found under fresh irrigation water conditions (0 mM NaCl), but not in case of saline irrigation water. Considering enhanced nutrient acquisition capabilities of the root system the artificial inoculation of symbiotic microorganisms could be considered as an effective alternative to improve growth of pea plants under either fresh or saline irrigation water conditions.

Characterization of 21 common bean (*Phaeolus vulgaris* L.) genotypes for biological nitrogen fixation under drought

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Drought stress limits common bean (*Phaseolus vulgaris* L.) production worldwide. Tolerance to drought is becoming an increasingly important factor due to climate variability. In addition, since common bean is a legume, it fixes nitrogen through symbiotic processes with rhizobia and, in turn, aids in lessening the use of inorganic nitrogen fertilizers. The objective of this study was to characterize 21 common bean genotypes for biological nitrogen fixation (BNF) under drought. These 21 genotypes were composed of 4 types: type IIa, IIb, IVa, and IVb. Due to their different growth habits, each was posed as an experiment. Thus, 4 experiments were conducted under two environments, screen house and field. The results showed that drought reduced the BNF capacity of genotypes and inoculation improved performance of most genotypes in the screen house but had no significant difference for genotypes under drought in the field. Nodule effectiveness of genotypes also reduced with drought. The genotypes DRK57 and A286 had high nodule number, mass and BNF under drought at mid pod fill in both screen house and field. The genotypes CIM-Climb-01-03-40 and CIM-Climb-01-03-34 also had high BNF under drought. Thus, the identified superior genotypes may be used for further breeding and research in BNF.

Drought stress tolerance in the early vegetative stages of field pea at biochemical and molecular level

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Drought stress leads to a creation of reactive oxygen species (ROS) in plants. Plants have developed antioxidant defense mechanisms in order to neutralize the effects of ROS. The aim of this research was to determine the molecular basis of drought stress tolerance in 7 field pea varieties at the early seedling stage in controlled conditions (phytotron chamber). Control plants were irrigated at the optimal level of soil water regime (36% substrate water content). Soil drought was induced by the termination of irrigation up to 18% (moderate drought), and 9% substrate water content (severe drought). The activity of antioxidative enzymes: SOD, APx and GR was measured spectrophotometrically. Total RNA was isolated from stressed and non-stressed plant leaves. The gene expression levels of genes encoding antioxidative enzymes were examined by semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) technique. Arabidopsis 18S rRNA was used as internal control. The activity of antioxidant enzymes was changed in all pea cultivars under osmotic stress compared to control. In the tolerant cultivar Trezor activity of all three enzymes was increased, while in the sensitive cultivar Javor their activity is decreased. The molecular analysis can explain changes in antioxidant enzyme activity. The reaction of the tested cultivars to ROS was the result of upregulated expression of the tested genes in tolerant cultivars i.e. downregulated expression in sensitive cultivars. The obtained results show that biochemical and molecular analyses for antioxidant enzymes (SOD, APx and GR) can be useful for testing tolerance to drought stress in field pea genotypes.

Performance of grain legumes genotypes at 15 sites in Europe

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Pan-European field trials with grain legumes were carried out at 15 European sites, well distributed within wide perimeter including UK, Estonia, Serbia, Greece and Portugal. Totally 106 cultivars belonging to 5 legume species were tested – 51 pea cultivars, 30 faba bean cultivars, 6 chickpea cultivars, 4 grass pea cultivars, 13 white and 2 yellow lupin cultivars. At each site four randomized blocks containing small plots with harvested area about 10 m2 were established according to a local practice. Each cultivar was tested at minimally four sites within a climatic zone. A 2-year study was conducted to evaluate genotype performance on the field and yield potential of planted crops and cultivars under targeted environmental conditions.

Due to the testing of a variety of genotypes in pea and faba bean the control cultivars were selected for comparison. Chickpea was tested only in Mediterranean climatic zone. Grass pea was tested in Greece, Austria and Serbia. White lupin was tested at 5 sites, in Greece, Portugal, France, Czech Republic and Serbia, yellow lupin was tested only in Portugal. 2 year´s results demonstrated strong genotype x environmental interactions. The most yielding genotype per each crop has differed according to a locality and year, in most cases the yield was higher in the first year of testing. In second year the yield was influenced by the occurrence of pests and diseases. The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/ 2007-2013) under the grant agreement n°FP7-613551, LEGATO project.

Emissions of greenhouse gases in legume-cereal crop rotations in the north of Portugal

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Extra inputs of nitrogen and carbon to cropping systems provided by legumes related to their ability to fix atmospheric N through symbiosis with rhizobia may enhance greenhouse gases (GHG) emissions due to increased soil microbial activity. Experiments were carried out at Vila Real, north of Portugal, during three years (Summer 2014 – summer 2017) aiming to assess the effects on the emissions of nitrous oxide (N2O) and methane (CH4) associated with the introduction of cowpea in short rotations (succession cropping) with triticale for forage production. Cowpea was grown from May to September and triticale from October to May. In addition, experiments include treatments to evaluate the effect of removal of legume residues or their incorporation into the soil after harvest. Triticale crops grown as monoculture not fertilized or fertilised with 80 kg mineral N ha-1 were used for comparison. Incorporation of cowpea residues, instead of their removal at harvest, represented an extra input of N into the soil ranging between 15.9 and 60.9 kg ha-1. The highest annual emission of N2O (1.92 kg N-N2O ha-1 yr-1) was measured in the monoculture of triticale fertilized with 80 kg mineral N ha-1; this value was statiscally higher than the values obtained when cowpea residues were incorporated or removed or when triticale was cropped under monoculture without N fertilizer, with no differences among them. The soil acted as a sink for CH4 in all treatments, but the highest sink effect was observed when cowpea residues were incorporated, 0.81 kg C-CH4 ha-1 yr-1, which represented about four times more soil CH4 absorption than when cowpea residues where removed. Although, there was no differences in the soil CH4 absorption between the treatments of cowpea-triticale and the treatments of cereal monocultures. Results showed that the introduction of the cowpea crop did not produce any harmful effect in terms of GHG emissions.

Chemical characterization and dry matter digestibility of some varieties of faba beans and their straw

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Chemical analyses, enzymatic and Tilley and Terry digestibility were used to describe the feeding values of fifteen feed samples, from which seven faba beans of different varieties representing local cultivars and foreign ones grown in Albania and eight faba pods and peas straws representing under utilized ones in ruminant feeding. All samples were analyzed for their chemical content, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), acid detergent insoluble nitrogen (ADIN) and neutral detergent insoluble nitrogen (NDIN) and digestibility of dry matter. The under-utilized feeds, pea straw and faba pod showed similar chemical content for main Weende parameters. Average protein content of faba beans resulted 31.9 % changing with varieties 28.3%-36.5 % with the highest value in foreign ones. There were not significant differences between DMD of faba pod and pea straw determined with each in vitro method. The dry matter digestibility values determined with Tilley and Terry method for all feeds included in the study resulted higher than DMD determined with enzymatic method. (94.0 vs. 84.8 for faba beans and 59.85 vs 40.65 for straws.). The results of DMD determined by two “in vitro” methods were strongly correlated. According to R 2-value (0.95) the DMD determined by Tilley and Terry method could be predicted from enzymatic test. Faba beans, their pods and pea straw could be a good feed resources for ruminant animals and must not be under valuated.

Anti-nutritional factors in soybean

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Soybean is at the moment an important crop cultivated worldwide due to its high protein and fat content and also other nutritional value. So the profitability of its growing is increasing every year in Europe. But there is also a „dark side“ of this plant, what reduces the quality of it. There are many controversies around different soybean foods and feed whether they are good or bad for human and animal health. Soybean contains anti-nutritional factors, which inhibits the absorption of certain nutrients like for example minerals and also inhibits the digestion of other nutrients. The aim of this presentation is to show the controversy of this famous *Fabacea* plant based on recent literature review

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