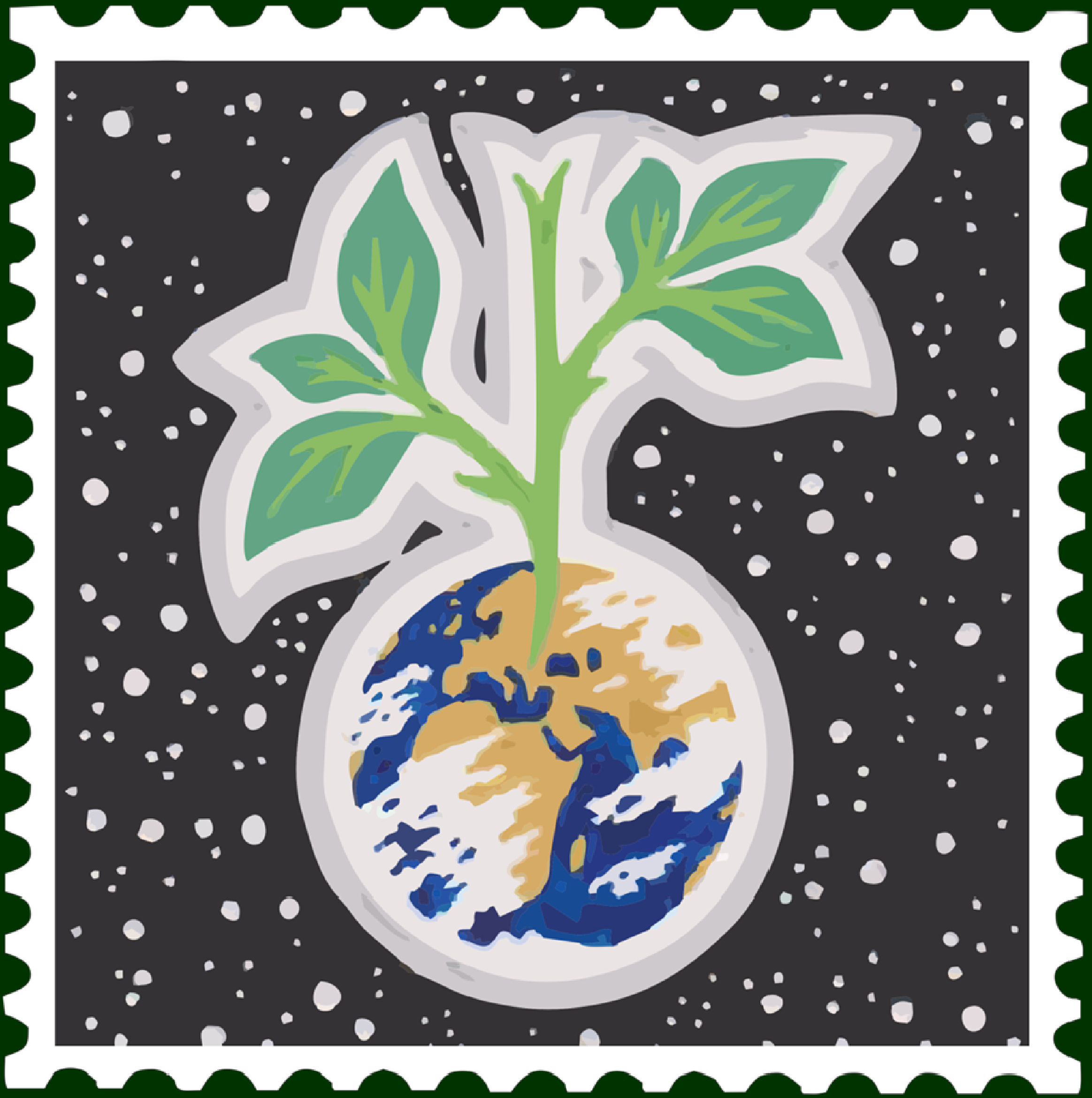

BOOK OF ABSTRACTS

First Legume Society Conference
2013: A Legume Odyssey

9-11 May 2013, Novi Sad, Serbia

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Book of Abstracts

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International Legume Society
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2013

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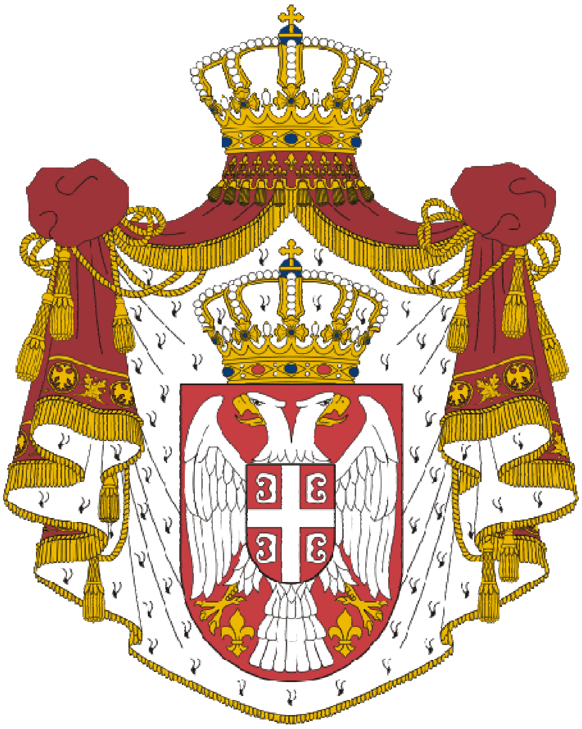

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of the Province of Vojvodina

Programme

Session 1

Achievements and challenges in crop legume research

KEYNOTE LECTURE

Achievements and challenges in grain legume research

Julie Hofer[1], Carol Moreau[2], Matthew Hegarty[1], Tina Blackmore[1], Martin Swain[1], Noel Ellis[1]

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This presentation will discuss the genetics and genomics of grain legume crops. The main reasons for being interested in grain legumes will be presented including a discussion of their role in food, feed and the provision of environmental services. The achievements to date will be summarised from a broad perspective, largely discussing the state of comparative genomics, sequence data available and systematic genetic resources that have been developed. This summary will identify the challenges before us, which will be discussed from the point of view of science policy, as well as technical and scientific problems. Finally some recent applications of new sequencing tools to address genetical questions in pea will be presented.

KEYNOTE LECTURE

Achievements and challenges in improving temperate perennial forage legumes

Paolo Annicchiarico[1], Brent Barrett[2], E Charles Brummer[3], Athole Marshall[4]

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The expected move towards more sustainable crop-livestock production systems implies wider cultivation of perennial forage legumes in temperate regions. Alfalfa (*Medicago sativa* L. subsp. *sativa*) is the main perennial legume in most of these regions, but white clover (*Trifolium repens*) and red clover (*Trifolium pratense*) are dominant in specific regions and farm systems. Although genetic progress for disease and insect resistance has been achieved, all of these crops have shown lower rates of yield improvement than major grain crops. This result can be accounted for by lower breeding investment, longer selection cycles, impossibility to capitalize on harvest index, outbreeding mating systems associated with severe inbreeding depression and lack of exploitable male sterility systems, and high interaction of genotypes with cropping conditions and crop utilizations. Increasing yield, persistence, adaptation to stressful conditions (drought; salinity; grazing) and compatibility with companion grasses are major breeding targets. We expect genetic gain for yield and other complex traits to accelerate due to progress in genetic resource utilization, genomics resource development, integration of marker-assisted selection with breeding strategies, and trait engineering. The richness in adaptive genes of landrace and natural population genetic resources will be fully untapped through an ecological understanding of plant adaptive responses and improved breeding strategies. Useful genetic variation from secondary and tertiary gene pools of *Medicago* and *Trifolium* is being increasingly accessed. Genome sequencing projects in alfalfa and white clover will enrich physical, linkage and trait maps. Genome sequences will underpin fine mapping of useful loci and subsequent allele mining, leveraging the synteny of these crops with the model species *Medicago truncatula.* Genetic markers will be used for paternity analysis, to dissect quantitative traits, and to develop a toolbox of functional markers for major disease and insect resistances and other traits. Recent developments of genotype-by-sequencing to generate genome-wide markers at little cost make genomic selection for adaptation and forage yield possible for these crops, complementing marker-based selection for other traits. Under current regulatory policies, transgenic approaches may be limited to a few breakthrough traits related to herbicide tolerance and forage quality. The key challenge in the future of applying genomics technologies is the seamless integration with breeding system logistics, and opportunities and pitfalls in the design of future breeding programs are discussed.

KEYNOTE LECTURE

Soybean Improvement: Achievements and Challenges

Joseph Burton, Lilian Miranda

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There have been major achievements in soybean improvement. Productivity has more than doubled in the past 20 years with average yearly gains of 30 kg ha^{-1}. It is estimated that more than 50% of that gain is due to genetic improvement. A consensus map of the genome has been developed with 5,500 markers and the genome has been sequenced. Herbicide tolerant soybean varieties have been developed and their popularity among farmers has resulted in their widespread use in the United States, Brazil, and Argentina. High oleic acid soybean varieties have been developed and are being produced. These provide very stable oil for use in frying applications. Soybeans are being developed with more bioavailable phosphorous in protein meal, and others with higher sucrose and less poorly digestible carbohydrates. Soybean varieties are marketed as being resistant or tolerant to a number of pests and diseases including *Phytophthora* root and stem rot, sudden death syndrome, *Sclerotinia* root rot, soybean cyst nematode, and root knot nematodes. Resistant QTL for these have been mapped. Varieties with resistance to iron deficiency chlorosis have been developed and are available for production. The challenge will be to maintain the genetic gains in seed yield as diseases change and new pests emerge. Global warming may alter abiotic stresses and decrease the genetic adaptation of varieties to their usual production environments.

Session 2

Legume genetic resources and phylogenetic relationships

KEYNOTE LECTURE

Present and future use of legume phylogeny, genomic knowledge and genetic resources diversity for science and breeding

Petr Smýkal

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The *Leguminosae* family, the third largest flowering plant family with 800 genera and over 20,000 species, has provided the largest number of humanity's most important crops, such as chickpea, common bean, faba bean, lentil, pea, soybean or mung bean. Grain legumes, accompanied cereals and formed important dietary components of early civilizations all over the world. Nowadays, legumes contribute about one-third of humankind's direct protein intake, serving also as an important fodder for animals and source of oils. However legumes are not just only field crops, but also economically important trees such as *Acacia*, tamarind or black locust, not to forget ecological impact of legumes. As in other crops, the demand for productivity has resulted in a limited number of high-yielding varieties, at the price of the loss of locally adapted ones. To prevent the extinction, *ex situ* conservation was pioneered by Vavilov (1926), resulting in large collections nowadays. However, beside duplications these are dominated by cultivated forms. There is an urgent need to systematically sample the genetic diversity in wild relatives that was only partially captured during the domestication, since natural habitats are being lost due to increased human pressure and ecological threats. The availability and quality of eco-geographical information is essential for more ecological approach to germplasm management. Crop wild relatives are also important from phylogenetic perspective, applied in interspecific crosses to increase the diversity, as the majority of allelic variation is predicted to occur outside of the crop itself. Genetic technologies enable the synthesis of introgression lines, with the alien genomes use more precise and efficient. In spite of the existence of vast collections, their use for crop improvement has been limited as accessing genetic diversity was a challenge until now. Over the last decade all major collections have been characterized and representative core collections formed. However, although the concept was developed 25 years ago, it still has not been properly applied to facilitate the identification and use of novel genotypes. Searchable databases are required as indispensable tools for the clients of gene banks to search for accessions that meet multi-trait criteria. Current challenge is phenotypic analysis and systematic screens for specific traits, while tremendous progress in omics knowledge has put horizons even further. New DNA sequencing and genotyping technologies provide the power to interrogate thousands to millions of diagnostic polymorphisms, across hundreds to thousands of genotypes, providing a rational basis to identify and select among genotypes and incorporate diversity in desired genetic background. Improvements in marker methods have been accompanied by refinements in computational methods to allow association mapping, linking genetic diversity with trait manifestation. With knowledge of phylogeny and genetic syntheny this can be applied to related species. It is likely that even novel species might be rapidly domesticated as illustrated by narrow-leave lupine. The challenge is to provide sustainable yield under suboptimal conditions, which are encountered in majority of land. Once agricultural policies recognize again the value of legumes as protein crops as well as nitrogen fixers, and invest also to respective research, there should be bright future for them.

Genetically affinity and ß –ODAP contents of Turkish grass pea populations

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Ondokuz Mayis University, Samsun, Turkey

In 12 provinces grass pea has been cultivated for a long time in Turkey. Throughout these provinces 52 grass pea farmer population seeds were collected from 52 different districts. There is only 1 released grass pea cultivar in Turkey, but there hasn't been commercial seed sale of this variety. 52 farmer populations and 1 released cultivar were grown according to randomized block design with 3 replications at experimental area of Ondokuz Mayis University, Agricultural Faculty. Beside the agronomic and chemical measurements and observations, genetically affinity levels among and inside the genotypes were determined by ISSR method. The data obtained from genetically analysis were processed by using NTSYSpc (Numerical Taxonomy and Multivariate Analysis System) and a dendrogram has been generated. Similarity coefficients among the genotypes ranged from 0.43 to 0.82. As similarity coefficients between some genotypes collected from adjacent provinces were higher than 0.80 showed us, some farmers has been exchanged grass pea seeds. ß –ODAP analysis was done in grass pea seed also, by using capillaryelectrophoresis. ß –ODAP proportion of the genotypes was determined between 1.12 and 9.96 mg g^{-1}.

Preservation and molecular characterization of 13 central Balkan landraces of faba bean (*Vicia faba* L.)

Jelena Aleksić[1], Bojana Banović[1], Aleksandar Mikić[2], Branko Ćupina[3], Bojan Zlatković[4], Snežana Anđelković[5], Vesna Maksimović[1]

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An increasing interest in cultivation and improvement of faba bean (*Vicia faba* L.), an important food and feed grain legume whose *ex situ* genetic resources comprise cultivated forms only, triggers both search for novel genotypes and their molecular characterization. Due to the poor representation of faba bean genotypes from the central Balkans in worldwide *ex situ* collections, we collected seeds of 13 faba bean landraces from this region, preserved them at the French National Institute for Agricultural Research (INRA) *ex situ* collection and used them also for amplification of ten nuclear microsatellites (simple sequence repeats – SSRs). Given the self-pollinating nature of faba bean and low heterozygosity levels in this crop, we assumed that relatively low number of alleles may be expected in each landrace. Therefore, we first demonstrated that all alleles obtained upon amplification of ten SSRs in 11 individuals from one landrace could be detected when DNAs from these individuals were pooled and such a bulk DNA was used as a template for PCR reactions, and then tested all SSRs in bulk DNA templates from each out of ten remaining landraces comprising six to 15 genotypes. Although such a cost-effective approach has certain drawbacks, it enabled us to acquire first insights into levels of genetic diversity and genetic structuring of a novel and untapped genetic material. We detected 203 alleles at ten loci, 20.3 alleles per locus and abundant private alleles (44). PCA and cluster analysis based on Simpson's similarity coefficients revealed that genetic differentiation of landraces mainly coincided with their geographic distribution despite relatively small sampling area (c. 1000 km^2). Our findings support the view that the central Balkans represents a promising source of novel faba bean genotypes.

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In situ **preservation of local landraces of faba bean (*Vicia faba* L.) and utilisation of their food products in central regions of Serbia**

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Today, faba bean (*Vicia faba* L.) in Serbia is almost completely replaced with *Phaseolus* beans and has become a neglected crop with no official data. A recently launched action of the Institute of Field and Vegetable Crops and the Faculty of Agriculture in Novi Sad is aimed at the identification of the regions in Serbia where faba bean, known in Serbian as *bob*, could still be found. This was essentially enhanced by the multilateral project ECO-NET 18817 *Search for new genetic variability in pea (*Pisum sativum*) and faba bean* (Vicia faba *L.) to support the development of sustainable agricultures*, with duration in 2008 and 2009 and involving partners from France, Bulgaria, Russia, Serbia and Republic of Srpska. The main regions in Serbia where faba bean is cultivated are the last section of the Southern Morava and its joint mouth with the Western Morava into the Great Morava, as well as in neighbouring regions in southeastern Serbia and northwestern Bačka. Each household usually maintains its own faba bean landrace, with rare exchanges with the others. It is usually produced to fit the needs of a household, along with other pulses such as common bean or pea (*Pisum sativum* L.). Faba bean in central Serbia is sown in late February or early March, with (30 x 20) cm distribution and several plants in one hole. A part of the harvested seed is put aside for the next sowing. The faba bean is usually prepared during the Christmas fast, lasting six weeks, when according to the ancient rules of the Christian Orthodox Church, no meat, eggs or dairy products are allowed. The aspic made of faba bean is especially popular at the feasts called in Serbian *slava*, dedicated to the patron saint of a family. By early 2012, more than 40 faba bean landraces from Serbia and few from Srpska have been collected, with the joint action continuing and enriching the achieved results.

FABA BEAN ASPIC A LA SERBE: Soak dry faba beans in warm water and leave for 24h. Peel and wash them, then soak in cold water overnight. In the morning, add some salt, few laurel leaves and 2 or 3 spoons of oil. Cook faba beans slowly until soft. Leaving out laurel leaves, make a paste and continue to cook in the same water. It is done when a small amount of the paste quickly hardens on a plate. Add small garlic pieces into the paste and pour it in shallow plates to set. Sprinkle with sweet pepper powder and pour some oil on top, cut into cubes and decorate with parsley leaves before serving.

Acknowledgements:
The projects of the Ministry of Education, Science and Technological Development of the Republic of Serbia, the bilateral project *Characterisation of faba bean genetic resources (*Vicia faba *L.) to support genetic improvement of this agronomic legume* (FABAGRALE) between Serbia and France and within the programme *Pavle Savić* (2012-2013), the bilateral project *Joint strategies for legume breeding for stress resistance in Spain and Serbia* between Serbia and Spain (2012-2013) and the project *Sustainable preservation of indigenous South East European legumes and their traditional food and feed products* (SEELEGUMES) within the EU SEE-ERA.PLUS Net programme (2010-2011).

Lupinus **Collections Database**

P Barzyk[1], W Swiecicki[2]

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The total number of accessions in collected databases, 13964 originates from centers in 10 countries (ESP – 1843 acces., FRA – 254 acces., DEU – 2767 acces., CZE – 107 acces., NLD – 69 acces., AUS – 4665 acces., POL – 1169 acces., PRT – 1743 acces., SVK – 54 acces., USA – 1293 acces.,). 96 percents of these resources are gathered in 6 centers, 57 percents in European centers but very valuable are accessions in UWA CLIMA (collecting expeditions) and USA DAPUL (New World lupins), 71 percents are accessions belonging to lupin crops, i.e. *L. albus*, *L. angustofolius*, *L. luteus* and *L. mutabilis*. Very important and urgent would be collecting expeditions for some wild lupins, eg. *L. digitatus* and *L. princei*. No accession of *L. somaliensis*is protected. Collected databases are very differentiated. A common/simple structure is suggested for preliminary and easy inventory of collected resources (Institution code, Accession number, Genus, Species, Subtaxa, Accession name, Donor, Donor institution code, Country of origin, Status of sample). Unfortunately, the information on South American lupin resources is still not available.

Assessment of Useful Germplasm Resources for Breeding at Mung Bean

Creola Brezeanu[1], Petre Marian Brezeanu[1], Silvica Ambarus[1], Teodor Robu[2], Maria Calin[1], Tina Oana Cristea[1]

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A particularly important treasure for each country is the volume and the genetic value of species, subspecies and plants populations, called germplasm (plant genetic resources). Variability of plant forms today is the result of human activity, by using in breeding wild forms, changing areas and acclimatization of plants in other than their geographic areas. Currently, the collection, study and conservation of genetic resources are topical issues which concern us. The aim of the study is to save precious resources that are endangered, by collecting local native populations, old, primitive varieties, which are under risk in order to reduce genetic erosion and save entire material obtained by breeding, and also to introduce in culture a species that is less known in our area. Promising genotypes were identified (superior plant characteristics, such as: seed's size, plant's height, number of pods per plant, number of seeds in pod, resistance to drought and pathogens and early maturation). The study presents all this items for a ten promising *Phaseolus aureus Roxb.* varieties used in breeding at Vegetable Research and Development Station, Bacau.

A contribution of the project SEELEGUMES to the re-discovery of *Pisum elatius* subsp. *elatius* in Serbia and FYR of Macedonia

Branko Ćupina[1], Bojan Zlatković[2], Svetlana Antanasović[1], Vuk Đorđević[3], Aleksandar Mikić[3]

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During 2011 and 2012, a project ERA-168/01 *Sustainable Preservation of Indigenous South East European Legumes and Their Traditional Food and Feed Products* (SEELEGUMES) has been carried out within the EU SEE-ERA.NET Plus programme, gathering together 19 partnering organisations from 14 European countries. One of the greatest achievements of the project was a re-discovery of numerous crop wild relatives of cultivated annual legumes in local floras, with a specific emphasis on 'tall' pea (*Pisum sativum* L. subsp. *elatius* (Steven ex M. Bieb.) Asch. & Graebn.). During 2011 and 2012, five expeditions have been made to the upper flow of the river Pčinja and on the slopes of the mountain Kozjak, where a population of *Pisum sativum* L. subsp. *elatius* was monitored *in situ*, from the stages of full bloom in late May 2011, full seed maturity in late June 2011, autumn emergence in October 2011, spring revival in March 2012 and the next reproductive stage in early June 2012. A morphological and chemical analysis of the forage and grain dry matter confirmed a considerable potential of *Pisum sativum* L. subsp. *elatius* for cultivation, while a prominent resistance to low temperatures has also been assessed. Two more populations of *Pisum sativum* L. subsp. *elatius* were found in FYR of Macedonia, namely in the vicinity of the villages Vataša and Bogdance. Generally and with certain regret, it may be concluded that many populations of *Pisum sativum* L. subsp. *elatius*, recorded in early 20th century, disappeared, as well as that in some cases *Pisum sativum* L. subsp. *elatius* was mixed with field pea (*P. sativum* L. subsp. *sativum* var. *arvense* (L.) Poiret).

Please visit the SEELEGUMES project web site: http://polj.uns.ac.rs/~seelegumes/index.html

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Where does Portugal stand in a world of diverse common bean?

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Common bean (*Phaseolus vulgaris* L.), with a history of more than five centuries of cultivation, is a popular and widely grown crop in Portugal due to its high organoleptic and nutritional value. To evaluate genetic diversity, 65 representative Portuguese landraces were selected from the common bean collection at INIAV germplasm bank (PRT005) and from a collecting mission to the Portuguese central-northern regions low-input farms. In order to place the Portuguese landraces in the context of the world bean diversity, 20 foreign wild and cultivated accessions (1 from Turkey, 1 from the USA and 17 from Central and South America from CIAT seed bank and 1 from Angola) were included in this study. 85 accessions were screened with 23 microsatellite markers uniformly distributed throughout the common bean genome, including a marker for phaseoline diversity analysis. Genetic distance values suggest high variability amongst Portuguese bean, while low variance within landraces indicates a high degree of uniformity within each landrace, reflecting their predominantly self-pollinated nature. While there seems to be no evident connection between geographic and genetic distances amongst Portuguese beans, a clear population structure, with 3 separated clusters, was identified, being most of the Portuguese landraces placed closer to the Andean domesticated gene pool. Those clusters are in accordance with the phaseoline patterns found among the bean landraces. With these results, we expect to deepen the understanding of the existing diversity in the common bean Portuguese germplasm, and pave the way for future improvement efforts based on this valuable and unexplored resource.

US-China collaboration on evaluating cool season legume genetic resources

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The mission of the USDA-ARS National Plant Germplasm System (NPGS) is to provide the genetic diversity necessary to sustain and improve crop productivity, not only in the US but for the entire world. As a vital part of NPGS, the USDA-ARS, Western Regional Plant Introduction Station (WRPIS) acquires, maintains, evaluates and distributes germplasm and associated information to researchers worldwide. Among the 92,800 accessions currently held at WRPIS, 52,800 (~57%) are grain legumes (bean, pea, chickpea lentil, faba bean and lupine) and forage legumes (alfalfa, clover and lotus). In order to identify desirable accessions for use in breeding legumes resilient against abiotic stresses, we initiated a collaborative project to evaluate cool season grain legume germplasm under a non-funded collaborative agreement between US and China. WRPIS supplies available accessions and Chinese scientists evaluate the responses of these accessions to cold, heat, salinity and drought stresses at various research locations with ideal conditions. In the past winter, we evaluated 1,294 pea and 286 faba bean accessions for winter survival in Qingdao, China. Approximately 52% of the pea and 92% of the faba bean accessions were completely killed by the cold. The percentage of surviving plants in each of the remaining accessions varied greatly. The resulting data will be entered into the Internet-based GRIN (genetic resource information network) database accessible to everyone in the global research community. We plan to expand the evaluation to other species like chickpea and lupine. Genomics tools will also be developed to expedite legume crop improvement.

Evaluation of Lentil Germplasm for Arsenic Uptake

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Arsenic contamination of groundwater, surface water and land is a public health issue across the globe. Arsenic can be taken up from soil by plants and accumulate in vegetative and reproductive plant tissues where they pose a human health threat through consumption, inhalation or touch. Different plant species and genotypes with species have varying abilities to detoxify, sequester or remove arsenic from their cells. A common mechanism contributing to low arsenic uptake in crops is the shutdown of phosphate transporters designed to function in low soil P conditions ("high affinity phosphate transporters"). The studies described here sought to address cultivar and germplasm variation for arsenic uptake in lentils and the relationship of that to phosphorus. Lentils (*Lens culinaris*) are an important source of dietary protein worldwide especially in the Middle East, Asia, and North Africa. Major lentil-producing countries that have agricultural lands impacted by toxic levels of arsenic include China, India and Bangladesh. Differences for arsenic uptake have been tentatively identified in lentil germplasm.

Portuguese traditional varieties of common bean (*Phaseolus vulgaris* L.) – An approach to beans diversity through phenolic composition study!

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Common beans (*Phaseolus vulgaris* L.) represent one of the most important crops worldwide, including many different varieties. In Portugal, represents 80% of the grain legumes intake, being grown all over the country but with special incidence on central northern regions. The local traditional varieties, not as productive as the commercial ones, present high social-economic impact for rural communities. Until now, these varieties were not chemically characterized relatively to phenolic compounds. Mostly concentrated in seed coats, but also present in cotyledons, phenolic compounds, generally classified as flavonoids (flavones, flavonols, flavan-3-ols, dihydroflavonols, flavanones, isoflavones) and non flavonoids (phenolic acids and tannins), are secondary metabolites related with plants protection against fungal parasites, herbivores, UV radiation, oxidative cell injury allowing growth and reproduction of plant. These compounds are well known for their contribution to organoleptic characteristics of beans and also for the benefits concerning human health. Within SOLIBAM (Strategies for Organic and Low-input Integrated Breeding and Management) project, funded by 7th Framework Programme, 32 different Portuguese inbred lines of whole seeds beans were analysed. Total phenolic content, total flavonoids content and antioxidant activity (ORAC) were determined in the methanolic extracts of grounded dry beans. Extracts were analysed by LC-MS/MS and several different compounds were identified based on their molecular ion and fragmentation pattern. Quantification was performed when standards were available. Similarities between samples were determined using multivariate analysis.

A combined archaeobotanical and palaeogenetic analysis of charred pea (*Pisum sativum*) seeds from an Early Iron Age storage pit at the hill fort settlement Hissar, Leskovac, southeast Serbia

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A unique example of 2,572 carbonized pea seeds was recovered from the 11th century B.C. deposits at Hissar, a multilevel settlement of the Brnjica cultural group (1,350-1,000 B.C.) in southeast Serbia. Five hundred seeds maintained pea-like hilum, only few had preserved intact smooth-surfaced testa and the majority of seeds had no seed coat. Fortunately, only few seeds of other pulses were observed in the sample and their identity was clearly determined. Applying the exclusion principle, all the "naked", 3-4 mm large seeds, mainly broad ellipsoid and less often globose, with flattenings or concavities, were determined to correspond to cultivated pea (*Pisum sativum* L.). Additionally, a high thousand seed weight of charred seeds (24.4 g) suggested cultivated status. To confirm our finding, we processed two samples with molecular tools. A sequence analysis of four chloroplast DNA loci (*trnSG, trnK, matK* and *rbcL*) in total length of 1329bp, showed intermediate position to cultivated *P. sativum* and wild *P. sativum* subsp. *elatius*. The level of the detected mutations in the DNA chain proved that genuine ancient DNA, non-contaminated with the modern pea DNA, was analyzed. On the basis of morphological and molecular data, we conclude that the material of the study was not so much wild pea, but rather represents an early domesticated pea. This is the first report of successful ancient DNA extraction and analysis from any legume species so far.

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Genetic diversity of the autochtonous *Phaseolus* bean germplasm originating from five South east European countries

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Genetic differentiation of 167 *Phaseolus vulgaris* L. accessions originating from five South east European countries was performed using 13 SSR markers. Analysis included 21 accessions from Bosnia and Herzegovina, 15 accessions from Croatia, 73 accessions from Macedonia, 38 accessions from Serbia and 20 accessions from Slovenia. Calculated mean number of alleles per locus was 9.8. The average polymorphic information content over all loci reached value of 0.710 where the most informative locus was GATS91 (0.926). These results showed that selected set of SSR markers are highly informative and applicable for studies of genetic diversity within germplasm collected in the five South east European countries, which was also confirmed with the calculated value of probability of identity (1.831×10^{-14}). High mean value of expected heterozygosity (0.731) and Shannon's information index (1.486) reflects high genetic diversity within accessions from five countries, where accessions from Macedonia are genetically the most uniform, due to 17completely identical accessions included. Analysis of molecular variance showed only 8 % genetic variability among accessions of *P. vulgaris* from different countries which indicates their common origin. These results were also confirmed by principal coordinates analysis where first three coordinates explains cumulatively 77% of genetic variability. Based on genetic distance, UPGMA clustering method and Bayesian clustering analysis, the accessions from five countries are generally divided into four genetic groups.

Forage yield in some legume crop wild relatives

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Since the abundance of genera and species within the family of legumes (*Fabaceae* Lindl. (syn. *Leguminosae* Juss. и *Papilionaceae* Giseke)), there is a large number of annual legume crop wild species having a great significance in improving their cultivated relatives. Species, such as red-yellow (*Pisum sativum* Sm.) or Ethiopian (*Pisum abyssinicum* A. Braun) peas play an important role of introgressing the resistance to pea weevil (*Bruchus pisorum* L.) and numerous diseases to cultivated pea (*Pisum sativum* L.). On the other hand, there is a certain number of annual legume crop wild relatives that showed a considerable potential for forage production. Two of them is large-flowered vetch (*Vicia grandiflora* Scop.) and narrow-leafed vetch (*Vicia sativa* subsp. *nigra* (L.) Ehrh.), both characterized by extreme winter hardiness, earliness and, in many populations, high and quality forage yield. The main obstacle in successful pre-breeding activities in these two wild vetches major problem that needs to be solved is indetermined stem growth and non-uniform maturity, leading to low and economically non-reliable seed yield. Similar performance, although much less studied so far, has French vetch (*Vicia serratifolia* Jacq.), one of the closest botanical relatives of faba bean (*Vicia faba* L.), with high forage yield and much better resistance to pod dehiscence than two previously mentioned vetch species. Although often considered cultivated crop, both Hungarian (*Vicia pannonica* Crantz) and hairy (*Vicia villosa* Roth) still suffer from many undesirable agronomic traits that may be solved by more detailed evaluation of the existing wild populations in many European floras. Among vetchlings (*Lathyrus* spp.), there are yellow (*L. aphaca* L.) and red (*L. cicera* L.) vetchlings that are characterized by a relatively short growing season and satisfying forage yield, enabling them appropriate to fit into many modern crop rotations. A half-domesticated relative of cultivated pea, 'tall' pea (*P. sativum* L. subsp. *elatius* (Steven ex M. Bieb.) Asch. & Graebn.), may produce up to 50 t ha^{-1} of fresh forage.

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Legumes in Serbian folklore

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Legumes such as chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* Medik.), pea (*Pisum sativum* L.) and bitter vetch (*Vicia ervilia* (L.) Willd.) have been present in the everyday life of both Neanderthal and modern humans. Their primary role was food, but they were also regarded as plants with both medicine and supernatural properties. There are many records related to faba bean (*Vicia faba* L.) and other grain legumes in ancient Greece and Rome, as well as to common bean (*Phaseolus vulgaris* L.) in Aztec, Maya and Inca civilisations. Serbs are a South Slavic and originally Indo-European ethnic group, located mainly in Serbia, Montenegro, Bosnia and Herzegovina and other neighbouring countries. The folklore of Serbs resembles those of other Slavic peoples and is intermingled with the one by the Pre-Slavic Balkan tribes. Plants are generally extensively present in the Serbian folklore, comprising cultivated crops, wild plants and numerous trees and shrubs. Like in the popular beliefs of other Indo-European peoples, faba bean had a strong chthonic character, representing a dish that was cooked and dedicated to the dead, especially to the ancestors of a family. It was also used and is still sporadically used in rural areas for divination. Faba bean has also been linked with fertility and was believed powerful enough to make women conceive by simply being consumed. In recent times, the role of faba bean was completely or fully replaced by common beans, following the advance of the latter in the agriculture of the Balkan peoples. Lentil shared certain features with faba bean, especially as a chthonic food, but was mostly regarded as a medicine. Yellow sweet clover (*Melilotus officinalis* (L.) Lam.) was regarded as a mighty protection from any disease, especially when held in wreaths above the house door. Red clover (*Trifolum pratense* L.) was also a talisman, especially the plants with four leaves, against any evil or disease, for 'unlocking any door' or finding out any secret, as well as in practical magic acts related to love, that is, to make someone falling in love with its owner. The last century witnessed a rapid degradation of many rural areas in Serbia and neighbouring countries, leading to an irreversible loss of traditional ways of life, including popular beliefs. Recent trends of reviving local communities could contribute to preserving many traditions, such as rich and unique folklore.

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Paleolinguistics and crop history of ancient Eurasian grain legumes

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The goal of a preliminary paleolinguistic research was to identify the roots in diverse Eurasian proto-languages directly related to grain legumes and producing the words denoting the same in modern Eurasian languages. Six Proto-Indo-European roots were attested, namely _arnk(')-_ ('a leguminous plant'), *_bhabh-_ ('field bean'), *_erəg_^{w}_[h]-_ ('a kernel of leguminous plant', 'pea'), _ghArs-_ ('a leguminous plant'), *_kek-_ ('pea') and *_lent-_ ('lentil'). So far, no Proto-Uralic or Proto-Kartvelian roots have been identified, except for the hypothetical Proto-Uralic *_kača_ ('pea'). On the other hand, there are two Proto-Altaic roots, *_bŭkrV_ ('pea') and *_ẓịăbsa_ ('lentil'). The Proto-Caucasian roots *_qŏr'ā_, denoting pea, and *_hōwł(ā)_, denoting bean and lentil, and the Proto-Basque root *_iłha-r_ ('pea', 'bean', 'vetch') may originate from a common Proto-Sino-Caucasian _*hVwłV_, denoting bean, within the hypothetic Dené-Caucasian language superfamily. The Modern Maltese preserved the memory of two Proto-Semitic roots, *_ʹadaš-_ ('lentil') and *_pūl-_ ('field bean'), while the third originally Proto-Afroasiatic root related to grain legumes is *_mang-_, denoting both millet and lentil. The origin of the Old Chinese word *_shok_, originally denoting grain and seed and later beginning to denote soybean, is in the Proto-Sino-Caucasian root *_ṣtHweḳĔ́ (~ -ḳ-)_, meaning 'chaff'. The presented evidence demonstrates that the most ancient Eurasian grain legume crops were well-known and widely grown by the ancestors of all modern European nations. The attested lexicological continuum confirms the existence of the millennia-long bonds among the peoples of Eurasia to the mutual benefit. This research will hopefully encourage future interdisciplinary and concerted actions between plant scientist dealing with crop evolution and biodiversity, archaeobotanists and paleolinguists.

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Beauty will save the world, but will the world save beauty? The case of highly endangered *Vavilovia formosa* (Stev.) Fed.

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Vavilovia formosa (Stev.) Fed. is a relict, endangered species from the highlands of Caucasus and Near East. It was described in 1812, with often uncertain status and finally recognised as a separate genus of the tribe Fabeae Rchb. Our informal international group was established in 2007 to revive the interest in this species since it had been seriously neglected for decades. This review gives an overview of the accumulated knowledge on *V. formosa* and presents the results of its most recent multidisciplinary research. Three expeditions were made to two locations in Armenia in 2009, providing the material for anatomical, morphological, chemical and molecular analysis. Unlike previous attempts, *ex situ* conservation in Yerevan and *in vitro* propagation, important for potential *interspecies* hybridisation, are successful. Molecular tools were used to clarify the taxonomical position of *V. formosa*, often considered the closest to the extinct ancestor of the whole tribe. The analysis of four informative regions of chloroplast and nuclear DNA showed that *V. formosa* belonged to the same clade with *Lathyrus* and *Pisum* and with a distinct status. Preservation and maintenance of *V. formosa* remains the only basis for further development of all other scientific aspects, especially breeding and uses in agronomy.

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Variability of protein and oil content in soybean genotypes from MRIZP collection

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Seed protein and oil content are two main seed quality traits in soybean. Numerous studies confirm negative correlation between seed protein and oil concentration. Short season soybean varieties have higher protein concentration, while late cultivars have better oil content. The objective of this study was to evaluate 135 soybean accessions different maturity groups (00,0,I,II,III) on the basis of their protein and oil concentration and to identify the genotypes with the best protein: oil ratio within each group. The accessions from soybean collection of the Maize Research Institute Zemun Polje (MRIZP) were analyzed for protein and oil content with a NIR spectroscopy. Cluster analysis using combined data of two traits was performed for genotypes of each maturity group. Categories of genotypes based on protein (low protein-LP, medium protein–MP, high protein-HP) and oil content (low oil-LO, medium oil–MO, high oil-HO) were identified. Proportion of categories differed among groups. The majority of HPHO varieties were found in maturity groups 00 and 0 (5 and 3 cultivars, respectfully). Mid season group (I) was mostly consisted of MPMO and LPHO cultivars, with exception of two MPHO genotypes. Within the maturity group II, no medium protein cultivars were found, while 5 genotypes had oil content higher than 21%. Maturity group III was characterized by low protein cultivars, with either medium or low oil content, excepting two cultivars with high oil concentration. Although groups II and III encompassed only low protein varieties, one cultivar from each group has been identified as HPHO. Soybean collection of MRIZP contains valuable sources of genetic variation for the soybean breeding program in the future.

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Morphological diversity in a pea world collection

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The importance of field pea (*Pisum sativum* L.) as a protein source supports scientific studies aimed to seek out and collect germplasm in temperate and sub-tropical regions and to investigate the species biodiversity for major agronomic traits. We assembled from various donor germplasm institutions a world collection encompassing mainly landraces from each of 20 agrogeographical regions worldwide. This material and 11 recent control varieties were evaluated in Lodi (Northern Italy) for a number of agronomic traits in one field experiment including 250 entries with 2 replications. Correlation analyses highlighted, *inter alia*, the negative relationship of days to flowering or to maturity with harvest index, and the inverse relationship of plant height with seed size. Variation among geographical regions ($P < 0.01$) emerged for days to flowering and to maturity, straw yield, plant height, harvest index and seed weight. Variation within regions was mostly significant, e.g. for flowering time (for which only France and Nepal did not show variation at $P<0.05$). We observed variation among accessions for several morphological traits such as flower colour (43% white, 51% purple, 5.6% mixed and 0.4% pink), integument colour (54.3% absent, 29.2% brownish green, 11.1% reddish brown and 5.4% brown), cotyledon colour (91.4% yellow and 8.6% green) and black hilum (25.2%). The variation in protein content is under investigation.

Ecogeography and distribution of forage legumes in Turkey

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Many economically important forage legumes species are naturally grown in flora of Turkey. The genera *Astragalus, Coronilla, Lathyrus, Lotus, Lupinus, Medicago, Melilotus, Onobrychis, Pisum, Trifolium, Trigonella* and*Vicia* are distributed in Turkey. *Astragalus* L. with 438 species is generally considered to be the largest genus in the family Fabaceae in Turkey. *Coronilla* L. contains three species in Turkey. *Lathyrus* L. is one of the largest genera and included 64 species distributed in Turkey.*Lotus* L. is represented by 20 species in Turkey. *Lupinus* L. includes six species in Turkey. *Medicago* L. consists of 51 species in Turkey. *Melilotus* L. contains 11 species in Turkey. *Onobrychis* Miller with 55 species is distributed in Turkey. *Pisum* L. comprises distinctly different two species, *P. sativum* L. and *P. fulvum* Subth & Smith, and all taxa of *P. sativum* including var. *sativum,* var. *arvense,* var. *elatius,* var. *humile* (syn: var. *syriacum* or var. *pumilio*), and var. *brevipedinculatum* are distributed in the flora of Turkey. *Trifolium* L. is found in Turkey with 106 species. *Trigonella* L. with 32 species are distributed in Turkey. *Vicia* L. is represented by 62 species in Turkey. The species of these genera are distributed from coastal region to alpine vegetation. Variation in the cultivated species, wild endemic species and progenitors grown in Turkey suggest that Turkey is one of the most important variation centers and cradle of forage legumes and also origin center.

Evaluation of bean and snap bean populations collected on western part of Fruska gora Mt.

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Seed of old varieties is an important source of genetic variability and plants adaptability. Broad genetic diversity is the base for successful breeding processes and adaptations to various environmental conditions and pathogens. In last five to six decades disappearance of old cultivars has been accelerated, mainly due to introduction of new cultivars based on principles of modern genetics into agricultural production. There was a cessation of cultivated plants previously used, and agricultural production and human diet is based on four main crops: wheat, corn, rice and potatoes. Large arable areas have been planted with single genotype of newly created cultivars resulting in great genetic impoverishment, especially, of the most cultivated plants. In that way, certain traits, particularly those connected with high yield, have been favored. Thus, the maintenance and evaluation of old cultivar's seeds have been recognized as extremely important for all of humanity and its future. The research presented in this paper has been conducted on the territory of southwestern Fruska gora Mt. as a part of the project „Za Vojvodjanskog paora" (2011-2012) financed by Provincial Secretariat for Science and Technological Development, Republic Serbia. Considering the extreme importance of Fruska gora Mt. for Vojvodina Province and the ongoing Master Plan for the mountain's Sustainable Development, idea of the project was to collect seeds and planting material of crops, vegetables and wild plants and to examine the state of the mountain's genetic resources. Of all the collected samples during the project, 14 samples of snap beans and 21 samples of beans have been analyzed in this paper. For all the accessions, 1000-seed mass, seed color, seed shape and phaseolin type was determined. Seed color was determined visually. Based on the ratios of: (i) seed length and seed width, and (ii) seed thickness and seed width, collected seed samples were classified into five basic groups of seed shape: round, ellipsoid, cylindrical, semi-flat and kidney-shaped. Seeds of collected bean samples have three, while snap bean seeds have four different shapes, and none of them was round. Seed coats of bean accessions were of five different colors, while in the snap bean group seed coats were of three different colors. Seeds of collected bean and snap bean accessions were predominantly white and cylindrical in shape. Mass of 1000 seeds ranged between 104.9 and 634.96 g. Averaged 1000-seed mass of bean accessions was 379.44g. Snap beans had smaller seed, with 247.98 g of 1000-seed mass in average. T phaseolin type dominated, while S type of phaseolin was present in six bean samples, and in snap bean accessions numbered NK2/12 and NK40/12. T phaseolin type was mostly present in seeds of snap beans that were white, while no significant correlation between seed color and phasolin type was determined in the beans.

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Composition of storage protein in pea interspecific hybrids

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Pea species *Pisum fulvum* is used in interspecific hybridization with *P. sativum* as a source of new agronomic traits. Polymorphism of storage proteins in 18 accessions of *Pisumfulvum*(Vavilov Research Institute of Plant Industry)with compare to *Pisumsativum* variety Stabil was studied. In experiments 1D SDS-PAGE was used. Presence of bands and their color intensity on electrophoretic spectra was taken into consideration. Summarized index of polymorphism was 0.39. Electrophoretic spectra of *P. sativum* variety Stabil (*af*), lines 109b (*af*), PAP (*aftl*), accessions of *P. fulvum* I609881 and I609885 and interspecific hybrids *P. sativum* × *P. fulvum* were studied. Hybrids of F_1 had bands of both parents. Unique bands of *P. fulvum* storage proteins were identified in interspecific hybrids F_1 109b × I609881 and Stabil × I609881. Accession of *P. fulvum* I609881 had a unique band (~107kDa) which was inherited in F_1 and F_2.Hybrids F_2 were segregated on restricted number of bands. Genetic segregation on protein bands of convicilin (~70 kDa), α-legumin (~35-43 kDa) and β+γ subunit of vicilin (~25-30 kDa) was observed in hybrids F_2 PAP × I609885. Monogenic codominant inheritance of convicilin and β+γ subunit of vicilin was determined. Non-allelic inheritance of α-legumin was identified.

Some agronomic properties of colored domestic bean (*Phaseolus vulgaris* L.) populations collected from Kelkit valley in the Black Sea reigon of Turkey

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Conservation and collection of domestic materials is very important in the world. Especially, pressure in the agricultural field and consideration in breeding programs by determining their properties are among the most primary factor for agricultural sustainability. Kelkit Valley is a historical place belonged to domestic people, cultural property and 6000-year historical development. It is located on the geographical transition region. It has altitude and climate diversity so this region has rich natural resources and biodiversity. Bean is one of the most liked legume cultivars and has a rich variety. Bean which is among the field products grown this region soils is the crucial product for dry bean farmers. Diversity of domestic dry bean populations has increased due to different climate diversity and fertile soils of region and also especially sugar type forms have distinguished with their tastiness in domestic market. In this study, it was aimed to collect the domestic bean populations grown in Kelkit Valley to determine some agronomic properties and to take the material for variety development programs. Colored domestic bean populations were collected from four cities, nine counties and thirty seven villages in Kelkit Valley boundaries. These materials were planted in May 2008 in Samsun which is located in coastal region of Black Sea having mild climate. Number of days to % 50 flowering, 50 % pot setting, plant height, pod length, seeds per pod, pods per plant, seed number per plant, seed yield per plant, 100 seed weight were determined. Frequency distributions were formed using the whole observation data obtained from each genotype. Number of days to % 50 flowering, 50 % pot setting, plant height, pod length, seeds per pod, pods per plant, seed number per plant, seed yield per plant, 100 seed weight were ranged from 39-62 days, 43-75 days, 45-238 cm, 6.4-13.6 cm, 2.6-6.6 number, 7-43 number, 17-106 seeds, 6.93-49.94 g and 19.31-53.69 g respectively. As a result of this study, it was obtained that some genotypes could use in breeding studies and some genotypes could also use for dry grain and fresh consumptions.

Production and evaluation of interspecific derivatives of *C. lanceolatus*

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Cultivated pigeon pea has a narrow genetic base. Wild relatives play an important role in the efforts to broaden its genetic base. In this report, a successful wide-cross between *Cajanus lanceolatus*, a wild relative from secondary gene pool, native of Australia, with desirable traits such as frost and drought resistance, and cultivated pigeonpea is reported. A range of F_1 progeny were obtained and the resultant F_1 hybrids set mature pods/seeds. Hybrids had intermediate morphology sharing traits of both the parents. All the F_1 hybrids flowered profusely. Some of the hybrids were completely male sterile and some were partially fertile with the pollen fertility ranging from 35% to 50%. Male sterile F_1 hybrids were backcrossed with different short-duration and medium-duration cultivated pigeonpea varieties which resulted in a new source of A_9 CMS line. Cytological analysis of the fertile F_1 hybrids revealed high degree of meiotic chromosome pairing between the two parental genomes. Meiotic analysis of sterile F_1BC_1 hybrids (CMS plants) revealed that the break-down in the microsporogenesis was at the post meiotic stage after the formation of tetrads. Fertile plants formed regular bivalents with normal disjunction except for occasional asynchrony at meiotic II division. All interspecific derivatives were evaluated for pod borer (*Helicoverpa armigera*) resistance under natural infestation at ICRISAT. Biochemical basis of resistance was also identified by studying the levels of defence proteins active against trypsin, chymotrypsin and trypsin-like enzymes of *Helicoverpa armigera* mid-gut proteinases. Proteinase inhibitor profiles differed in the activity units in parents and interspecific derivatives. The details of the study will be discussed.

Perspectives of interspecific hybridization in the genus *Lens* Mill.

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Hybridization of cultivated lentil *Lens culinaris* Medik. was successful in crossing with wild species *L. orientalis* (Boiss) Schmalh. and *L. tomentosus* Ladizinsky. Hybrids in cross of *L. culinaris* (cvs. Rauza, Obraztsov Chiflik 7) with *L. orientalis* ILWL7 were obtained by conventional cross, but F_1 plants were germinated *in vitro* on a nutrient media in order to overcome seed dormancy. Ovule rescue technique was used to recover interspecific embryos in cross of *L. culinaris* (cvs. Obraztsov Chiflik 7, Svetlaya, Vekhovskaya 1) with *L. tomentosus* (ILWL90, ILWL120). F_1 seeds of both interspecific crosses had red cotyledons, and F_1 plants had violet flowers typical of the wild paternal species. PCR-analysis also confirmed hybrid nature of recovered seeds. Later it was found that seeds could fully develop without help of ovule culture in cross of *L. culinaris* with wild accession *L. tomentosus* ILWL120. Highly productive plants with large seeds were regularly selected in F_2 – F_7 generations, that led to creation of breeding lines recombinant in flower, seed coat and cotyledon coloring, with new character combinations. After some cycles of selection lentil lines were closely related to the cultivated species *L. culinaris* in plant habit. The best breeding lines were highly productive and had high protein content. Seed yield of the best lines in 2012 field trial was at the level of 2.0 t/ha, whereas yield of maternal cv. Rausa was 1.9 t/ha. Thereby interspecific hybridization in the genus *Lens* could be a powerful tool in increasing of genetic diversity and breeding of the cultivated lentil.

Achievements and future perspective for gene transfer from wild relatives to the cultivated species in cool season food legumes

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The following cultivated crop species, chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* Medik.), pea (*Pisum sativum* L.) and faba bean (*Vicia faba* L.) in the genera *Cicer* L., *Lens* Miller, *Pisum* L. and*Vicia* L., respectively; are main diet and calorie sources for human in most part of the world. Selection processes for higher yield and quality characteristics during domestication have resulted in narrowing of the genetic variation in the cultivated crop species of the cool season food legumes. Wild *Cicer, Lens, Pisum* and *Vicia* species do not consist of useful variation for morphological characteristics and protein content, but they also possess sources of resistance to biotic and abiotic stresses. The aims of the present study were to review (i) for resistance to abiotic and biotic stresses on wild species of *Cicer, Lens, Pisum* and *Vicia*; (ii) on achievements and future perspective for gene transfer from wild species to the cultivated species in cool season food legumes.

Characterization of faba bean (Vicia faba L.) landraces of Turkey

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Seventy five faba bean (*Vicia faba* L.) accessions in germplazm collection at the National Gene Bank of AARI and 6 registered varieties (Eresen87, Filiz99, Kıtık2003, Histal, Lara ve Seher) were characterized in Menemen/İzmir. The populations and varieties were grown in lattis design in 2 rows of 4 m length with two replications. Spacing between and within rows were 45 cm and 20 cm respectively. The trial was evaluated with PCA (Principle Components Analysis). Twenty six characteristics were observed based on the Faba Bean Descriptors of UPOV and IPGRI. Observations were recorded of 5 plants. The average, minimum and maximum values of the quantitative characteristics were determined and large variability was observed in faba bean populations. This variability especially was observed in 100 grain weight (35-139 g), pod length (3,8-16,8 cm) and maximum number of ovules per pod (3-8). The data for 26 traits were evaluated with PCA. The Eigen values of three PC were between 2,1 and 3,1. These components constituted 31 % of the total variation. The single plants were selected in these populations for different breeding purposes to develop inbred lines.

Determination of Kunitz trypsin inhibitor types in soybean (*Glycine max*) and wild soybean (*Glycine soja*)

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Among the antinutritional factors present in the soybean seed, the main ones are the protease inhibitors. Soybean seeds contain two major protease inhibitor classes - the Kunitz (KTI) trypsin inhibitor and the Bowman-Birk (BBI) trypsin–chymotrypsin inhibitor. Approximately 80% of the trypsin inhibition is caused by KTI. Soybean KTI has several polymorphic types, which are controlled by codominant multiple alleles at a single locus. Of these types, *Tia* and *Tib* are predominant types. In total 10 soybean varieties (7 cultivated and 3 wild) was analyzed. In order to determine the type of KTI present, PCR-RFLP using restriction enzyme *Mse* I (*Tru1* I) was performed. *Tib* type was found in cultivated as well as in wild soybean varieties. Using a pair of primers for KTI3 gene fragment of about 700 bp was amplified in all analyzed varieties, including the Kunitz variety, lacking active trypsin inhibitor. DNA extracted from the Kunitz variety and other varieties mixed equally and used for SNP analysis. SNP detection was based on a type of heteroduplex mismatch cleavage by a single-stranded specific nuclease from celery extracts named CEL I. Upon digestion of formed heteroduplexes with CEL I enzyme, it generated two fragments of 500 bp and about 250 bp. This study clearly demonstrated that CEL I extracted from celery could detect SNPs in KTI gene of soybean.

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Native legumes in the flora of Pčinja valley (South Serbia) – species diversity and distribution pattern

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The flora of Pčinja valley, as situated in southern Balkan Peninsula, is comparatively rich in native legume species, representing important genomic and breeding resources for legume crops and forages. According to their taxonomical diversity, legume family belongs to the group of most important plant families in the flora of Serbia, and at the same time the second best represented in the flora of Pčinja valley. Considering elements different in origin and ecological requirements this family comprises impressive list of 100 species and subspecies that represents more than 40% of their total number in Serbia. At the same time such a large number of representatives are distributed across the area of hardly 0.17% of whole country area. The genus Trifolium, referring 32 taxa, is the most abundant among all other genera in the flora of the valley. The following genera: Vicia (15), Lathyrus (11), Medicago (7), Chamacytisus (5) and Astragalus (4) are also considerably rich at the family level. The life forms spectrum points at ecological group of annual representatives taking the largest portion of the all legume flora of valley. That is reasonable according to the strong Mediterranean, but also Pontic and even Oriental influences to the flora of southern Serbia. Spreading toward central Balkan Peninsula, special attention is given to Mediterranean and north Macedonian taxa, mostly forming their northern range limits in Pčinja valley. Spatial distribution of selected groups of the species as limitedly distributed mediterranean elements (*Vicia melanops, V. laeta, Ornithopus compressus, Trifolium phleoides, T. cherleri, T. sylvaticum*) or rare and endemic legume species (*Trifolium trichopterum T. velenovskyi, T. medium* subsp. *balcanicum*) is disscused. The presence of wild growing crop relatives (*e.g. Pisum sativum* subsp. *elatius, Lens nigricans, Lathyrus sativus, Medicaco falcata*) in the flora of the valley could represent a valuable source of desirable traits for the introgression into cultivated species, such as tolerance to abiotic and biotic stress is of special relevance. Examination of their actual diversity and distribution pattern was one of the aims of the studies conducted.

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Session 3

Legumes in foods and impacts on human health

KEYNOTE LECTURE

The health benefits of plant proteins in human nutrition: focus on the genus Lupinus

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On the base of numerous clinical studies performed in the preceding years, in 1999 the American Food and Drug Administration has approved the health claim that soy protein is useful to reduce the risk of cardiovascular disease, since it is able to control the serum cholesterol level. After this fact, numerous teams around the world have started to investigate the health benefits deriving from the consumption of pulses, such as chickpea, common bean, faba bean, pea, lentils, etc. Our group has investigated in particular *Lupinus albus* and *Lupinus angustifolius,* since their protein contents and sequences are similar to soy proteins. We have analyzed some specific nutrients, such as polyunsaturated fatty acid and tocoferols, or anti-nutrients, such as quinolizidine alkaloids, and have dedicated much effort to study the possible health benefits derived from the consumption of lupin proteins. We have demonstrated with experimental and clinical studies that lupin foods may be useful for decreasing total and LDL-cholesterol and for controlling blood pressure. A rabbit model of atherosclerosis was subsequently used for demonstrating that lupin protein is also useful for slowing down the formation of atherosclerotic plaques. In parallel, the mechanism of action at molecular level was investigated, demonstrating that specific peptides are responsible for the observed activities. These data may be the basis for further successful studies on other legumes. These are relevant results, considering that there is a growing demand for innovative ingredients for the formulation of functional foods and dietary supplements.

A study of the fatty acid composition of *Trigonella caerulea* (L.) Ser. seed oil

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Trigonella caerulea (L.) Ser. is an annual plant of the *Trigonella* genus in the *Leguminosae* family. The dried powdered leaves and flowers are used as a spice in soups and potato dishes. This herb is also used in cheese making. The purpose of this work is the analysis of the fatty acid composition of seed oil of *Trigonella caerulea* (L.) Ser. which have been cultivated in the Republic of Belarus. Oil was extracted with boiling hexane in the Soxhlet extractor for 10 hours, and then hexane was deleted. For GC analysis the derivatisation of the samples of oil for generation of methyl ethers of fatty acids was spent. The chromatograph Crystal 5000.1 with a quartz capillary column 60 m long was used. The oil of *Trigonella caerulea* (L.) Ser. content was 5.16%. It has been established, that oil was mainly composed of linoleic (42.27%), α-linolenic (23.91%), palmitic (12.05%), oleic (10.95%), stearic acids (1.95%). *Trigonella caerulea* (L.) Ser. oil was rich unsaturated fatty acids (nearly 78% of the total fatty acids), and polyunsaturated fatty acids accounted for 66.3% (mass percentage) of the total amount. Oil of *Trigonella caerulea* (L.) Ser. can be added in cosmetic products as natural analogue of vitamin F.

Functional properties of protein isolates prepared from soybean, pea and adzuki

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The aim of this work was to investigate functional properties including solubility, emulsifying and foaming properties of native and thermally treated adzuki, soy and pea protein isolates. To avoid differences that may be caused by the method of preparation, all isolates were prepared by isoelectric precipitation using the same procedure. All investigated properties were determined in the wide range of pH values (3.0-8.0).The lowest solubility at all pH values were obtained for isolates of adzuki. Depending on pH value, solubility of native adzuki isolate was ranged from 3.28% (pH 5.0) to 69.66% (pH 8.0) and at lower pH values (pH 3.0; pH 5.0) this parameter was approximately two times lower than the solubilities of soybean and pea isolates. However, native isolate of adzuki had better emulsifying properties at pH 7.0 compared to soy and pea protein isolates, whereas at other pH values emulsifying properties were in the range of soybean and pea protein isolates. Also, native adzuki protein isolates showed foam capacity comparable to soy and pea protein isolates at pH 5.0, 7.0 and 8.0, but foams formed with this isolate were less stable. Thermal treatment generally reduced protein solubility of almost all investigated isolates, whereas the impact of thermal treatments on emulsifying and foaming properties was variable. According to our results appropriate selection of legume seed as well as variety could have great importance in achievement of desirable functional properties of final products.

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Seed composition, genetic variability and heritability of important seed ingredients of *Lupinus angustifolius* L.

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In order to get information on the usability of narrow-leafed sweet lupins (*L. angustifolius*) in human nutrition, the seed composition of new varieties was analysed. Based on these results, genetic parameters like variability, heritability and genetic advance of important seed quality traits were estimated. To achieve this, 42 new genotypes and 8 German cultivars of *L.angustifolius*, grown in randomized block design with two replications in four different locations during 2010 and 2011, were characterized according to their contents of protein, soluble carbohydrates, oil, non-starch polysaccharides, alkaloids, as well as fatty acids and amino acids. The results showed that there is not much variability within the German breeding lines concerning the content of sugar, non-starch polysaccharides, oil and protein, but large variation of the alkaloid content was detected, ranging from 52.4 to 1482.2 μg/g dry matter. The alkaloid content showed the highest heritability of 96.1%, indicating that this character can be effectively improved. The lowest heritability was observed for protein content (68.4%) and oil content (77.8%). The genotypic coefficient of variation was near to the phenotypic coefficient of variation for all characters, suggesting a highly significant effect of genotype on phenotypic expression with only a small effect of environment. However, it has to be taken into account that in the set analysed only little variation was found, limiting genetic improvement based on this set of genotypes.

Determination of L-DOPA (L-3, 4-dihydroxyphenylalanine) content of faba bean (*Vicia faba* L.) genotypes that sown in autumn

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Faba bean which can be sown in autumn at humid and temperate climate and it reflects all of properties of legume crops such as soil improvement and benefit of human health, also contains L-Dopa (L-3, 4-dihydroxyphenylalanine) which is used to cure Parkinson's disease in elderly community is a rare plant. We believe that consumption of faba bean will expand with put forward of this propety. The study was conducted in Samsun province which located in coastal of Black Sea and the north of Turkey to determine of L-Dopa content of faba bean genotypes. Twenty two genotypes (4 cultivars and 18 lines) were sown on november in randomized complete blocks desing with 3 replications. Samples were taken from leaves at vegatative period, flowers and fresh pods at generative period of faba bean and determined L-Dopa content by HPLC (High Performance Liquid Chromotography). According to the result of variance analysis, the differences among genotypes for L-Dopa content of flowers and fresh pods were significant ($P<0.01$). L-Dopa content of leaves were ranged from 10.88 to 33.41 mg kg^{-1} but this difference wasn't a statistically significant. L-dopa content of flowers and fresh pods varied between 10.95 and 96.37; 4.16 and 52.28 mg kg^{-1} respectively. L-Dopa content of flowers of 20 genotypes except a cultivar and a line were founded same statistically. Avarage L-Dopa content of leaf, flower and pod of faba bean were 19.36, 75.87 and 25.27 mg kg^{-1}, respectively. The most common form of consumption of faba bean in Turkey and treatmet of for Parkinson's disease in some countries is fresh pod. But in this study showed that L-Dopa amount in flower is higher ($P< 0.01$) than leaf and pod according to t test. If agronomic characters of 7 lines selected from in this study are well, they can be recommended to nominated for registration due to high L-Dopa content.

The Swedish Legume Academy (Baljväxtakademin)

Camilla Gard

The Swedish Legume Academy, Sweden

The Swedish Legume Academy is a non-governmental organization, formed in 2009. The aim of the Academy is to raise awareness of the benefits of legumes, promote cultivation of and increase the consumption of legumes. In addition the Academy aims to contribute to the development of new food products with legumes and serve as a forum for people interested in legumes. The webpage (www.baljvaxtakademin.se) includes a blog, a calendar and legume related information about cooking, nutrition, health, cultivation, environment and culture. In March 2013 the Swedish Legume Academy had 75 members, 780 followers on Facebook and 391 followers on Twitter (@baljvaxtakademi).

Nutrition content in legume based food products

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The consumption of cooked legumes in Sweden is around 12 grams per person and day, according to the latest national food survey Riksmaten 2010-11 by the Swedish National Food Agency (NFA). The survey shows that the consumption of vegetables, fiber, folate and iron was low, while the intake of sugar and saturated fat was high. Data from a nutrient analysis project in 2008 by the NFA show that legumes have several nutritional benefits. Legumes are vegetables with high content of protein and fibre and low content of fat and sugars. Several of the legumes were good sources of folate and iron. The Nordic Nutrition Recommendations is currently being revised. The draft proposal for the chapter "Sustainable food consumption" stresses the low climate impact in the production of legumes and concludes that the consumption of legumes need to increase to reach a sustainable diet.

Seed color effect on nutritional composition of dry bean and path analysis

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This study was conducted to determine the relations between seed color (the L, a and b values) and nutritional composition of dry bean genotypes. A total of 39 dry bean genotype seeds which are widely grown in Turkey were used as material. Color tests were made with three replications on 50 seeds of each genotype by color meter. According to the correlation analysis, the color of the seed coat and cotyledon showed insignificant correlations with nutritional composition. For the present study, it can be concluded that the color of common bean seeds does not give sufficient information about mineral composition. Path analysis resulted that the copper (Cu) and phosphorus (P) are the important nutrients in the statistically importance level of 5% with the positive correlation values of 0.239 and 0.277 respectively. These elements are important to increase the protein ratio in common bean genotypes.

Determination of physicochemical properties of local pea genotypes in Turkey

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Pea is plant that has the most yield and usage diversity in the legumes and grown mostly in developed countries. Pea is originated from a geography that Anatolia is also located in it. Conservation and evaluation of local materials is the most important agricultural values of a country. New varieties to be developed should be resistant to stress conditions, has good agronomic characteristics and also high quality characteristics. The aim of this research was to determine the variety candidates by identifying quality characteristics of the local pea materials which morphological identification was done. Forty local lines and four varieties were used in this study. Sowing was done in winter (13.11.2008) and early spring (25.02.2010) in Samsun conditions for quality characteristics can be influenced by planting time. Genotypes were harvested dry. Color, grain number that not absorb water, cooking time, dry matter losses at cooking, starch and amylose ratio in the grain were investigated. Variance analysis was done according to split plot experimental design in the SPSS.13 package program. Twenty two of genotypes were light-colored and the rest was dark-colored. Dark-colored genotypes should be evaluated as fodder, to darken the cooking water, have high grain number that not absorb water (24.84) and high starch ratio (33.39%). Determined differences between sowing times showed that winter sowing was suitable when using dry grain as human food, early spring sowing was suitable in the case of canning and frozen foods. On average fragmentation degree, cooking time, grain numbers that not absorb water, dry matter losses at cooking, starch and amylose ratio in the grain of light-colored genotypes were found as 33.10%, 46.25 minute, 3.39, 11.67%, 32.94%, 23.94%. These values were found respectively as 12.09 %, 36.93 minutes, 24.84, 11.58%, 33.39%, 23.08% in the dark-colored genotypes. Three lines of light-colored genotypes due to low amylose ratio, two lines of light-colored genotypes due to low cooking time (19 minute) and fragmentation degree have been selected as variety candidate to be developed for canning and dry consumption.

The impact of yeast fermentation of blue and yellow lupin seeds on their chemical composition

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Optimizing the technology of lupin seeds fermentation by yeast was provided. Three varieties of lupin were selected: two of blue lupin - Graf, characterized by a low content of alkaloids, Karo with a high content of alkaloids and one of yellow lupin - Lord with low content of alkaloids. To the study three species of yeast were selected *Saccharomyces cerevisiae, Kluyveromyces lactis* and *Candida utilis*. The grinded lupin seeds were autoclaved and inoculated by yeast. Fermentation was provided in anaerobic conditions during 48 or 72h. The fermented products were dried in 50ºC. 18 dry preparations in 3 replications were obtained. In fermented samples the proximal composition, amino acids profile, pH, antinutritional factors concentration and microbial status were analyzed. The results indicated that fermentation by yeast increased protein content in dry matter of products in comparison to the raw seeds, for var. LORD from 2.2 to 5.5 units %, for Graf from 2.6 to 7.0 % units, and for Karo from 2.5 to 4.5 % units, respectively of yeast type. Crude fibre content increased not significantly but crude fat and antinutritional factors content decreased during fermentation. The pH of fermented products was about 5.5 – 6.1. Amino acids composition of protein was similar.

***Lathyrus sativus*: To eat or not to eat?**

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In its long history as a cultivated crop, grass pea (*Lathyrus sativus* L.) has received praise and blame. In the Pharaonic era, grass pea was part of funeral offerings found in the pyramids. In drought prone areas of Ethiopia and the Sub-Indian continent, grass pea is considered a life-insurance crop. Already in antiquity, the link was made between overconsumption of grass pea seeds and a crippling neurological disorder, later coined neurolathyrism. Overemphasis on suspected toxicity of the seeds has led to neglect of the exceptional positive agronomic properties of the plant. In normal situations, neurolathyrism is virtually non-existent. The etiology of neurolathyrism had been oversimplified as being caused by a single metabolite and factors such as oxidative stress, mineral content, the total diet, emotional stress and deficiency of essential amino acids were neglected. Recent epidemiological and pharmacological studies have indicated the importance of essential amino acids and oxidative stress in the incidence of neurolathyrism. This same metabolite is also present in Ginseng and patented in China as a haemostatic drug. The drought tolerant grass pea is the survival food for the poor during drought-triggered famines. It is the most efficient nitrogen fixer among commercial legumes, grows on poor soil and yields even better in moderate salinity. Grass pea can become a wonder crop if it can shed the double stigma of being a toxic plant and the food for the poorest of the poor. Potential strategies will be discussed.

Potential of chickpea (*Cicer aeritinum* L.) as an oil crop

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The production of vegetable oils has been registering a constant increase of production and soybean is the largest source of vegetable oil in crop production globally. In addition to soybean, other legume species have the potential, in addition to the basic product-protein, to be a source of vegetable oil. One of these species is the chickpea. It is necessary to analyze the content and composition of the oil to determine the potential of chickpea as a source of oil. Four different genotypes of chickpea from the collection of the Institute of Field and Vegetable Crops in Novi Sad were tested in 2010. Oil and protein content were measured and it was found that the highest oil content was in genotype 3 (5.43%), and highest protein content in genotype 4 (24.15%). Fatty acids composition in chickpea seed is very specific, and the presence of rare fatty acids, such as capric and lauric, and high content of saturated fatty acids - palmitic. The obtained preliminary results indicate that chickpea is of potential use as a source of oil intended for specific types of manufacturing industries, primarily in the pharmaceutical and confectionery industries, as well as cold-pressed oils, which could justify oil extraction through their value, as a by-product in the production of proteins.

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Content of essential fatty acid of Estonian soybean variety Laulema

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Soybean is globally important crop providing oil and protein. View of the quality of foodis essential to look not only oil and protein content but also fatty acid composition.Much attention is paid to omega-6 and omega-3 fatty acids, to their equilibrium in food. Linolenic acid (LA) is an unsaturated omega-6 fatty acid and alpha-linolenic acid (ALA) is an unsaturated omega-3 fatty acid.Human body does not synthesise these two essential amino acids, and therefore the best way to replenish amino acids is to eat the foods that contain them.In 2011 the Estonian first soybean variety Laulema was tested in trial with four early maturing varietiesof Lithuanianand Russian origin, and in 2012 withfifteen varieties from Latvia, Lithuania, Russia, Ukraine and Sweden.In 2011LA of Laulema was 53.18% of total fatty acids, which is 0.19% below from trial average, in 201254.89%, which exceeded 0.44% of trial average. In 2011 ALA of Laulema was 12.85%, which was 2.34% higher than the average andin 201216.8%, which exceeded 17.02% of test average. To increase the level of fatty acids,two preparations were applied in 2012: the Effective Microorganisms (EM) (*Rhodopseudomonas palustris, Rhodobacter sphaeroides,* etc.), at sowing time(1.5 l ha^{-1}),and RuterAA (free amino acids 8.4%, organic matter 18%, N5.5-$P_2O_5$5-K_2O3.5;micronutrients), in eight leaves growth stage(2 l ha^{-1}). LA content increased in the variant with RuterAA 4.23% and 3.66% with EM. ALA content decreased in the variant with RuterAA 10.65% and with EM 10.06%.

Functional properties of adzuki (*Vigna angulata*) flour

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For a long time legumes have been recognized as a valuable and low cost source of high quality protein products such as flour, concentrates and isolates. Nevertheless, the application on an industrial scale has only soybean proteins and to lesser extent pea protein products, in part due to insufficient information relating to functional properties of proteins of other legumes. Adzuki bean (*Vigna angularis*), like other legumes, is an excellent source of high quality proteins, fiber and several other nutrients. The aim of this work was to investigate functional properties including emulsifying and foaming properties of adzuki protein flour and to compare with those prepared from soybean under the same conditions. These properties were investigated at four pH values (3.0; 5.0; 7.0; 8.0). According to our results, adzuki flour had better emulsifying properties than soybean flour. At all investigated pH values, except at pH 3.0, values of emulsion activity index of adzuki flour were higher than in the case of soy flour. Also, at all pH values adzuki flour formed more stable emulsions than soybean flour. On the other hand, at higher pH values foaming properties (foam capacity and foam stability) of adzuki flour were inferior to soy flour.

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Simple cooking and in vitro gastrointestinal digestion improves antioxidant activity of legume seed flour extracts

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Antioxidant activities and phenolic contents were studied in methanolic and water extracts of non-processed, cooked and in vitro enzymatically digested seed flour. Antioxidant activities of total protein hydrolysates and small peptide fractions (MW<3kDa and MW<3kDa) isolated from seed were also determined. Study showed that cooking and enzymatic digestion strongly enhanced release of phenolic compounds. Scavenging activity against 2,2-diphenyl-1-picryhydrazyl radical (DPPH$^{\bullet}$), hydroxyl radical and hydrogen peroxide were also increased. Protein hydrolysates especially MW<3kDa fraction possess significant antioxidative potential. Taken together our findings indicate that in methanolic and water extracts of cooked and digested seed flour small peptide fraction, beside phenolic compounds, can also significantly contribute to free radical and hydrogen peroxide scavenging activity.

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Potential of white lupin (*Lupinus albus* L.) as an oil crop

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White lupine has a long history of cultivation and wild-growing forms have been preserved in Greece until nowadays. Seeds of white lupine are rich in proteins but also contain significant amounts of oil making it an interesting source of edible seeds. The demand for new types/sources of oil is constantly rising following the rising demand for food diversification and quality, thus giving an opportunity for less used crops to enter the market. Soybean is the largest source of vegetable oil in crop production globally but in addition to soybean, other legume species have the potential to be a source of vegetable oil. One of these species is the white lupine. To determine the potential of white lupine as a source of oil it is important to analyze oil content and composition. White lupine genotypes from the collection of the Institute of Field and Vegetable Crops in Novi Sad were tested in 2010. Oil and protein content were measured and two different genotypes had the highest oil content of 11.27%, and the highest protein content of 37.31%. Fatty acids composition in white lupine seed was marked with the presence of rare fatty acids, such as capric and lauric, and a high content of oleic acid. The obtained results indicate that white lupine can be of use as a source of oil intended for specific porpoises, primarily in the processing industries, as well as cold-pressed oils, which could justify oil extraction through their value.

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Antioxidant profile of alfalfa (*Medicago sativa* L.)

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Alfalfa (*Medicago sativa* L.) is one of the most important species of the Leguminosae (Fabaceae) family. Besides being important food for animals, this species is rich source of phytochemicals that play an important role in human health. The alfalfa contains many phenolic compounds such as coumesterol, apigenin, luteolin, quercetin, and isoflavonoids that have a positive effect on the menopausal disorders such as osteoporosis or breast cancer. All these compounds are known as natural antioxidants because of their ability to neutralize free radical species giving them a hydrogen atom. In this study we have determined the antioxidant potential of ethylacetate extracts (different solutions) on several radical and non-radical species such as: DPPH (2,2-diphenyl-1-picrylhydrazyl), $O_2^{-\bullet}$ (superoxide anion radical) , NO • and H_2O_2. All measurement are based on spectrophotometric methods, where RSC (radical species capacity) was determined in percentage: RSC (%) = 100 × (A_{blank}-A_{sample}/A_{blank}). From the RSC values, there were obtained IC_{50} values, which represented the concentrations of the ethylacetate extracts that caused 50% neutralization and it was determined by linear regression analysis. All results were compared with the commercial synthetic antioxidant BHT (*terz*-butyl hydroxytoluene) as positive control. Ethylacetate extracts was shown the best capacity on the neutralization of DPPH radical, because its IC_{50} value (11.29 µg/ml) is similar to the IC_{50} of positive control BHA (IC_{50}=11.08 µg/ml), while at $O_2^{-\bullet}$ (IC_{50}=12.90 µg/ml) and H_2O_2 (IC_{50}=11.28 µg/ml) was showed moderate activity. The lowest activity showed by NO • radical (IC_{50}=30.30 µg/ml). Generally, extract shows good antioxidant activity, which indicates that it would be useful to extend the research to the field of pharmacy and medicine.

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Improvement of soybean food safety: Reducing heavy metals and food allergens

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Exploiting genetic variation in food safety characters is an important option for soybean (*Glycine max* [L.] Merr.) breeding in order to improve the overall quality of soyfoods. For instance, in soy-oil based products, reduction of linolenic acid has contributed to reduced trans-fatty acid formation during food processing. At present, soybean genetic variation is being utilized to reduce the uptake of cadmium and to select hypo-allergenic soybeans. Based on a major QTL for cadmium accumulation in the seed mapped with microsatellite markers, soybean genotypes with either high or reduced cadmium uptake were grown in pots with different cadmium levels or in field experiments at three locations, and seed cadmium content was determined analytically. Cadmium accumulation was reduced by about 50% in genotypes carrying the allele for low cadmium uptake at the *Cda1* locus, whereas other genotypes had higher cadmium concentrations which partly exceeded the maximum level of 0.20 mg kg^{-1} tolerated for food uses in European Commission regulation 1881/2006 and WHO guidelines. In addition, the reduction of soybean allergenicity is a highly desirable goal in the food industry, as soy protein is present in many food products. A Chinese soybean landrace from a genebank collection has recently been identified to carry a null allele at the P34 protein locus, which is the immunodominant soybean allergen. Therefore, microsatellite markers are currently identified which allow for the introgression of the P34 null allele into early maturity germplasm. Thus, soybean breeding can significantly contribute to various aspects of food safety.

Antioxidant capacity of yellow and black soybean seed coat and dehulled bean in relation to their distributions of isoflavones, anthocyanins and phenolic acids

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Recently, attention has been given to the significant potential of soybean in the prevention of chronic diseases such as atherosclerosis, coronary heart disease and cancer. Given that oxidative damage represent one of the mechanisms leading to chronic diseases many researches have been focused on the antioxidant compounds in soybeans. In this study, the total and individual phenolic profiles and their contribution to antioxidant capacities of seed coats and dehulled beans of black and yellow soybeans were investigated. Black seed coats had the highest content of total phenols, flavonoids and anthocyanins, in addition the highest catechin and protocatechin contents. On the other hand, dehulled beans from yellow soybeans had the highest levels of ferulic and *p*-coumaric acid content, and free and bound isoflavone content. The present study demonstrated that anthocyanins predominantly accounted for the difference in polyphenols between black soybeans and other yellow soybean genotypes. According to our study, the average value of black seed coat samples for the anthocyanins content was 13955.01 mg CGE/kg. Due to the highest phenolic index, the black seed coats from soybean genotypes Black Tokio and Cornaja had the highest ABTS$^{•+}$ scavenging activity (597.46 and 486.15 mmol Trolox/kg, respectively). The results suggest that the black seed coat, as well as dehulled bean from yellow soybeans would potentially provide sources of natural antioxidants that may play a crucial role in the prevention of oxidation-related diseases.

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Session 4

Advances in legume breeding concepts and tools

KEYNOTE LECTURE

Breeding annual legumes for sustainable agricultures must target for new and more complex variety ideotypes

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Although yield and total biomass produced by annual legumes remain major objectives for breeders, environment-friendly, resource use efficient including symbiotic performance, resilient production in the context of climate change, adaptation to sustainable cropping systems (reducing leaching and glasshouse gas emissions), adaptation to diverse uses (seeds for feeds foods, non-food, forage or green manures), and finally new ecological services such as pollinator protection, imply the development of innovative genotypes, definition of new ideotypes and acceptance of their commercialisation. Taken as a whole, this means more complex and integrated objectives for breeders. Several illustrations will be given of breeding such complex traits for different annual legume species. Genetic diversity for root development and for ability to establish efficient symbioses with rhizobia and mycorrhiza can contribute to better resource management (N, P, water). Shoot architectures and phenologies can contribute to yield and biotic protection (weeds, disease, parasitic insects). Long winter cycles or short cycles, tolerance to biotic or abiotic stresses, are key features for the introduction of annual legumes in low input cropping systems. Adaptation to intercropping requires adapted genotypes. Improved health and nutritional value for humans are key objectives for developing new markets. Modifying product composition often requires the development of specific varieties and sometimes the need to break negative genetic correlations with yield. A holistic approach in legume breeding is important for defining objectives with farmers, processors and consumers. The varietal structures may be more complex, combining genotypes, plant species and associated symbionts. New tools to build, evaluate and register them are important.

Grain yield stability of guar [*Cyamopsis tetragonoloba* (L.) Taub.] genotypes under various environments

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Guar genotypes vary in their response to different environmental conditions. Six genotypes namely S-2536, S-3112, S-4055, S-4540, S-5274 and BR-99 were tested at seven locations throughout Pakistan during cropping season of Kharif 2012 to study their grain yield stability. Analysis of variance and stability were computed. Variance due to genotypes, environments and G*E interaction were found to be significant. Linear and non-linear components of G*E interaction were also present. Three stability parameters were computed to judge the stable and superior genotypes of guar. On the basis of these parameters, the top yielding genotype S-5274 did not exhibit general adaptability and was considered as unstable due to high deviation from regression. The genotype BR-99 showed the stable performance over all locations followed by genotype S-3112.

Breeding research for abiotic stresses tolerance in faba bean (*Vicia faba* L.)

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Rapid powerful approaches for identifying quantitative trait loci (QTL) and DNA markers tightly associated with drought and frost stress tolerance are prerequisites for marker-assisted selection and breeding of faba bean (*Vicia faba* L.) for areas prone to such stresses. Our faba bean breeding research focuses mainly on winter types. We use SSD lines (our so-called association set, A-set), a verification-set (V-set; subset of A-set), a first mapping set (K-set), and a set of back-cross families developed from some of the A-set lines. These aforementioned genetic materials are being used to map drought and frost stress tolerance separately and jointly using AFLP, RAPD and SNP-markers (altogether about 900 markers). The phenotyping of drought stress tolerance is being conducted using rain-out-shelters and chemical desiccation in the field, applying drought stress in pot trials (greenhouse) with juvenile plants, and determining physiological parameters in leaf disc/single leaf tests. On the other hand, for frost stress tolerance, the phenotyping is conducted in the field as well as in so-called frost chamber (juvenile plants in pots). Under frost stress, loss of leaf turgidity ($h^2=0.84$) was correlated with disposition to survive ($r=-0.76^{**}$). Moreover, wilting score ($h^2=0.52$) was correlated with dry matter content ($r=0.55^{**}$) under drought stress. A main outcome of our project will be a list of useful, well-marked QTLs for drought and frost stress tolerance in winter faba bean. Moreover, we are currently identifying reliable screening tools to phenotype these traits and we are identifying highly promising parental lines for applied breeding purposes.

Yield and quality performances of new soybean (*Glycine max.* L. Merrill) varieties improved in Turkey

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In this study was used 9 soybean varieties improved in Turkey and 3 soybean varieties (Blaze, Nova, Progen 375 as control group) in foreign origin. The experimental areas were conducted with randomized block design and three replications, two locations in Black Sea Region located in northern Turkey. New varieties were improved by Research institues and Universities of Turkey (Nazlıcan, Türksoy,Adasoy, Ustun1, Atakisi, Arısoy, Ataem-7, Erensoy, Umut 2002and represented 3rd, 4th and 5th maturity groups. Plant height ranged from 112.8 cm to 195.9 cm, first pod height 8.5-21.0 cm, pod number per plant 91.7-189.2, seeds per pod 2-3, branches per plant 1.0-5.43, seed yield per decare 335.5-570.7 kg, biological yield per plant 90-175 g, seed yield per plant 37.4-102.5 g, harvest index 28.0-40.8 % and 1000-kernal weight 125.0-235.0g. Protein and oil contents varied between 39.6-43.1 % and 16.1-18.5 %, respectively. Furthermore, positive relationships were determined between pod number per plant and biological yield (r= 0.835**), between pod number per plant and seed yield per plant (r= 0.637*) and between seed yield (kg/da) and harvest index (r= 0.581*). Improved varieties Erensoy and Atakisi gave higher seed yield from foreign varieties in Terme location and improved varieties Ustun1, Turksoy, Ataem 7 and Atakisi in Bafra location. However, it was concluded that this research is still continuing in different locations in order to determine the effects of the climate and other enviromental factors on yields and qualities of soybean varieties improved in Turkey.

Seed and forage potential of cowpea (Vignaunguiculata L.Walp) genotypes

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Nine cowpea genotypes (two released cultivars and seven lines) were evaluated for forage and grain yield, some agronomic parameters and quality features at two locations in the Middle Black Sea region of Turkey for two years. Experiments were established within May and arranged in a randomized complete block design with 3 replications in both years and locations. Genotypes were evaluated for plant height, first pod height, branches per plant, main branch diameter, pod length, seed number per pod, thousand seed weight, seed yield, forage yield and CP, ADF, NDF, P, K, Ca, Mg and some micro element contents. Forage yield was significantly ($P<0.01$) affected by genotype, year and location and ranged from 6.03 to 7.94 t ha^{-1} among genotypes over the years and locations. Crude protein proportions ranged between 170.2 and 185.2 g kg^{-1}. Although there were no significant differences among genotypes for ADF, NDF, Mg and P contents, P, Mg, Ca, and K contents in all genotypes were higher than animal needs in both locations. Thousand seed weight ranged between 138.7 and 233.2 g. Seed yield ranged from 1,010 to 1,420 kg ha^{-1}. In general, average values for most variables studied were higher in Samsun than at Kavak location.

Seed yield and yield components of different narbon vetch (V*icia narbonensis* L.) genotypes in Mediterranean climate

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Population growth, urbanization, and income growth in developing countries are causing an increase in demand for food of animal origin. Number of animals must be raised in order to increase milk and meat production. Feed requirements of animals play an important role in livestock production. Legume seeds are a major source of plant-derived proteins and are economically important for worldwide feed and food. Narbon vetch (*Vicia narbonensis* L.) is a leguminous species with the potential to become an important grain and straw crop for animal feed due to its drought and cold tolerance, pest resistance, high green biomass and seed yields and ability to fix nitrogen. A study was conduct to investigate seed yield and yield related characters of 15 narbon vetch genotypes and 1 local accession in typically Mediterranean climate. Experiments were carried out in the Akdeniz University, Faculty of Agriculture experimental fields located at the Mediterranean coastal region of Turkey in 2002 and 2004. The experimental plots were arranged in a randomized complete block design (RCBD) with three replications. Seed yield ranged from 1450 kg/ha to 3855 kg/ha and dry mass ranged from 2880 kg/ha to 8812 kg/ha. A significant positive correlation was observed between seed yield and plant height, number of stems, number of pods per plant, number of seeds per plant, dry mass and harvest index. Results in this study demonstrated that narbon vetch has the potential to play a more prominent role in the agriculture of regions within Mediterranean type environments.

Breeding a red clover variety for grazing use

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Cultivated red clover (*Trifolium pratense* L.) is almost exclusively used for mowing. It grows too tall to fit as a companion to typical pasture grasses, and it does not tolerate intensive grazing. For this reason, white clover (*Trifolium repens* L.) is the usual species of choice as a pasture legume. However, white clover has some disadvantages. It tends to have unbalanced high protein content, and it often dominates pastures to an undesirable degree when little nitrogenous fertilizers are applied, such as in organic agriculture. These considerations led us to start developing a red clover variety for grazing use. Breeding goals were persistence under grazing for at least 3 years, a rapid regeneration after grazing through strong tillering, compatibility with pasture grasses such as perennial ryegrass or fine-leaved tall fescue, and an acceptable yield potential for admittance to national variety lists. An ecotype population of red clover originating from a horse pasture in the Swiss Jura region was intercrossed with breeding material of the cultivated, persistent "Mattenklee" type, followed by a backcross with Mattenklee and 3 generations of individual plant selection for prostrate growth and strong tillering. The resulting variety was tested under grazing and successfully passed official variety testing in Switzerland and in France. It was registered under the denomination Pastor in 2010 (CH) and 2011 (F). Pastor is well suited for mixtures with fine-leaved tall fescue and with perennial or hybrid ryegrass.

Evaluation of normal and micronutrient dense lentil (*Lens culinaris* Medikus) cultivars for nutritional security and cropping intensification in mid and far western Terai of Nepal

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Lentil (*Lens culinaris* Medikus) is the main pulse in Nepal often referred as the "poor man's meat" for its rich protein. Biotic and abiotic stresses are the major threats of the lentil production and productivity. Micronutrients are especially required for adolescent and pregnant women in sufficient quantum. Therefore research in collaboration with International Center for Agricultural Research in Dry Areas (ICARDA), Syria and National Grain Legume Research Program (NGLRP), Rampur has identified very promising micronutrient rich lines i.e. iron, Zn, and ß-carotene etc. Twelve to twenty four lentil genotypes of diverse origins were evaluated in the Coordinated Varietal Trial (CVT), Regional Elite Varietal Trial (REVT), Participatory Varietal Selection (PVS) and micronutrient trials at the station and the outreach research (OR) sites. During the three experimentation seasons (2009/010-2011/12), using a randomized complete block design with three to four replicates, the genotypes were tested for variation, performance and suitability for growing under rainfed environments. Significant genotypic differences were detected for major traits of each season and combined data over the year and may be due to their different genetic backgrounds. The tested genotypes were varied in their interaction with the prevailing environmental influences and exhibited different responses. Lentil genotypes ILL6467 (1306 kg/ha) produced the highest grain yield followed by Shital (1289 kg/ha), ILL6819 (1147 kg/ha) and LN00136 (1116 kg/ha) among the tested genotypes, respectively. Likewise, genotypes ILL6467 (Iron-94 mg/kg, Zinc-57 mg/kg), ILL 7723 (Iron-91 mg/kg, Zinc-59 mg/kg), Khajura-1 (Iron-94 mg/kg, Zinc-59 mg/kg) and RL13 (Iron-101 mg/kg, Zinc-57 mg/kg) contain rich micronutrient content in the seed and performed very well in rainfed environment of Nepalgunj.

Enhancing the productivity of soybean through the exploitation of host resistance breeding against the virus diseases in Nepal

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Soybean is a miracle legume crop of Nepal with 9% out of total legume production and ranks 2nd after lentil (MOAC, 2010/11). Being a highly nutritious legume crop aids to food & nutritional security and sustainable soil management in the system niches. It has great potential for commercial cultivation in terai/inner terai, as soybean is becoming one of the important ingredients of poultry feed which is well thriving in Nepal. However, the national productivity of soybean was very low due to vulnerability to virus diseases. Mungbean Yellow Mosaic Virus (MYMV) an important disease of soybean in terai/ inner terai region of Nepal. White fly (*Bemisia tabaci Genn.*) transmission of MYMV has been a serious threat to soybean production. Yield losses up to 21% have been reported due to MYMV in soybean. Farmers have been using a variety of fungicides indiscriminately, which has serious hazardous effects on environment and human health. Durable resistance to MYMV is scanty in soybean. Released varieties have more or less smaller seed size, susceptibility to different foliar diseases Like YMV and longer maturity period (135-145 days), which farmers usually do not prefer. Chemical control measures are not environmentally friendly. Under such situation use of resistant varieties and manipulations in cultivation practices are important options for the management of grain legume diseases. In this context, the main objective of the study was to identify high yielding and resistant soybean germplasm suited for the existing cropping system. For this, many local landraces and exotic lines were evaluated at Rampur. Three years research results (2009-2011) revealed that soybean genotypes CM9125, G8754, LS-77-16-16, SB0065 and SB0095 showed resistance to MYMV and Chaing Maw 60-63, CM9133, Dhankuta and SJ-4 were moderately resistant. These genotypes were promoted in participatory trials in farmers' fields and in seed increase programs at the farm level of different eco-systems. In addition, legume breeders using the lines as a parent material in the crossing program at the station.

Genetic variation in biomass traits among Indian lentil cultivars

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The concept of harvest index has been considered as a tool in interpreting crop response to different environment and climate change. Generally, pulses are known to produce sufficiently high biomass, but very little grainyield, resulting in poor harvest index. Such a behavior in them seems to be a part of their builtin mechanism tailored to offset natural stresses. Lentils also produce disproportionately large vegetative growth relative to grain yields. On the contrary, species domesticated for commercial purposes are expected to produce optimum vegetative growth to support maximum grain yields. In the recent changing climate scenario, it has been observed that biomass production in lentil reduced drastically in lentil and, as a result, yield component traits are affected. Therefore, the cultivars with high biomass and high harvest index will be important to realize maximum grain yield. In the present study, 36 commercial cultivars released in India were evaluated for higher biomass and harvest index both in macro and microsperma types for their potential use in lentil improvement. Considerable variation was observed for harvest index ranging from 38% (L 4076) to 63% (Barahia Local). However, nine other varieties also had shown harvest indeces of over 55%. Among these, Asha was the earliest flowering (< 60 days) while HUL 57 and L 4147 were late flowering types (> 80 days). On the basis of seed size, DPL 62, IPL 406, DPL 15, PL 234 and NDL 1 were the large seeded varieties, whereas Barahia Local, Asha, HUL 57, WBL 77 and L 4147 were the small seeded types with high harvest index. The range for biomass per plant was observed between 3.4 g to 40.9 g. Eight cultivars were identified with biomass above 20 g per plant. Among these varieties, IPL 406, IPL 315 and PL 234 were early maturing types in the large seeded group while L 4147, HUL 57, Asha and WBL 77 were in the small seeded group. In the changing climate scenario, lentil breeders have to concentrate on developing high biomass lines with higher harvest indices within the desired maturity group and seed size. Therefore, three lentil genotypes viz. L 4147, IPL 406 and PL 234 were suggested for their potential use as an agronomic base in lentil improvement.

Learning from past: Implication of neutral variability changes during soybean breeding on further breeding process

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Historically, soybean breeding began with a small number of genotypes and can be seen as a recurrent selection for yield. Analyzing allele frequencies in ancestral and elite population for particular region, it is possible to find neutral alleles with significant different frequencies between two populations. Each allele, which has a different frequency in the elite and ancestral population, indicates selection effects in the vicinity of a given locus. Exploring neutral variability in ancestral and elite population, it is possible to find soybean genomic locations which are under selection, actually affecting the yield increase. These parts of the genome are associated with agronomical important genes and can be used in marker assisted selection. Ancestral population was established based on pedigree analysis, which includes last available ancestor. Elite population consist of high yielding varieties that are grown in Central and Eastern Europe, breed at Institute of field and vegetable crops, Novi Sad. Analysis of variability in ancestral and elite soybean population with 50 microsatellite markers, indicate several genomic regions which are under selection. Deviation in allele frequencies from expecting values calculated by pedigree, indicate significant decreases, increases and fixation of alleles frequencies on several SSR loci. Also, strong population differentiation (Fst) found between elite and ancestral population for several loci. Some of genomic regions that possess signatures of selection are consistent with previously reported yield QTLs. Also, some genomic regions, previously reported as yield QTL region, do not show evidence of selection. Those regions are potential targets for further marker assisted selection. It is observed, as expecting, reduction in gene diversity in elite population. Surprisingly, is also observed increment of heterozygosity in elite population. That indicates that elite lines still have certain degree of variability within varieties, which can exploit for further selection. Overall, analysis of changes in neutral diversity can be useful in practical breeding programs, and can be used in marker assisted selection.

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Natural and induced mutations that modify seed composition and visual traits in *Pisum sativum* L. (pea) may be exploited for improved food and feed uses

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Improvement of seed quality traits in pea (*Pisum sativum* L.) by marker-assisted breeding relies on characterizing the genetic control of the relevant traits and identifying novel sources of variation within candidate genes. Germplasm resources, including EMS and fast neutron mutagenised populations, are being exploited for novel variants of genes that impact on seed metabolism and nutritional quality. Among these, mutations in genes involved in the control of starch/sugar metabolism are being studied, together with genes encoding seed proteins, in particular those proteins that have antinutritional properties. In the first group, novel variants for genes encoding starch-branching enzyme, ADP-glucose pyrophosphorylase and other genes involved in sucrose metabolism have revealed differences in the contents of starch and a range of sugars within seeds. In the second group, mutations affecting the accumulation of pea seed albumin 2, trypsin inhibitor and lectin have been identified, among additional seed protein mutations. The combination of null mutations for such proteins offers improved prospects for increasing the yield of highly digestible protein for feed. For example, seed protein content is not reduced when albumin 2 is absent, most likely as a consequence of the higher expression of a class of legumin genes. In order to stabilize desirable seed visual traits, variation within genes that are induced during late seed maturation may be exploited. The characterization of such resources will assist greatly with the adoption and selection of superior alleles of relevant genes in the future breeding of improved legume food and feed crops.

Association of SNP markers with pea grain protein and grain yield traits in Italy: preliminary results

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Grain yield and quality are the main economically important traits in breeding of field pea (*Pisum sativum* L.). The objective of our work was investigating trait-marker associations in elite pea germplasm, with the final aim to develop marker-assisted selection procedures for these traits. The genetic base for this study was represented by three connected recombinant inbred line (RIL) populations obtained from paired crosses between three genotypes (the varieties Attika, Isard and Kaspa) which featured different geographical origin and outstanding adaptation to Italian cropping environments. At least 86 F_7 lines per RIL generated by single-seed descent were evaluated for grain yield, plant height, lodging resistance and grain protein content in a spring-sown field experiment in Lodi. The same material was genotyped by 384 SNP markers. Values for protein content, estimated by NIRS, showed range values at least as large as 6% in each RIL population. SNP genotyping helped in building a genetic consensus map and detecting QTLs related to all phenotypic traits. Further work is on-going for enlarging both the evaluation environments for phenotyping and the set of SNP markers for map saturation.

PlantsProFood – New varieties of narrow-leafed lupin for a broad application in human nutrition

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Narrow-leafed lupin (*Lupinus angustifolius*) represents a resource of proteins with high nutritional value for humans. To make use of this potential for a broad range of food applications the regional network 'PlantsProFood' was established in the Northern part of Germany. Lupins shall be promoted as a protein resource for food purposes, like ice cream, sausages, bakery products or pasta. The network, consisting of four research institutions and ten local companies, aims at processing the value chain from the development of (I) new varieties, (II) new processing approaches towards (III) innovative and healthy food. Key for these efforts are high and stable kernel yields of narrow-leafed lupins. This may be accomplished by plant breeding provided that sufficient genetic variability for relevant traits is available. To broaden the genetic variability of advanced lupin breeding materials an EMS (ethyl methanesulfonate) treatment of cv. 'Boruta' was performed. Phenotypes with a striking novel growth type, like vigorous growth or high branching compared to the wildtype 'Boruta', were identified and devised to homozygous and stable M lines. The yield potential of the M lines was estimated under field conditions and revealed in some cases significantly increased kernel yields. For genetic analyses, crosses of the respective M lines with gene bank accessions as well as with the wildtype were performed. Segregation analysis of F2 populations indicated a monogenic-recessive inheritance of novel growth types. Promising M lines are going to be subjected to an analysis of differentially expressed cDNA between mutant lines and the wildtype to develop molecular markers for the mutant traits.

Analysis of *Lupinus angustifolius L.* seed yield and its stability across different environments

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Ten traditional and unbranched cultivars of narrow-leafed lupin (*Lupinus angustifolius* L.), differentiated in terms of morphological structure and phenological phases, were studied. Field experiments were conducted at Wiatrowo and Radzików in 2011 and 2012 in–randomized complete block design. Assessment of seed yield and its stability for various cultivars in different environments were the main goal of the research. Weather conditions were characterized using the Sielianinow factor. The results were analyzed statistically. Highly significant (a=0.01) differences between cultivars of lupin were observed. The analysis of series of trials across environments was performed and stability of cultivars assessed. The stability of cultivars was different. Analysis of variance for localization showed that among lupin the Neptun, Regent, Zeus, Dalbor and Kadryl cultivars yielded above average for the species. Analysis of interactional deviations showed that the most stable lupin were the Boruta, Kalif and Zeus cultivars. Based on correlation analysis, it was found that there were differences between the high and low cultivars of lupins (the length of the flowering phase). The length of the generative phase was correlated with the Sielianinow factor in all the studied cultivars. In case of regression analysis, seed yield and seed yield components were related to the Sielianinow factor. Multiple regression was applied in order to determine the relationships between seed yield and its components and the Sielianinow factor in two different phases (flowering and generative). Account of differences between the cultivars in terms of the discussed traits increases the value of the determination factor in all the analyzed traits, which suggests the need to focus attention on cultivars.

Variability of chosen grass pea accessions (*Lathyrus sativus* L.) in terms morphological traits and the chemical composition of seeds

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The grass pea (*Lathyrus sativus* L.) is one of the oldest cultivated species and was known as far back as 8000 BCE. In total there are 15 species of *Lathyrus* in Poland, although only one of them, the grass pea, has any economic significance. The history of grass pea cultivation in Poland is not known. According to Milczak in Poland (in the Podlasie region) grass pea first appeared as a weed accompanying lentils as far back as 17th century, at the time of the Tatar settlement. In 1991, on the basis of selection from the local grass pea population, Milczak developed two promising lines denoted "Der" an "Kra" which, after a series of multi-year field experiments, were registered as original cultivars – Derek and Krab. This study investigated both these two Polish cultivars and 29 European grass pea accessions (*Lathyrus sativus* L.) from Italy, Spain, France and Germany grown in the same soil-climatic conditions (an experimental station) in Poland. The morphological traits of plants of particular accessions were studied during the vegetation season, as well as their seed morphology and nutrient content. The results indicated a great differentiation between the analyzed accessions in terms of some morphological characteristics. This was particularly visible between Polish small-seeded cultivars and large-seeded accessions derived from the Mediterranean Basin. Polish cultivars, as well as all the remaining accessions, were characterized by a high protein and low fat content, with large differences in fatty acid composition. However, for the seeds of all accessions containing anti-nutritional compounds – neurotoxins and tannins, a large differentiation was noted among accessions. This enabled selection of those accessions, derived from different parts of Europe, with the most promising nutritional value. These may constitute valuable initial material for breeding purposes leading to the release of new and improved grass pea cultivars.

Adaptation studies of some chickpea genotypes in Moroccan conditions

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This study was carried out to identify adapted (suitable) and high yielding genotypes of chickpea (*Cicer arietinum*) for Dryland Moroccan regions. In this endeavor, 40 chickpea genotypes (from different countries and selected randomly from the ICARDA chickpea germplasm collection) were evaluated during 2009/2010, 2010/2011 and 2011/2012 planting seasons at Jemâat Shaim research station. The experiment was carried out in alpha-lattice design (8*5) with two replications. During the season, characters such as number of days from sowing to flowering and to maturity, ascochyta blight score, and plant height were recorded or determined. Data on weight of one hundred seeds and yield were recorded from the middle 3 m of the plots to avoid border effects. The results of present study showed that mean square of genotypes for all characters were significant. FLIP 84-92C, FLIP 81-71C, FLIP 98-121C and FLIP 93-58C were selected for their high seed yield. FLIP 85-1C, FLIP 97-503C, FLIP 97-677C, FLIP86-5C, FLIP 87-8C and FLIP 86-6C were selected for their large seeds. FLIP 98-121C had the tallest plants and was selected for its high seed yield and large seed size. All of those genotypes selected, were also resistant to ascochyta blight. It was assumed that the selected genotypes could be proposed for catalogue registration or be used in breeding programs.

Cluster analysis in common bean genotypes (*Phaseolus vulgaris* L.)

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Phenotypic observations in the real farm conditions have importance for the plant breeding programs. In the present study, the common bean genotypes that are widely grown in Turkey were subjected to cluster analysis according to their phenotypic evaluations. Cluster analysis for the field performance of 35 promising common bean genotypes showed 4 main groups. Distance was ranged from 0.99 to 9.05 values. Hierarchical cluster analysis is a useful guide to evaluation of different genotypes. In the present study, analysis to determine distances among the used genotypes clearly separated into the bean groups. A dendrogram obtained which was based on the matrix of relationship between the genotypes. It can be concluded that cluster analyze can be useful to give information about selection of the promising genotypes for breeders.

A genetic study of the hybrid progenies between common (*Pisum sativum* L.) and red-yellow (*Pisum fulvum* Sm.) peas

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Red-yellow or tawny pea (*Pisum fulvum* Sm.) has become an object of increasing interest for breeders in the last decade, since it has been proven to be a source of a partial or complete tolerance to various biotic stresses, such as the attack of pea weevil (*Bruchus pisorum* L.). Crossings were made between red-yellow pea and four distinct agronomic types of common pea, namely with fasciated stem, determinate stem growth, afila leaf type and strongly developed funiculus. Each of these four traits was chosen because of its supposed positive impact on grain yield. A genetic analysis included monitoring of the main grain yield components, such as main stem length, number of fertile nodes, number of pods, number of grains and thousand grains mass, as well as the inheritance of those four morphological traits and the degree of the pea weevil attack in F_1 and F_2 hybrid generations. The results have shown that all grain yield components are controlled by numerous genes, since the average values in both hybrid generations were between those of the parents, as well as that the morphological traits are inherited as in common pea. They also confirmed that there were probably three independent genes controlling pea weevil resistance.

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Ideotypes in breeding vetches (*Vicia* spp.)

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Vetches (*Vicia* spp.) are one of the oldest annual legume crops used in animal feeding throughout the temperate regions of Europe, Near and Middle East and North Africa. The most important species are bitter (*V. ervilia* (L.) Willd.), Hungarian (*V. pannonica* Crantz), common (*V. sativa* L.) and hairy (*V. villosa* Roth) vetches. They are used mostly in the form of fresh forage, forage dry matter, forage meal, silage, haylage, mature grain and straw, as well as for grazing. Generally, breeding programmes on bitter vetch, common vetch and Hungarian vetch are more advanced in the countries of South Europe, Asia Minor and Near East, while hairy vetch is becoming more and more interesting as green manure in North America and Japan. If cultivated for forage, an ideotype of a vetch plant should be characterised by slender stems with determinate growth, a number of photosythetically active leaves of at least 15 in full flowering and large leaflets. Determinate growth is one of the essential traits, since it prevents excessive lodging and an economically significant loss of lower leaves that, in a lodged stand, easily degrade. Closely related is uniform maturity, in terms of both concurrent flowering and pod and seed development, unlike the wild type in all vetches, especially in hairy vetch, notorious for its indeterminate stem growth and extremely prolonged time of flowering and seed maturing, with flowers, young pods and shattered seeds at the same time in one plant. A good model for a vetch cultivar ideotype may be fund in bitter vetch that, despite short stems in comparison to other vetches, extremely well uses the volume of a stand and is able to produce considerably high forage yields. A separate aspect in vetch breeding is achieving to combine all desirable traits related to forage yields with high and reliable seed yield, necessary in commercialising a developed variety. In vetch cultivars aimed at using in non-ruminant feeding, an emphasis is also on seed chemical composition, with significantly reduced content of various anti-nutritional factors.

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Developing lines of less-widespread warm season legumes for cool climates

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In most Western, Central and Southeastern European countries, it is cool season legumes, such as pea (*Pisum sativum* L.), faba bean (*Vicia faba* L.), chickpea (*Cicer arietinum* L.), lupins (*Lupinus* spp.), grass pea (*Lathyrus sativus* L.), vetches (*Vicia* spp.), lucerne (*Medicago sativa* L.) and clovers (*Trifolium* spp.) that are mostly grown. Two warm-season exceptions are common beans (*Phaseolus* spp.) and soybean (*Glycine max* (L.) Merr.), cultivated mostly in Southern and Southeastern Europe. During the last decade, a programme has been launched at the Institute of Field and Vegetable Crops and assisted from the Faculty of Agriculture of the University of Novi Sad aimed at breeding and agronomy of the less-known warm season legume crops in this part of Europe, with a specific emphasis upon pigeon pea (*Cajanus cajan* (L.) Huth), hyacinth bean (*Lablab purpureus* (L.) Sweet), adzuki bean (*Vigna angularis* (Willd.) Ohwi & H. Ohashi), black gram (*Vigna mungo* (L.) Hepper), mung bean (*Vigna radiata* (L.) R. Wilczek) and cowpea (*Vigna unguiculata* (L.) Walp.). The first step was the establishment of a collection of each of these species, with characterising the most peculiar morphological and anatomical traits and evaluating the most important agronomic traits such as forage and grain yield, quality and stress response, as well as the photoperiodical reaction. Some of pigeon pea accessions were able to produce forage yield of more than 100 t ha^{-1} of fresh forage, but remained in full flowering until very late autumn and were barely able to bring forth seeds. On the other hand, selecting the lines of all the said warm season annual legume crops led to the development of the first lines suitable for both forage and grain production in cool climates and temperate regions, such as Serbia, with a northern latitude of 45°. The ultimate goal of this complex programme are cultivars that would be able to fit into various existing crop rotations, providing forage in mid- and late summer, when the traditional annual legumes have already left the field and thus provide the local animal husbandry with quality forage in the driest period of a year.

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Breeding autumn-sown annual legumes for temperate regions

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Annual legumes are one of the fundamental crops of the agriculture of Serbia. Until recent times, only pea (*Pisum sativum* L.) or vetches (*Vicia* L.) were autumn-sown in Serbia and other neighbouring Balkan countries. During the last decade, the first seminal steps in the development of autumn-sown grain legumes were made, with emphasis on dry pea, faba bean (*Vicia faba* L.) and dual-purpose vetches. The winter forage pea cultivars developed in Serbia are generally characterised by prominent winter hardiness and a rather extended growing season, usually beginning with sowing in early October and ending either by cutting for forage production in late May or harvesting seeds in mid-July. One of the strategic advantages of recently released cultivars of autumn-sown dry pea is their significantly improved earliness. The Serbian winter dry pea cultivar NS Mraz, newly registered in Serbia in 2011 and developed from hybrids between French and Serbian autumn-hardy materials, is regularly at least a week earlier than winter barley, so many farmers will not have to choose between pea and cereals due to limited harvesting resources. The initial material for pre-breeding autumn-sown faba bean in the conditions of Serbia consisted of both collected local landraces of Serbia and populations from France and Germany. The preliminary results of their agronomic performance showed that they have a great potential for high grain yield in the conditions of Serbia. Promising results in breeding winter hardy cultivars were achieved with several vetch species, as well as with lentil (*Lens culinaris* Medik.) and grass pea (*Lathyrus sativus* L.), and will be continued with more species including barrel medic (*Medicago truncatula* Gaertn.).

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Genetic diversity assessment in soybean cultivars using morphological and molecular marker data

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Eighteen soybean genotypes from three different breeding institutions were evaluated using morphological and molecular marker data. Thirteen qualitative traits were chosen for morphological characterization. RAPD molecular marker analysis was used to deterimne genetic diversity and relatedness between soybean cultivars. Diversity of qualitative traits was described by Shannon index and its average value was 0.766. Polymorphism information content (PIC) was calculated for RAPD data with average value of 0.389. Genetic similarity was determined using simple matching coefficient for all types of data and it ranged from 0.278 to 0.888 for phenotypic and from 0.740 to 0.972 for molecular markers. Genetic similarity matrices were submitted to cluster analysis using UPGMA method in NTSYS-pc software. Relative positions of the soybean cultivars in dendrograms based on qualitative and RAPD data were rather different. Results of cluster analysis based on molecular data were in better agreement with pedigree data. Both systems (molecular and morphological) together provide different and complementary information. Genetic relationships estimated with both molecular markers and phenotypic traits can reveal sources of desirable characteristics in closely related individuals.

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Correlation and path analysis for yield and yield components in common bean genotypes (*Phaseolus vulgaris* L.)

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Determination of breeding criteria is quite important for plant breeders. The present study was carried out to determine yield and its components which are affecting seed yield and to study the relationships between yield components and other characteristics. A total of 42 common bean genotypes that are being widely grown in Turkey were used as material. Direct and indirect effects of the yield components on seed yield were analyzed using path coefficient analysis. Seed yield (kg da^{-1}) was most affected by biologic yield (84.6%), harvest index (65.5%) as positive and negatively affected by main branch per plant (28.4%) and flowering day (13.3%), respectively. Correlation analysis showed that seed yield (kg da^{-1}) was effected by biologic yield (0.8224**), harvest index (0.2913**) as positive and negatively affected by flowering day (-0.3256**), first pod height (-0.2473**), plant height (-0.2406**) and pod per plant (-0.2272*), respectively. According to the path analysis, it is important the biologic yield, harvest index, number of main branch per plant and days to flowering, due to direct effect to increase seed yield on selection studies.

Deploying genomics tools for improving resistance to leaf rust through marker- assisted backcrossing in cultivated groundnut (*Arachis hypogaea* L.)

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Rust (*Puccinia arachidis)*, is a major devastating fungal disease of groundnut (*Arachis hypogaea*) in semi-arid tropics regions of the world resulting in significant yield loss. It has been witnessed recently that integration of genomics tools with conventional breeding leads development of improved cultivars in less time and with increased efficiency. However, availability of genomics tools such as validated tightly linked markers to traits of interest is prerequisite to improve resistance in cultivars through marker-assisted breeding. In this context, a major QTL flanking with four SSR markers (IPAHM103, GM2079, GM2301, GM1536) for rust resistance contributing phenotypic variance up to 82.62% has been identified in TAG24 x GPBD4.This QTL and associated markers were validated in a wide range of germplasm and also including another RIL mapping population (TG26 x GPBD4). Then subsequently, this QTL for rust resistance was introgressed from GPBD 4 into three elite groundnut varieties (ICGV91114, JL24, TAG24) from GPBD 4 through marker-assisted backcrossing (MABC) approach. Among the four SSR markers, IPAHM103 behaves as a dominant marker while the remaining three markers were co-dominant in nature and hence, we deployed them in selection of heterozygotes/homozygotes among segregating backcrossed progenies. In summary, a total of 200 introgression lines (117 progenies of BC2F5 and 83 of BC3F4) with homozygous resistance allele were developed and evaluated. These introgression lines have shown par resistance than donor, GPBD4, for rust resistance and other agronomic traits. The promising lines with improved rust resistance and desirable yield will be further subjected to multilocational trials for varietal release.

Agronomic and morphological characters of newly registered Reyhan and Peksen vegetable cowpea cultivars in Turkey

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In Turkey, cowpea (*Vigna unguiculata* (L.) Walp.) is mainly grown in Aegean and Mediterranean regions for fresh pods and dry seeds. Cowpea breeding program was started in 1997 at The University of Ondokuz Mayis, Samsun, Turkey. During this breeding program, 12 advanced cowpea lines developed from 27 local cowpea populations of Turkey. Until April 2011, registered vegetable cowpea cultivars did not exist in Turkey. In the results of breeding program and subsequent two locations registration experiment during 2005 and 2006, L3 and L13 lines were registered as the first vegetable cowpea cultivars with the name of Peksen and Reyhan. Over two years study, cowpea cvs Peksen and Reyhan gave 18.0 t ha^{-1} and 10.99 t ha^{-1} fresh pod yield, while dry cowpea cvs Karagoz-86 and Akkiz-86 used as controls gave 10.23 and 7.47 t ha^{-1}. It may be concluded that new vegetable cowpea cultivars may be a good alternative vegetable crop and it can be successfully grown in Black Sea Region of Turkey as much as in Aegean and Mediterranean regions. The main purpose of this paper is to introduce of newly registered vegetable cowpea cultivars, Peksen and Reyhan, and to present their important agronomic and morphological characters.

Agronomic evaluation and quality characterization of lentil accessions (*Lens culinaris* L.) in a Mediterranean environment

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Lentil (*Lens culinaris* Medik.) is an important grain legume cultivated in the Mediterranean region and used for human nutrition. An extensive differentiation of lentil over millennia has permitted the evolution of many landraces. These ecotypes often are the result of centuries of selective choices by local farmers and are characterized by high genetic variability and high adaptation to different environmental conditions. Presently, lentil is mainly cultivated in marginal areas of Central and Southern Italy and in Sicily small cultivation has permitted the evolution of local landraces. An understanding of the genetic relationships and diversity of lentil germplasm, in relation to landraces collected in traditional cultivation areas of Sicily and from other countries, is important in attempting to widen the genetic resource in the region. 30 accessions of lentil obtained from FAO collection and 10 local ecotypes were evaluated for agro-morphological-qualitative traits in internal hilly environment of Sicily. A wide range of diversity of almost all of detected traits was recorded for the lentil accessions. Some accessions were characterized by a good adaptation to the semi-arid environment of internal environment of Sicily as shown by the comparison of its yield performance with that of populations native to Sicily or to other countries. The study of agronomic important traits, as plant height, showed less variability, even though some accessions could be used to adapt the crop to modern production techniques such as mechanical harvesting. The evaluation of this collection has shown appreciable results that could foster future breeding programmes.

Development of large seeded chickpea lines resistant to Ascochyta blight

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With the aim of developing chickpea cultivars including blight resistance and large seeds a crossing program between 5 resistant advanced lines with medium-large seeds and different sources of resistance were initiated in year 2005. Those advanced lines were originally derived from crosses between resistant accessions from ICARDA and the Spanish large seeded (around 0.50 g per seed) and susceptible local cultivar 'Blanco Lechoso'. A F_2 population with a total of 909 plants was obtained joining F_1 derived from all possible crosses between the 5 advanced lines. Early generation testing method of F_2-derived lines was employed for large seed selection until $F_{2:5}$ families. The best individuals from the best $F_{2:5}$ families were selected and advanced selecting for large seeds until $F_{5:8}$. Finally, in 2011/2012 agronomic season, 30 $F_{5:8}$ advances lines were sown following a randomized block design with two repetitions and parents as controls. Eleven lines with larger seed size than the best parent (0.44 g per seed) were selected (ranking from 0.49 g to 0.57 g per seed). These results suggest that transgressive segregation is present in chickpea for seed size. In order to test the presence of QTLs for ascochyta blight in the selected advance lines, allele-specific markers for QTL_{AR1} (CaETR) and QTL_{AR2} ($SCY17_{590}$) were employed. Both QTLs were present in all selected lines.

Selection for protein content in high protein vs. food grade crosses of soybean

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Soybean (*Glycine max* [L.] Merr.) is increasingly utilized for soyfood production in Central Europe. While conventional soybean seeds are typically composed of 40% protein and 20% oil, a higher protein content of 42-45% is required in food grade soybeans for producing soy drinks, tofu, spreads or food additives. In addition, larger seed size and increased sucrose content as compared to conventional soybeans are desirable characters for many soyfood applications. As most of the soybeans utilized at present have not been selected for food grade characters, there is need to introgress specific germplasm into early maturity backgrounds. A high protein line and a major food grade cultivar were each crossed to two early maturity cultivars adapted to Central Europe, lines from each cross were selected for either high or low seed protein content and subsequently tested for variation in agronomic and seed quality characters in three years. Seed protein content was in the range from 430-490 g kg^{-1} in high protein crosses and from 400-440 g kg^{-1} in food grade crosses; in contrast, lines from food grade crosses were higher in sucrose content and 1000-seed weight. Selection for increased protein content reduced both oil and sucrose content in three out of four crosses and resulted in a larger 1000-seed weight in the food grade crosses. Heritability estimates of seed quality characters were higher in high protein populations than in the food grade crosses but suggest a good potential for introgressing desirable characters into adapted germplasm.

Effect of genotype and environment on yield and crude protein content in narrow-leafed lupin

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Lupinus angustifolius L. is the most important cultivated lupin species in Central Europa, especially in Germany. Currently the only breeding program in Germany is located in North-East Germany at Saatzucht Steinach. Yield parameters of 50 varieties and breeding lines (eight with restricted branching and 42 with normal branching) of narrow-leafed lupin (*Lupinus angustifolius* L.) were measured in Mecklenburg-Pomerania over three years at three locations to demonstrate the effect of locations (l), years (y), genotypes (g), and genotype x environment interaction on yield and crude protein content. The locations, years, genotypes and the genotype x environment were clearly significant for each parameter. The trials demonstrate, that environment has a greater influence on yield (significant at < P = 1%). For grain yield, the strong interaction between site and year was caused by the extreme environment of Bornhof. The highest yield was found in 2011 at the site Groß Lüsewitz. Yield was more affected by site than by year. The crude protein content was also significantly affected by site and year. Between the year 2010 and 2012 less differences were found among the sites. Yield was negatively correlated with crude protein content. The differences in crude protein yield reflect the differences in yield indicating the stronger impact of grain yield than of protein content.

Grain legumes genetic enhancement through pre-breeding

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Narrow genetic base of grain legumes is one of the limitations in genetic enhancement for yield, quality traits and resistances to biotic and abiotic stresses. Wild relatives have enhanced levels of resistance/tolerance to multiple stresses, and provide important sources of genetic diversity for crop improvement. ICRISAT genebank conserves about 1350 accessions of wild relatives of chickpea, pigeonpea and groundnut from 53 countries. Though the importance of these wild species as new and diverse sources of variations is well known, their exploitation for improvement of cultivated species is mainly hindered due to various cross-incompatibility barriers as well as due to linkage drag, thus making it a time-consuming and resources-demanding research endeavor. To overcome this, pre-breeding provides a unique opportunity to the breeders through the introgression of desirable genes from wild germplasm into genetic backgrounds readily used by the breeders with minimum linkage drag. At ICRISAT, wild accessions of *Cicer*, *Cajanus* and *Arachis* possessing high levels of resistance to multiple stresses have been identified. Further, for groundnut improvement, several amphiploids and autotetraloids have been synthesized by using various A- and B- genome species to create genetic variability for use in the breeding programs. Pre-breeding activities using wild relatives and popular cultivars have been initiated at ICRISAT to develop new genepools in chickpea, pigeonpea and groundnut with high frequency of useful genes, wider adaptability, and a broad genetic base. Further, the availability of molecular markers will greatly assist in reducing linkage drag and increasing the efficiency of introgressions in pre-breeding programs.

Introgression lines of wild *Pisum fulvum* in cultivated pea (*P. sativum*) genetic background, as a tool to broaden genetic diversity.

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Plant evolution under domestication has altered numerous traits, including self-pollination which reinforced fertility barriers between wild and cultivated populations, facilitating fixation of the desired genotype. Consequently, domestication bottleneck has resulted in high degree of relatedness between varieties, which was further pronounced in modern breeding programs, leading to narrower genetic base of cultivated germplasm, prone to pests and diseases. The study of genetic diversity preserved in pea collections showed that although wide diversity is captured among cultivated material, wild material provides yet broader diversity. We report development of 105 chromosome segment substitution BC_2S_3 lines (CSSL) containing genomic segments of phylogenetically the most distant wild pea (*Pisum fulvum* WL2140) in the cultivated pea (*P. sativum subsp. sativum* WL1238) genetic background. Selected polymorphic 28 microsatellite and 44 gene-specific markers covered all seven linkage groups (LG) of pea 4.45 Gbp genome at 2 to 82 cM spacing, with mean of 15.4 cM, delimiting 78 chromosomal regions. The heterozygozity was detected in 533 (8%) cases while recurrent *Pisum sativum* parent in 4552 (69%) and introgressed segments of *P. fulvum* in 1551 (23%) of 40 cM in average, covering 20 – 70% of given linkage group. There were 5 to 14 segments per line, with mean of 9.6. These lines have been phenotyped for 14 traits (including branching, height, node, pod and seed numbers) showing often transgression. A CSSL Finder analysis was used to reveal genetic and graphical relationships and select lines. Establishment of such permanent introgression library will allow phenotypic characterization of unlimited number of target traits, which, coupled together with higher density markers, will provide means for QTL and gene identification and subsequent incorporation in desired genotypes ultimately leading to better performing pea varieties.

Yield relationships between F_2 and F_3 soybean lines differing in homozygosity for the presence of the Kunitz trypsin inhibitor

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An F_2 population from the cross combination between Kunitz-free soybean variety Laura and common grain type variety Afrodita was developed. Satt228 marker, tightly linked to Kunitz trypsin inhibitor (KTI) locus, was used to screen 92 individual F_2 plants in order to identify homozygous and heterozygous genotypes for KTI alleles. The genotypic ratio was 1:2:1 (Ti TI : Ti ti : ti ti constitution) which is expected for a character determined by a single mendelian gene. The seed yield of individual F_2 plants, as well as yield of their F_3 progenies, was measured. In order to determine yield relationships between F_2 and F_3 soybean lines, simple correlations and Spearman's rank correlation was calculated. Dependences between yield of F_2 and F_3 soybean lines was not found into whole three genotypic classes. Because of that early generation selection should not to be rigorous. An SSR marker Satt228 can be useful tool for early Identification of homozygous and heterozygous genotypes in breeding for Kunitz-free soybean varieties, with no potential risk for loosing high yielding genotypes. Presence or absence of KTI did not influence yield relationships between two early generations (F_2 and F_3) of soybean.

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Analysis of some nutritional and antinutritional compounds in dry seeds of grass pea and field pea cultivars from Croatia

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Field pea (*Pisum sativum* var. *arvense*) and grass pea (*Lathyrus sativus*) are important high protein crops used as feed. Legume seeds are also rich in many other nutrient compounds including starch, sugar, dietary fiber and micronutrients (vitamins and minerals.) They are also a rich source of many bioactive non-nutrient compounds including phenolic antioxidants. Besides their beneficial effects, some phenolic compounds, especially condensed tannins in feed, are considered as antinutritional factors for ruminants, owing to their capacity for binding to macromolecules such as fiber and proteins. Seed mass and diameter are valuable parameters used in breeding, as well as seed mineral composition, since significant quantitative trait loci were identified for Ca, Fe, Mn, K, Mg, Ni, P and Zn. Thus we conducted this study in order to determine some parameters, in three cultivars of previously non-tested field pea and five grass pea cultivars from Croatia, which are important for breeders. We determined seed mass, diameter and proximate mineral composition as well as content of soluble sugars and total phenolic content in dry seeds of these cultivars. Cultivars differed significantly in total phenollics and mineral composition.

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Genes *ti* (terminal inflorescence) and *rb* (restricted branching) in legume breeding

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The flag leaf finishes vegetation growth in cereals. In subsequent plant development exclusively generative organs are formed – ears, flowers and grains. In legumes, the appearance of generative organs does not end vegetative growth. In favourable atmospheric conditions, plants form successive nodes (pea, field bean) or lateral branches (lupins) with new flowers, pods and seeds. It causes a longer vegetation period and non-uniform seed maturity at harvesting. The above was a reason to look for plant characteristics controlling an end of vegetative growth. Respective characteristics/genes were selected from collections and as induced mutations. Genes *det* in the pea and *ti* in the field bean cause terminal inflorescence on the top of the stem while a homologous row of the gene *rb* in four lupin crops (*L. angustifolius, L. albus, L. luteus and L. mutabilis*) blocks lateral branching. These genes were incorporated into breeding programs in Poland and the first cultivars were registered: the field bean cv. Tibo (1990), yellow lupin cv. Manru (1990), narrow-leafed lupin cv. Bar (1991) and Wersal (1995) and white lupin cv. Katon (1999). Progress in the breeding of a new cultivar model (in comparison to traditional ones) of the above mentioned legume crops over the past 20 years will be presented. The results of field trials by the Research Center for Cultivar Testing will be used in the analysis. It is worth mentioning that appropriate growing technologies should be applied to these new cultivar ideotypes.

Adopting high yielding cultivars for enhancing legume productivity in rainfed farming system

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In arable and permanent croplands, South Asia has the largest area because India tops all East and South Asia & Pacific countries, with 202.83 m ha. Rainfed mixed farming covers the largest area within the subcontinent and is confined to India. This system covers 147 million ha, with about 59% under cultivation. Arid and rainfed mixed farming systems are associated with extensive poverty. High inequality of land distribution and declining average farm size pose the question. In one of the aspect of increasing sustainable and resilient production, building on best local practices while exploiting the latest research and technology and nurturing innovation, it focused not only on developing and making available improved technologies, but also on building the capabilities of poor rural people as producers and managers of increasingly scarce natural resources. In this context, the state of Madhya Pradesh- a largest legume producer was chosen for increasing the productivity of pigeonpea (*Cajanus cajan*). The aim is to raise and stabilize the incomes of poor rural people, so they can improve their food security. The dissemination of knowledge of adopting new high yielding, wilt resistant cultivars of pigeonpea, is changing yield levels through sustainability in yield. Pigeonpea is mostly grown as inter crop with soybean on residual moisture. The intercultural operations in soybean favours the crop growth of pigeonpea and also reduces the cost of inter culturing. After the harvest of soybean, the pigeonpea triggers its growth and cover the whole area like sole crop. The shaded leaves of soybean provides the mulching effect on surface soil, checking soil moisture evaporation, thus providing long lasting sub surface moisture to pigeonpea till its reproductive stage. The high yielding varieties like JKM 189, JA 4 and newly released hybrid RVICPH 2671 has increased the yield manifold in rainfed farming system and helped the marginal farmers in boosting their productivity of unit area.

Induction and inheritance of compound leaf and cone stipule in the cultivated chickpea (*Cicer arietinum* L.)

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The cultivated chickpea (*Cicer arietinum* L.) have mainly three different leaves, multipinnate/ bipinnate, simple/unifoliate and normal/fern leaves, produced by spontaneous or induced mutations. The present study was aimed to study the inheritance of two new induced characteristics. Two new induced characteristics, compound leaf like the genus *Lupinus* L. and cone stipule on internodes**,** were isolated in a single mutant chickpea in M_3 generation. Inheritance of the new characteristics was studied in the segregating populations of M_4 generation. Results indicated that the new characteristics were governed by two recessive genes. The new induced leaf and stipule characteristics were designated by *clcl* and *cscs* symbols, respectively. The segregated results suggested that these genes could be linked since they dependently segregated. The new genes may be useful for gene mapping in *Cicer* species.

Diversity of E genes among soybean maturity groups

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Soybean maturity groups are designated based on geographical areas where particular varietis are adapted. The main environmental factor affecting adaptation is photoperiod. The wide distribution of soybean is caused by diversity of the E genes that interact and control time to flowering and maturity. Dominant alleles delay flowering and their effect under different environments is stable. E2 gene encodes an ortholog of GIGANTEA gene while E3 and E4 control photoperiod insensitivity and encode phytochrome A. E genes are well characterized, but their distribution among germplasm and maturity groups is not quite known. A set of 78 genotypes, originating from North America and Europe was tested on presence of dominant or recessive allele of E2 and E4 genes. All observed maturity groups (from 000 to III) had low frequency of dominant allele of gene E2 ($p=0.22$). Recessive allele of this gene was present in all tested genotypes ($n=15$) in early maturity groups (000 and 00). In 0 and I maturity groups were noticed low frequencies of dominant allele whereas in later maturity groups, II and III, significantly higher frequencies are observed. Clinal variation of E2 gene frequencies among maturity groups was found. Significant differentiation between maturity groups in allele frequencies was indicated by g_{ST} values (0.439). Furthermore, fixation index ($F_{ST}=0.76$) demonstrated strong differentiation among soybean maturity groups, based on E2 gene. Diversity of E4 gene is very low and analysis of E4 gene distribution showed presence of recessive allele only in three genotypes belonging to the 000 maturity groups. This findig can be useful in breeding of phoperiod insensitive soybeans.

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Branching in red clover (*Trifolium pratense*): a morphological, physiological and molecular approach

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Red clover has several advantages in agriculture: the ability to fixate nitrogen, high seedling vigour and high nutritional value for feed and indirectly for food (high protein and polyunsaturated fatty acid levels). Plant architecture, which is under genetic and environmental control, may have a strong influence on agronomic important traits such as forage yield, re-growth capacity, seed yield and persistence in red clover. We have done an in-depth study of the architecture of a limited number of red clover genotypes with contrasting branching phenotypes. A detailed morphological analysis showed differences in the number of buds, the number of branches, the position of bud outgrowth and bud outgrowth percentage in the different genotypes. Also, differences were observed in the re-growth after cutting between the genotypes. In a physiological approach, isolated single node fragments were used to study the influence of branching hormones on bud outgrowth without having to deal with the complex architecture of a complete red clover plant (non-outgrowing main axis, many first-order branches). These results will be linked to expression levels of branching genes. In addition, we determined concentrations of endogenous strigolactones and auxins in intact plants of the various genotypes. The results demonstrate that both bud formation and bud outgrowth are important to explain branching differences in red clover. The importance of the strigolactone and auxin pathways as candidates for further analysis and molecular breeding for high yielding and more persistent red clover cultivars will be discussed.

Corolla tube lengths in red clover (*Trifolium pratense*) cultivars

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Red clover increases the yield of grass-clover mixtures by its capacity for nitrogen fixation. Today, red clover is mainly used as silage and as organic nitrogen source in organic agriculture. Tetraploid red clover cultivars produce more dry matter weight and have an increased disease resistance compared to diploid cultivars. However, the disadvantage of tetraploid cultivars is their poor seed yield. One of the proposed causes is a larger corolla tube length which would prevent most of bumblebees and honeybees from reaching the nectaries. To test this hypothesis we measured corolla tube length along with the individual seed yield after open pollination in single genotypes of 5 diploid and 5 tetraploid red clover cultivars. The corolla tube length varied between cultivars as well as within cultivars. Although there was a significant difference in corolla tube length between diploid and tetraploid genotypes, seed yield of individual genotypes was not correlated with the corolla tube length. The selection for smaller corolla tube lengths in tetraploids will not increase seed yield so other aspects influencing seed yield such as the number of flower heads, pollen viability, nectar heights need to be analysed.

Breeding of minor fodder crops for sustainable agriculture

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Breeding programmes of minor crops are very important for increasing the diversity in agriculture and for sustainable agriculture. Input materials in most cases are crop wild relatives of main cultivated forage species like alfalfa, red clover, white clover etc. Gathering, growing and breeding of crop wild relatives has a long tradition in the Czech Republic. In the workplace in Troubsko it has been done for 60 years. The result of this activity is 21 registered varieties of different minor crops (*Trifolium pannonicum, Lablab purpureus, Lathyrus sativus, Trigonella foenum-graecum* etc.) and many breeding lines included in the state registration trials (*Astragalus cicer, Trifolium alpestre, Galega orientalis* etc.). One of the results of breeding programme is unique variety *Trifolium pratense × Trifolium medium* (*Trifolium* X *permixtum* Neumann) – PRAMEDI. The variety was bred by interspecific hybridisation of the red clover (*Trifolium pratense* L.) cv. Tatra ($2n = 4x = 28$) and zigzag clover (*T. medium* L.) ($2n = 8x = 64$). It is a perennial legume species that is suitable as a substitution for red clover especially in dry and warm regions. The height, flowering time and quality are comparable to standard tetraploid red clover varieties. By the plants short underground rhizomes are frequent and the plants have better persistence.

Genetic advancement in seed yield of pulse crops in Madhya Pradesh, India

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Pulses are one of the important segments of Indian Agriculture after cereals and oilseeds. India is the largest producer and consumer of pulses in the world contributing around 25-28% of the total global production. About 90% of the global pigeonpea, 75% of chickpea and 37% of lentil area falls in India, (FAOSTAT 2010). The estimates for 2011-12 indicate that the total pulse production is 17.28 million tons from 25.43 million ha area majority of which falling under rainfed, resource poor and harsh environments frequently prone to drought and other abiotic stress condition. Pulses are least preferred by farmers because of high risk and less remunerative than cereals; consequently, the production of the pulses is sufficiently low. To meet the demand of pulses, India is at present importing about 3 million tons. Chickpea continues to be the largest consumed in this complex comprising of 45-50% of the total pulses production of India. Historically, Madhya Pradesh, a pulse bowl state of India enjoying the cultivation of diversified pulses *viz.* chickpea (*Cicer arietinum*), pigeonpea (*Cajanus cajan*), lentil (*Lens culinaris*), field pea (*Pisum sativum*), green gram (*Vigna mungo* L. Happer) and black gram (*Vigna radiata*). Chickpea is the major pulse crop covering 3.11 m ha area with average productivity in 1080 kg/ha. Pulse research in Madhya Pradesh though started in 1950s, but development of short duration and high yielding varieties having durable resistance against *Fusarium oxyporum* f.sp. *ciceri* in recent past has resulted in both horizontal and vertical spread of the crop. Chickpea variety develop in the university namely JG 11, JG 130, JAKI 9218, JG 16, JG 6, RVG 201, RVG 202 and RVG 203 in desi type and JGK 3 and RVKG 101 in kabuli type have covered the area not only in the Madhya Pradesh but also in southern and western India states. The average yields of these varieties is > 2.0 t/ha. The high yielding and medium duration varieties; JA4, JKM7, JKM 189 and TJT 501 of pigeonpea are popular in the state but the university is credited for development and release of world fist CMS based pigeonpea hybrid RVICPH 2671 in collaboration with ICRISAT, Pattancheru. This hybrid has sown the standard heterosis between 30 to 35 per cent. In lentil, microsperma type bold seeded (> 2.5 gm/100 seed weight) are grown. JL 3 and RVL 31 are recommended for cultivation which is short duration, high yielder and possess ability to tolerate drought. Green gram variety TJM 3 is short duration (60 days) bold seeded and high yielding variety (1.3 to 1.5 t/ha) having resistance against powdery mildew, YMV and bacterial blight. A large number of land races have been collected, evaluation and characterize which are utilizing in genetic amelioration of pulse crop for early maturity, better seed size, high nodulation ability, better seed yield than existing ones, resistance against major soil borne diseases and tolerance to high temperature and moisture stress during crop growth in order to fulfil the demand of growing Indian population.

Stereological analysis of petiole of forage and grain soybean cultivars

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The structure of petiole resembles the stem structure in most of the legumes and has higher potential impact on digestibility than leaf lamina. Petiole anatomy of forage and grain soybean cultivars was analyzed using stereological method. The aim was to obtain data about petiole structure, to assess the proportion of different tissues from its proximal to distal end and to compare petiole characteristics between grain and forage cultivars. Lignified petiole tissues were xylem and sclerenchyma. Their volume densities (*Vv*) were the lowest in distal petiole part, closest to the leaf blade, whilst *Vv* of epidermis and collenchyma were the lowest in proximal region. Xylem proportion gradually increased towards the petiole base, whilst the proportion of phloem and the number of vascular bundles significantly increased from proximal to distal petiole end, probably as the result of bundle division along the petiole length. Thin epidermal and collenchyma tissue, the small size of vascular bundles and small groups of sclerenchyma are favorable characteristics concerning petiole digestibility. 'Tyrone'could be singled out as the cultivar with the highest *Vv* of parenchyma and the lowest *Vv* of xylem and sclerenchyma, and significantly the lowest *Vv* of epidermis and collenchyma in petiole. Grain cultivars had higher *Vv* of phloem compared to forage cultivars, which enabled faster transport of photosynthates from leaves to grains. No significant differences were recorded between forage and grain cultivars in anatomical parameters connected to digestibility, so petioles do not reduce the potential of grain cultivars to provide the high quality forage.

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Session 5

Legume seed production, meeting market requirements and economic impacts

KEYNOTE LECTURE

Legume seed production, meeting market requirements and economic impacts

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Among the forage legumes alfalfa (*Medicago sativa* L.) is the most important crop with white clover (*Trifolium repens* L.) coming second. The major seed producers of alfalfa is USA (California, Idaho and Washington), Canada and France, whereas white clover seed predominantly is produced in New Zealand, USA (Oregon) and Denmark. Seed yield traits has not been of highest priority in forage legume breeding programmes, however, with the increasing demand for plant products for food, feed and bioenergy a high seed yield potential is required to allow for the commercial success of new forage legume varieties. Agronomic research in white clover seed production was carried out in the Department of Agroecology, Aarhus University, Denmark, where the influence of harvest methods on seed yield and seed quality was evaluated in 2010-2012. In a field experiment performed in three consecutive years seed yield was determined and seed samples were identified for the analysis of germination in relations to seed colour. The traditional harvest method is swathing followed by combining after 3-5 days of drying. Precipitation during this period is likely to initiate germination resulting in high seed yield loss. Overall it was concluded that desiccation with MCPA and/or Reglone (a.i. diquat) did not negatively influence seed yield or seed quality and the necessary drying period may be shorter than 3-5 days. Detailed results from this and other experiments in forage legume seed production are presented during the talk

Agricultural innovative practices and impacts of the supply chain: An ex-ante study of the logistics of agricultural cooperatives to estimate the acceptability of durum wheat-grain legumes intercrops

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Diffusion of innovative agricultural practices like intercropping (growing simultaneously two or more species in a same field) raises the question of their acceptability within the supply chain. Intercrops like durum wheat-grain legumes have shown potential advantages for global yield, grain quality and pests reduction and could facilitate reductions in nitrogen supply and pesticide use. However, adoption of these new practices by farmers is linked to the logistics of agricultural cooperatives (abilities to the collection, grading and marketing of the two species). The aim of this work is to assess ex-ante the characteristics of the logistics of agricultural cooperatives likely to promote the adoption of new practices such as intercropping. It is based on a survey carried out in 2010 on the logistics of agricultural cooperatives in the Midi-Pyrenees region (SW France). The assumption is that the actors of the so-called "conventional" supply chains could accept such new practices according to their leeway to change their system. This study reveals a set of criteria such as transport, storage, grading or contracts and commercialisation which determine whether or not they could be compatible with these new practices. The cooperatives indicating that their logistics is *a priori* compatible are those oriented towards quality management. These cooperatives present a specific set of skills facilitating adaptation to a greater diversity of crops, allowing us to qualify their logistical organization as more resilient. The main obstacle to the diffusion of durum wheat-grain legumes intercrops within the “conventional” supply-chains remains the problem of sorting seeds.

Autophagy in embryo axes of yellow lupin (*Lupinus luteus* L.), white lupin (*Lupinus albus* L.), and Andean lupin (*Lupinus mutabilis* Sweet) germinating seeds

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Experiments were conducted on embryo axes which were isolated from imbibed seeds and culturedin *in vitro* for 96 hon liquid mineral medium in six trophy variants: on medium with 60 mM sucrose (+S) or without sucrose (–S) and on media additionally supplemented with asparagine (+S+Asn and –S+Asn) or sodium nitrate (+S+NO_3 and –S+NO_3). Ultrastructural observations were performed in embryo axes root meristematic zone cells. Sugar starvation (–S) caused a significant increase in cell vacuolizationin cells of embryo axes of all three investigated species (irrespectively of Asn and NO_3 nutrition).Inside vacuoles of starved cells (–S) many plasma membrane structure were observed. Simultaneously, a decrease in phosphatidylcholine level was observed. These results pointed on advanced autophagy.In cells of embryo axes fed with asparagine (–S+Asn) the decomposition of autophagic bodies was remarkably lover than in cells of non-fed with asparagine (–S) embryo axes. This allowed to more or less precise identification of cell components which were degraded during autophagy. Inside vacuoles of –S+Asn axes the following cell components were visible: cytoplasm, ribosomes, oil bodies, mitochondria, and spherical objects which were looked like peroxisomes. Ultrastructure of cells of axes fed with nitrate (–S+NO_3 and +S+NO_3) was very similar to –S and +S, respectively.

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Production and economic effects of various intensity of pea cultivation

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The field experiments were carried out in 2005 – 2008 in Złotniki Experimental Station, near Poznań, as a completely randomized design in four replications.Investigated factors:1) water variant: - irrigated – irrigation was applied when the water content in 0 -30 cm soil layer fell to 70 % of field capacity in the period of the greatest susceptibility of plants to water deficit, - non irrigated – natural water conditions; 2)cultivation technologies: - low-input – without phosphorus and potassium fertilization and chemical plant protection, - medium-input – with limited application of pesticides, phosphorus fertilization – 45 kg P_2O_5/ha, potassium fertilization – 65 kg K_2O/ha, -high-input – with full plant protection, phosphorus fertilization – 60 kg P_2O_5/ha, potassium fertilization – 90 kg K_2O/ha and foliar fertilization; 3) Nitrogen fertilization: O, 30, 60, 90 kg N/ha. It was stated that irrigation affected the yield of seeds interacting with cultivation technologies and nitrogen fertilization. Under irrigation conditions the medium- and high-input technologies, in comparison to low-input one, increased seed yield, respectively by 0,48 and 0,51 t/ha, whereas under non irrigated conditions by 0,13 and 0,23 t/ha. Under irrigation conditions the nitrogen doses: 30, 60 and 90 kg/ha caused a significant yield increase as compared to the object without nitrogen. Under non irrigated conditions nitrogen fertilization did not differentiate significantly seed yield. In the four-years cycle of research irrigation, on the average for other factors, increased the seed yield by 1,64 t/ha (124,2 %). In both water variants the most profitable turned out the low-input cultivation technology.

Leguminosis facing lock-in effects: enhancing food chain coordination to unlock?

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Facing agro-environmental issues, evolution of French agrosystems towards more sustainability is through the development of cultures such as protein pea (*Pisum sativum*). Whereas it was a staple nutriment for several livestock production in France during the 1980's, cultivated surfaces have been dramatically decreasing for two decades, despite the agroecological interests of this culture (N-fixation) and the nutritional interests of the raw material for various animal breeding (particularly pork and poultry industries). This diagnosis gets public researchers to question the conditions of emergence/re-emergence and development of new crops in cultural systems. This paper aims to present an original study on how the organisational forms of food chains may lead to generate "lock-in" mechanisms, preventing stakeholders of the upstream of the chain to adopt of a new crop in their system. Mobilising key concepts from transition theories and from neo-institutional economics, we analysed several agrofood chains organisational forms, highlighting the fact that French production system is "locked" around production paradigms that affect the ability of producers to diversify their rotations. The discussion deals with the relevancy of enhancing several coordination mechanisms (contracts, production specifications, labels) to provide remunerated outcomes for diversified crops, in order to, in fine, unlock the system.

The cost-effectiveness of production of leguminous plants in view of sustainable development

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The aim of the article is to present suggested cost accounting concerning the biological advantages of cultivation of leguminous plants for subsequent plants. Contemporary agriculture pursues maximisation of harvest in order to generate the highest possible income, frequently forgetting about the negative consequences of this approach. Sustainable development combines actions in three areas (i.e. environment, economy and society) to reduce the negative influence on natural environment. Considering the cultivation of leguminous plants from the standard gross margin accounting, between 2006 and 2010 the production of yellow lupine and narrow-leafed lupine in Poland was not cost-effective. The loss from yellow lupine ranged from 199.46 euros in 2009 to 359.96 in 2006. The main causes were: low and unstable yield, absence of market and in consequence, a low price (or even no price). In cost accounting when we take into consideration the biological advantages resulting from the lower content of nitrogen in the cultivation of subsequent plants, the cost-effectiveness of the cultivation improves. As results from the research by Szukała (2012), in 2012 yellow lupine bound 95.9 kg N/ha from the air. Of the total amount of nitrogen bound in post-harvest residue about 43.4kg N/ha was left, which in the equivalent of 127.6 kg of ammonium nitrate. This means that the costs of fertilisation in the cultivation of subsequent plants can be reduced by 115.57 euros or the profitability of low effective lupine cultivation can be improved.

The origin of the market of leguminous plants in Poland in terms of restitution of their production and widespread use for feeds

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The superior goal of the economic policy of every country is to guarantee vegetable protein security. In history in Poland the goal was achieved by using domestic sources of protein based on leguminous plants. However, the development of global trade caused a breakdown in the domestic production of this raw material. In 1989 the cultivated area was 309 thousand ha, but in 2002 it was only 19 thousand ha. The protein from domestic leguminous plants in feeds for poultry and pigs began to be replaced with imported soya protein, which was easily available and competitive in price and quality. The process led to the situation where more than 80% of the domestic demand for vegetable protein is covered with imported soya protein. In consequence, there is dependence on foreign sources of protein and uncertainty in domestic protein security. Thus, the problem of restitution of the production and market of domestic leguminous plants has become topical. It is a known fact that an increasing demand for domestic leguminous plants on the current global and very competitive market will require development of a legume-based protein product, which will be capable of quality and price competition with the omnipresent soya protein. As a result of the research, two possible procedures aimed at increasing the demand for domestic leguminous plants were developed. The scientists developed two models for increasing the demand on the Polish market.

Selection of elite molecular markers and their role in cultivar identification in subterranean clover (*Trifolium subterraneum* L.)

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Subterranean clover (*Trifolium subterraneum* L.) is reviving as the elite pasture legume in southern Australia for breeders, since its first molecular maps were published last year. Australian Trifolium Genetic Resource Centre (ATCRG) based at the Department of Agriculture and Food Western Australia (DAFWA), holds an estimated 8,000 accessions of *T. subterraneum*. This is the largest collection followed by a collection of 2,000 accessions in Spain. A core collection was developed using molecular and non-molecular data, downsizing the whole collection to about 1% of the original size. Molecular markers were then selected based on their level of diversity among the accessions of subterranean clover for screening the current cultivars of the species. A subset of elite markers showed considerable reliability in cultivar discrimination even amongst the cultivars with the most similarly looking seeds. These results are indicating the potential use of these markers for seed quality/purity and cultivar identification for seed companies before distribution to farmers. It may be concluded that a set of carefully selected and elite molecular markers will enable the seed industry to guarantee its subterranean clover seed quality.

Drought tolerance of *Vicia* sp. at germination stage

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Drought is considered the most important abiotic stress which limits growth and productivity of plants and degrades agricultural soils. Therefore, testing drought tolerance of different species, particularly in the early stages of development, is of great importance. The aim of the experiment was to examine the tolerance of vetch to water stress during the germination stage. Three species of vetch, *Vicia sativa* L., *Vicia villosa* Roth and *Vicia pannonica* Crantz, produced at the Institute of Field and Vegetable Crops in Novi Sad, were tested. The substrates for germination were moistened with the PEG solution of 0, 0.3, 0.6 and 0.9 MPa concentrations. As indicators of drought tolerance, germination and growth parameters (shoot and root length, fresh and dry weight of seedlings) were determined. With an increasing concentration of PEG, all the tested parameters were reduced, but the results showed the interaction between the species and drought level. Although *V. pannonica* had the lowest percentage of germination in the control (74%) compared to *V. sativa* (90%) and *V. villosa* (93%), within these species a minimum germination rate decreased with increasing levels of water stress was observed. Similar dependences have been obtained for the other tested parameters. The existence of significant differences in germination and seedling growth, in drought conditions simulated in the laboratory, suggest that these indicators can be used as criterion for ranking the species or cultivars most tolerant of drought stress at the germination and seedling stages.

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Preliminary validation of soybean germination test

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On the basis of experience from practice, it is observed certain level of disagreement between results of laboratory germination test (ISTA) and filed emergency of soybean. This research should evaluate and validate power of prediction of standard laboratory test of soybean seed germination. Experiment included three soybean varieties from three seed lots. Sample from each lot were sieve fractionated and obtained five sub-samples of different seed size. From each subsample, laboratory germination were determinates, and all subsamples were planted in the field for field emergency evaluation. It was observed influence of thousand seed weight (TSW) on results of laboratory germination test, where the highest germination was observed for TSW between 165 and 172g. This relation was not confirmed in field experiment. In order to sample the predictive power lab test, considered the absolute difference between the lab germination and field emergence (delta). All sub-samples has higher field emergency than predicted by laboratory germination. Although there are varietal specifics, it is observed that the lowest value of delta was for seeds that has TSW greater than 145g. This finding indicates that laboratory germination test has lower power of prediction in small seed samples. Also, higher delta values were observed when laboratory test show lower values. Standard laboratory test has lesser power of prediction in seed samples with lower seed vigor. These results indicate that is necessary to evaluate and validate laboratory test under different field conditions. This is important from practical aspect of soybean seed production, especially when soybean seed is produced under unfavorable condition.

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Assessing the potential of forage pea (*Pisum sativum*) cultivars for seed production

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Producing the pea (*Pisum sativum* L.) seeds with high quality and able to meet diverse market criteria is not an easy task. Like many other annual legumes such as vetches (*Vicia* spp.), pea is rather susceptible to lodging. This is extremely specific for its forage agronomic type, where abundant aboveground biomass and high forage yield means poor lodging tolerance and large seed loses. The aim of our research was a comparative analysis of ten forage pea cultivars dominant on the Serbian market, targeting seed yield, number and length of internodes, plant height, first pod height and number of branches. A small-plot trial was carried out at the Experimental Field of the Institute of Field and Vegetable Crops at Rimski Šančevi in 2011 and 2012, including the cultivars with normal and afila leaf types, with white and purple flowers and with determinate and indeterminate stem growth. The seed yield in the cultivars with normal leaf type and white flowers (5012 kg ha^{-1} and 5182 kg ha^{-1}) was significantly higher in comparison to the cultivars with indeterminate stem growth and purple flowers and insignificantly higher than in the cultivars with white flowers and afila leaf type. The average number of branches (1.3 plant^{-1}) was significantly greater in the cultivars with indeterminate stem growth and purple flowers as compared to the cultivars with determinate stem growth and white flowers. The average number of internodes, length of internodes and first pod length were highly greater in the cultivars with indeterminate stem growth (24.1 plant^{-1}, 6.6 cm and 101.6 cm). Overall, the number of internodes was 19.0 plant^{-1}, the length of internodes was 4.6 cm and the first pod height was 53.0 cm. The cultivars with determinate stem growth and white flowers proved to be the most suitable for seed production, with the leaf type insignificant.

Acknowledgements
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The seed value and vigour of narrow lupine depending on some agricultural factors

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Seed is one of the key factors in crop production. The quality of the propagation material sown is determined mainly by genetic factors, but agronomic and environmental factors also have great importance. Growing plants under different production systems may cause variation in the quality of seed harvested. Laboratory tests carried out at the Department of Agronomy, University of Life Sciences in Poznań in 2008-2010, on the seed derived from field experiments executed in the Agricultural Experimental Station Winna Góra belonging to the Institute of Plant Protection - National Research Institute. The aim of this study was to assess the value of sowing and the vigor of narrow lupine depending on the production system, sowing rate and variety. Evaluated parameters were: germination energy, germination capacity and vigor using: test seedling growth, seedling growth rate test, test Hiltner, test a comprehensive stress. Length of roots, number of roots, the weight of grain and seedlings were also determined. It was found that the conventional system significantly modified the sowing value of narrow lupine. Seed of conventional crops was characterized by higher parameters of energy capacity, germination capacity and vigor expressed in seedling growth test, Hiltner test, test comprehensive stress, vigor index. The density of sowing of narrow lupine without of germination of capacity did not influence the quality of sowing. Among the compared varieties, higher seed value and vigor were observed in Boruta variety.

The world's first commercial legume hybrid reaches farmers

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Hybrid technology has been exploited for large yield gains in many crops. The food legumes, however, could not be benefitted from this breakthrough. Recently, ICRISAT scientists have achieved a major breakthrough by developing the world's first commercial hybrid in pigeonpea (*Cajanus cajan* L.), a food legume of tropics and sub-tropics. The hybrid technology has provided an opportunity for breaking the decades-old yield plateau in pigeonpea. ICPH 2671 is the world's first pigeonpea hybrid, based on cytoplasmic nuclear male sterility system, was released by a Government agency in India in 2010. During 2009 and 2010, ICPH 2671 was evaluated in 2013 on-farm trials in five provinces and on average, this hybrid produced 40.6% more yield over the best variety. This hybrid is highly resistant to diseases such as fusarium wilt and sterility mosaic virus. ICPH 2671 performs superbly under both pure and intercropping systems in different environments. During the last few years, large-scale hybrid seed production technology has also been perfected. The cross-pollination of female parent is facilitated by a number of flying insects and hybrid yield of between 800 - 1000 kg/ha can easily be harvested. Due to high demand of hybrid seed and ease in its large-scale production, this hybrid technology has been adopted by a number of seed producers. Plans have been made by various Government and other agencies to promote hybrid cultivation on a large scale. We believe that the large-scale adoption of the hybrid will break the decades-old yield barrier and benefit millions of farmers.

Overcoming the problem of variety contamination in pigeonpea through genetic means

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Pigeonpea [*Cajanus cajan* (L.) Millsp.] is an important pulse crop of India, grown mainly as rainfed crop on about 4.2 m ha . Despite the largest producer (3.5 million tons) of pigeonpea India needs to import around 5 lakh tons of pigeonpea annually. It has been observed that the productivity of high-yielding disease resistant varieties generally decline over a period of time due to their genetic contamination. Unlike other pulses, the variety deterioration in pigeonpea is very fast (2-4 years) due to its peculiar floral morphology that permits 20-50 % natural out-crossing. Since most of the pigeonpea varieties are of medium to long (6-9 months) duration, the variety re-constitution is not a practical option. Hence, for quality seed production we need to depend on expensive selfing or use of isolation. Also, in India, > 75% farmers use their own seed and it is always open to contamination. To overcome this decade- long problem, a floral mutant in pigeonpea was developed at ICRISAT controlled by a single recessive gene from an inter-specific cross (*Cajanus cajan x C. lineatus*) which restrict the natural out-crossing to 1-2%. Keeping in view of the issue of genetic purity in pigeonpea, an effort has been made to incorporate selfing trait in the existing high yielding cultivars and new breeding materials. Developing farmer preferred pigeonpea varieties with this selfing trait would reduce the cost of seed maintenance and also stabilize their productivity by minimizing the hardship and yield losses associated with genetic contamination.

Uncertainty analysis of trypsin inhibitor activity determination using microtiter plate method

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Measurement of proteinase inhibitor activity of legume seeds is widely used since some of these exert toxic and antinutritional effects. Standard method assay for trypsin inhibitor activity (TIA) determination is based on the hydrolysis of benzoyl-DL-arginine-p-nitroanilide (BAPA) by trypsin and includes spectrophotometric measurement of the reaction products. Assaying of trypsin inhibitor activity is usualy performed using reaction mixture volumes of 4-10 ml, however, it was shown that microtitration method can also be used for TIA determination. Although the results obtained by microtitration method were consistent with those obtained by French Association for Normalization reference method (AFNOR), it was noted that any experimental error in absorbance values was increasingly magnified as the sample size was diminshed. The aim of this study was to analyse measurement uncertanty of TIA determination using microtiter plate method. Assaying procedure was conducted at 37 °C on sample (soybean variety Vojvođanka), positive and negative control, which were set in four replicats in each row of the microtiter plate, with preincubation time of 10 minutes and incubation time of 30 minutes. The trypsin inhibitor activity was expresed in number of trypsin units inhibited (TUI) per miligram of sample, taking into account the fact that one trypsin unit is defined as an increase of 0.01 absorbance units at 405 nm. Large number of experiments was conducted and uncertainty analysis was obtained using uncertainty budget. Values of calculated trypsin inhibitor activity were approximately 70 TUI/mg and estimated expanded measurement uncertainty (with coverage factor *k*=2) was aproxitemately 15%.

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Visualize to explore: Towards a different model of scientific information retrieval with emphasis on legume research

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In the era of ubiquitous computing and academic hyperproduction, researchers are often encountered with the problem of information overload. Scientific information retrieval systems such as Google Scholar or commercial bibliographic databases, usually display search results as textual lists. This form of representation is suitable for typical browsing tasks, but not for thorough search and exploration. Textual search interfaces usually do not provide users with the appropriate feedback on some relevant aspects of scientific communication, such as the relevance of used terminology, authors' field of expertise, or patterns of collaboration among institutions. This paper presents the basic features of the *SCIndeks Visual Search*, an alternative information retrieval system which implements different visualization techniques for presenting search results, with a specific emphasis upon publications, scientists and institutions related to legume research. Multidimensional scaling and conceptual maps model were used to visualize the relationships among descriptors and authors of scientific papers dealing with legumes referred in the *Serbian Citation Index*. The system of visual search was not designed to replace the existing information retrieval model, but rather to enrich the users' information retrieval experience, especially in the case of specific exploration tasks, such as resolving typical vocabulary problems (e.g. polysemy), or analyzing collaboration networks among researchers. Several examples are given to illustrate the advantages of visual search model in the field of legume research and agricultural sciences in general. The authors point out possible benefits of the information visualization as a mediator between the user's query and search results, i.e. between user's information need and information space of the document corpus, anticipating a significant enhancement of mutual awareness of each other's achievements in various topics of legume research.

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A new journal on the legume research horizon - *Legume Perspectives*

Legume Perspectives Editorial Board

Mike Ambrose, Paolo Annicchiarico, Birte Boelt, Beat Boller, Ousmane Boukar, Judith Burstin, Marina Carbonaro, Branko Ćupina, Vuk Đorđević, Gérard Duc, Noel Ellis, Sara Fondevilla, Bernadette Julier, Branislav Kovačević, Kevin McPhee, Aleksandar Medović, Aleksandar Mikić, Teresa Millan, Fred Muehlbauer, Ramakrishnan Nair, Pádraig O'Kiely, Diego Rubiales (Editor-in-Chief), Christophe Salon, Marta Santalla, Petr Smýkal, Frederick L. Stoddard, Wojciech Święcicki, Richard Thompson, Rajeev Varshney, Carlota Vaz Patto, Tom Warkentin, Christine Watson, Ping Wan, Daniel Wipf

Through the decades of research on various legume species and crops worldwide, its results have been published in an endless number of national and international journals and magazines dealing with various topics. It is certain that the articles on genetics, propelled by Mendel's pioneering work, are among the most numerous, but it is also true that those on agronomy, agro-ecology, or stress tolerance were produced rather abundantly. So far, there has not been a journal devoted specifically to legume science, except *Legume Research* published by the Agricultural Research Communication Centre, India. We have published our articles in several crop-specific journals, such as *Bean Improvement Cooperative Annual Report*, *Journal of Lentil Research*, *Lathyrus Lathyrism Newsletter*, *Pisum Genetics* or *Soybean Genetics Newsletter*: however, some of these no longer exist. A unique publication in the world of legume science, research and promotion was *Grain Legumes*, published by the European Association for Grain Legume Research (AEP), with 57 issues published from 1992 to 2011. With the official registration of a novel International Legume Society (LS) on 1st April 2013, an initiative was born among the researchers on all legume crop groups, including grain legumes, forage legumes, soybean, tree legumes, ornamental legumes or pharmaceutical legumes, and all legume research topics, ranging from genomics, genetic resources and breeding to agronomy, agro-ecology and stress tolerance, to establish a magazine-like journal, named *Legume Perspectives*. Following the tradition of *Grain Legumes*, each of its four issues per year will be devoted to a specific legume crop or research topic, with several brief but rather informative and updated reviews supplemented by a few research articles, and important announcements for the global legume community. We are deeply convinced that *Legume Perspectives* will serve well the members of the International Legume Society and all interested in legumes in general, and will successfully meet the criteria for an innovative, serious, useful, interesting and regularly issued publication.

Celebrating the golden jubilee of *Ratarstvo i povrtarstvo* and the diamond jubilee of the Institute of Field and Vegetable Crops

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The newly founded International Legume Society early this year is making its first steps towards the first Legume Society Conference, an event which aim is to connect legume researchers worldwide and establish well-linked legume community. Its host this year, the Institute of Field and Vegetable Crops (IFVCNS) is on its well-trodden path and celebrating its diamond jubilee, 75th years since its foundation in 1938. Another less known, but worth remembering anniversary is the golden jubilee of a journal *Ratarstvo i povrtarstvo* (Field and Vegetable Crops) published by the IFVCNS. Starting as *Zbornik radova* (Review of Research Work) in 1963, in the course of 50 years, the journal published 1651 papers in total, 402 of which are devoted to legume crops. The frequency of the research papers on legumes has steadily increased from 11.7% in the first decade, up to 27.3% till today. The most numerous papers were devoted to lucerne (10.4%), soybean (9%), vetches (7.5%), clovers (6.1%) and pea (5.8%). In total 144 legume species have been investigated in the journal. Out of 55 papers with at least one author out of Serbia and other ex-Yugoslavian countries, 45 papers were published in the last decade. The editorial board comprises 56 editors, a half of them are from Bulgaria, Canada, China, Czech Republic, Finland, France, Germany, Hungary, India, Iraq, Ireland, Romania, Russia, Spain, Turkey, UK and USA. The electronic issues of the journal are available at:
http://nsseme.com/en/about/?opt=casopisi/ratpov&cat=about.

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A treasury of the legume lore in the libraries of Serbia

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The largest number of books in Serbia is deposited at the National Library of Serbia and Matica srpska in Novi Sad, with an approximate total number of more than 6.4 million copies. This survey was aimed at assessing the number of the general publications on legumes in the libraries of Serbia, such as books, monographs and handbooks and excluding journal and conference articles and other simpler categories, by an online search of the COBBIS regional network. A vast majority of the publications specialised in grain legumes is devoted to soybean (*Glycine max* (L.) Merr.) with 71 titles. The oldest one is a book in Serbian by Nikola Angelov *Soja: kineski pasulj: budućа hrana čovečanstva* [*Soybean: Chinese Bean: The Future Food of the Mankind*] from 1929. The next one is common bean (*Phaseolus vulgaris* L.), with 41 titles. The oldest one is a book in Serbian by Miloš N. Lukićević called *Pasulj (grah), sočivo i grašak: naša najglavnija variva* [*Common bean, lentil and pea: Our major pulses*], dating from 1902. There follow pea (*Pisum sativum* L.) with 25 titles, faba bean (*Vicia faba* L.) with 10 titles, vetchlings (*Lathyrus* spp.), vetches (*Vicia* spp.) and cowpea (*Vigna unguiculata* (L.) Walp.) with 2 titles each and black gram (*Vigna mungo* (L.) Hepper) with 1 title. Among the perennial forage legumes, lucerne (*Medicago sativa* L.) is the most represented one, with 40 titles and the oldest one, a book in Serbian by D.Đ. Ćatić *Podizanje lucerišta* [*Establishing a lucerne stand*], published in 1925. The rest of the general publications on perennial forage legumes comprises 30 titles on red and other clovers (*Trifolium* spp.) and 1 on birdsfoot trefoil (*Lotus corniculatus* L.) and sainfoin (*Onobrychis viciifolia* Scop.) each. One of the most important pharmaceutical legumes, melilot (*Melilotus* spp.), is present in 7 titles, while there is 4 books on black locust (*Robinia pseudoacacia* L.), as one of the most significant tree legume species. Although not all of these 237 general publications on legumes are in Serbian language or written by Serbian authors, there is a strong, and perhaps expected, correlation between the number of the titles on individual legume species and their cultivation area and therefore economic importance in Serbia.

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And now for something completely pea (*Pisum sativum* L.) fferent: It’s... Legumes in “Monty Python’s Flying Circus”!

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Legumes have played a significant part in human diets from the very dawn of mankind and it is no wonder they entered culture in various ways. In the most recent times, legumes have become present in comedy television programmes, such as “Mr. Bean” by Rowan Atkinson. “Monty Python’s Flying Circus” is a widely known British comedy programme, where legumes appeared in numerous sketches. Common bean (*Phaseolus vulgaris* L.) was mentioned in the famous sketch “Spam” by a waitress, portrayed by Terry Jones, as an ingredient of a meal called “Spam, Spam, Spam, Spam, Spam, Spam, Spam, Baked Beans, Spam, Spam, Spam and Spam”. They were also immortalised by Terry Gilliam, who was devouring them laying on a sofa and constantly asking for more in the sketch “Most Awful Family in Britain”, with notorious and almost unintelligible screams “I want more beans!”. However, the most surrealistic appearance of legumes in “Monty Python’s Flying Circus” surely was in the episode “Dennis Moore” in which this highwayman, portrayed by John Cleese, robbed the rich, while driving in a coach called ‘Lupin Express’, took all the plants of garden lupin (*Lupinus pollyphylus* Lindl.) they had hidden, brought them to a family of the poor, being convinced lupins were what they essentially needed to survive, eventually buried the whole interior of their house with this beautiful, multicoloured ornamental species and finally realized he should have provided them with money or food. There is a possibility that the appearance of garden lupin in this sketch is historically inaccurate, since it almost certain took place in late 18th century, while garden lupin arrived in England from North America in the 1820s; but, does reality matters at all in something like *Monty Python’s Flying Circus*?

P.S. Eric the Half-a-Bee (the original Monty Python’s version, followed by a leguminous rearrangement)

A-one, two, a-one two three four. / Half a bee, philosophically, must *ipso facto* half not be. But half a bee has got to be *vis-à-vis* its entity - d'you see? But can a bee be said to be or not to be an entire bee, when half the bee is not a bee, due to some ancient injury? Singing! / La di di, one two three, Eric the Half-a-Bee. A, B, C, D, E, F, G, Eric the Half-a-Bee. Is this wretched demi-bee, half asleep upon my knee, some freak from a menagerie? No! It's Eric the Half-a-Bee. / Fiddle di dum, fiddle di dee, Eric the Half-a-Bee. Ho ho ho, tee hee hee, Eric the Half-a-Bee. / I love this hive employ-ee-ee, bisected accidentally, one summer's afternoon by me, I love him carnally. / He loves him carnally... / Semi-carnally. / The End. / Cyril Connolly? / No, semi-carnally. / Oh. / Cyril Connolly...

A-one, two, a-one, two, three, four. / Half-a-pea, morphologically, must *ipso facto* half *not* be. But half the pea has got to be, *vis-à-vis* its entity - d'you see? But can a pea be said to be or not to be an entire pea when half the pea is not a pea, due to some processing injury? / Sowing! / La di di, one two three, Eric the Half-a-Pea. A, B, C, D, E, F, G, Eric the Half-a-Pea. / Is this wrinkled demi-pea, half dormant upon my field, some freak from a bank of genes? No! It's Eric the Half-a-Pea. / Fiddle di dum, fiddle di dee, Eric the Half-a-Pea. Ho ho ho, tee hee hee, Eric the Half-a-Pea. / I love this trial employ-ee-ee, intercropped intentionally, one fine early spring's noon by me, I love him omically./ He loves him omically... / Semi-omically. / The End. / Cyril Connolly? / No, semi-omically. / Oh. / Cyril Connolly...

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In memory of Aleksandar B. Popović (1973-1999)

Session 6

Translational omics for legume improvement

KEYNOTE LECTURE

Use of translational genomics to identify genes important for legume seed filling

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Translational genomics, i.e., the transfer of genetic information from model species to cultivated crops, is on the brink of revolutionizing plant breeding. The recent publication of genomic sequences for several cultivated legumes is also accelerating this process. For pea, recent high-throughput RNA sequencing, and the prospect of a genome sequencing project, will further accelerate the transfer of information from the *Medicago truncatula* model to the cultivated crop. We have been using genomics approaches with Medicago as a tool to identify key genes determining seed yield and composition in closely related legumes. Analyses of the proteome and transcriptome of the component tissues of the developing seed revealed extensive compartmentalization of gene expression and metabolic activities. Using a TF (Transcription Factor) qRT-PCR platform and the Affymetrix Gene Chip, TFs specific for each seed tissue were identified, along with putative target genes. These TFs have been located on the *M. truncatula* genetic map and correlations between map positions of TF loci and QTLs for protein quantities and other seed phenotypes were detected. These correlations can be recently confirmed in numerous cases by the existence of similar QTLs at syntenic positions in pea. Two genes, both specifically expressed in the developing endosperm, have received particular attention. One of the genes encodes a DOF class transcription factor, whose mutant phenotype severely affects endosperm development. The second gene encodes an endosperm-specific subtilase (*SBT1.1*), which affects final seed weight. MiRNAs constitute another level of gene regulation whose importance in the developing seed is beginning to become apparent. We have recently started to compare the sRNA profiles of developing M. truncatula and pea seeds and preliminary results will be presented.

Transcriptional profiling of grass pea genes differentially regulated in response to infection with *Ascochyta pisi*

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Grass pea (*Lathyrus sativus* L.) is a robust crop, mainly due to its tolerance to drought and resistance to several diseases, being a good candidate for production in marginal areas. In order to increase the available genomic resources and knowledge on grass pea disease resistance, we studied the genetic alteration occurring, at the transcript level, when this grain legume is infected with *Ascochyta pisi.* This pathogen causes ascochyta blight, one of the most devastating legume diseases worldwide. For that, we have analyzed the leaf transcriptome profiles, generated by SuperSAGE, of a resistant grass pea accession during the first 24h after inoculation with *Ascochyta* sp. Transcript tags were annotated using cDNA libraries of *L. sativus* and *L. cicera* generated by RNA-seq. 29.468 sequence tags of 26bp were generated, where 82% were present in the *Lathyrus* cDNA libraries, 2% found only in the TIGR database, 1% only in the NCBI database and 15% tags with no hits. This study of the transcription profiles by SuperSAGE in response to infection has as goal the identification of candidates genes involved in the control of resistance to ascochyta blight in grass pea. In the future the genomic resources here develop will be use to map economically important resistant traits and deliver markers for breeding. Our future work will focus on the transcription profiling of candidate genes identified via SuperSAGE in larger sets of resistant and susceptible germplasm to deepen our knowledge on those genes conferring resistance to ascochyta blight.

Identification and characterization of cDNA genes *FLK* and *FVE* of the autonomous pathway of flowering *Lupinus luteus*

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The flowering time is one of the factors providing reproductive success. Four genetically defined pathways have been identified that control flowering: the vernalization pathway, the photoperiod pathway, the gibberellin or hormonal pathway and the autonomous pathway. All pathways control of flowering cooperate regulation of key flowering genes which name 'integrator genes' or 'central floral pathway integrators'. The autonomous pathway of flowering induction genes includes 7 genes. All these genes are negative regulators of *FLOWERING LOCUS C (FLC)* - main inhibitor of generative development. In this study, cDNA fragments *FLOWERING LOCUS KH DOMAIN (FLK)* and *HISTONE-BINDING PROTEIN FVE (FVE)* homologous genes were identified in *Lupinus luteus* genome. The sequences of these genes from other legumes species were searched in NCBI's database. DNA fragments of *Lupinus luteus FLK (LlFLK)* and *FVE (LlFVE)* genes were amplified in PCR on the cDNA template used degenerate primers. The predicted amino acid sequences were used to search conserved domain with BlastP program. One conserved RNA-binding domain – KH motif was found in LlFLK which confirmed it is the homolog for FLK in another plants. One conserved domain WD40 was found in LlFVE protein which confirmed it is the homolog for FVE in another plants. Identified cDNA fragments from *L. luteus* were the homolog for *FLK* and *FVE* in another plants as also confirmed by sequenced nucleotide and predicted amino acid sequences of these genes show high homology to known plants sequences of genes and proteins.

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Embryogenic callusogenesis in anther culture of pea

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Pea (*Pisum sativum* L.) is a recalcitrant species for haploid production. But in certain conditions pea microspores can be reprogrammed towards embryogenic development. Initiation of morphogenic calli in pea anther culture leads to regeneration of plants derived from somatic cells. Stress treatment of pea microspores and stimulation of their embryogenic development could help to produce haploid plants. In our researches cold (+4^{0}C) and heat (+35^{0}C) stress treatments were applied to buds and *in vitro* culture of isolated anthers, respectively. Anther culture of pea variety *Stabiltranslucent calli* were initiated in the presence of 2.4-D with use of cold stress treatment. Green globular embryos (2.5-3 mm) were formed on their surfaces after long (until 3 months) cultivation. In anther culture of cultivar *Visir green nodular calli* were initiated in the presence of 2.4-D with use of heat stress. Several successive transfers of these calli onto fresh medium were conducted. Then green nodular calli were transferred onto medium MSB supplemented with 4 mg/l BA and 1 mg/l NAA. In these conditions embryogenic tissues were converted onto morphogenic pathway of development. Proliferation of tissues was accompanied with appearance of hypertrophic embryos on different stages of development. Arrest of successive embryo development was observed on globular, heart and torpedo stages. There was no germination of these embryos. Shoot regeneration took place on tissue clusters with buds.

The genetic mechanism that controls the angle of lateral shoots: A new leguminous model from *Arachis hypogaea*

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The growth angle of lateral shoots, or branching habit, is an important aspect of plant architecture.While several studies of the growth angle of lateral shoots have been carried out in monocotyledons (e.g., tiller angle in rice), little is known about its molecular/genetic mechanism in dicotyledonous plants. We are studying this important aspect of plant development by using the well-defined leguminous model system of *Arachis hypogaea* (peanut). We chose peanut since it has four easily distinguishable branching habits (Prostrate, Spreading, Bunch and Erect) and because growth habit is an important agronomic trait of this legume crop. Our genetic infrastructure is comprised of several populations that segregate for the different branching habits. An initial examination of some of these populations revealed that the Spreading/Bunch trait is controlled by a single Mendelian gene. Subsequently, bulked segregant analysis was performed on completely Bunch and Spreading F_3 families. In order to facilitate the SNP detection process and candidate-gene analysis the transcriptome was used instead of genomic DNA. Young leaves and lateral shoot bases were sampled and bulked. RNAs were converted into libraries suitable for Illumina sequencing and reads were aligned against peanut reference transcriptome. Inter-varietal SNPs were detected, scored and quality filtered according to very strict criteria. Around 20 candidate SNPs were retrieved and further analyzed in segregating populations. One candidate gene, hypothetical membrane steroid-binding protein, that previously was shown to be related to branching habit in monocots, is currently being studied as the leading candidate gene for Spreading/Bunch trait in peanut.

Functional exploration of salt responsive *Phaseolus vulgaris* (common bean) genes using model organism *Arabidopsis thaliana*

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Phaseolus vulgaris is a widely cultivated leguminous crop that is highly susceptible to soil salinity. Pre-requisite for the development of salt-tolerant crops is the identification of key genetic determinants between tolerant and susceptible germplasm in response to salt stress. To this end a subtraction suppressive hybridization (SSH) cDNA library was constructed using mRNA from susceptible TR43477 and tolerant ISPIR genotype. The construction of the root library yielded around 30000 colonies and leaf library around 800 colonies including transcripts such as aquaporins, bZIP transcription factors, vacuolar ATPases. 7 genes from SSH library and 9 extra genes which are not from the SSH library but are known to be involved in salinity response were selected as candidate genes for functional study. For qPCR analysis GAPDH and Cyclophilin were validated as stably expressed reference genes for salt treatment experiments. qPCR analysis showed 10 out of 16 genes were significantly upregulated. ($p<0.05$). 5 genes including a vacuolar ATPase, Superoxide dismutase, proline synthetase, protoplastic integral protein and NHX Na-H antiporter were cloned into Gateway expression vectors. *Arabidopsis* orthologues for these genes were determined and T-DNA insertional mutant *Arabidopsis thaliana* lines for these genes were obtained from ABRC and NASC and verified for homozygosity. Overexpression of bean genes will be achieved in *Arabidopsis* lines using *Agrobacterium tumefaciens* based transformation. Phenotype alterations and recovery will be screened in vitro salt treatments after transformation of wild type and knock out lines are performed.

Induced mutations in the *TI1* gene encoding a major double-headed protease inhibitor in *Pisum sativum* L. can reduce significantly the inhibition of target enzymes

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Trypsin / chymotrypsin inhibitors in the seeds of many legume crop species are regarded as anti-nutritional proteins often leading to a requirement for heat-treatment of seed products prior to their use in feed. A TILLING resource developed in *Pisum sativum* L. (pea) was exploited to identify mutants in the major seed-expressed trypsin / chymotrypsin inhibitor gene, *TI1*, where the inhibition of either or both of the target enzymes may be reduced. Three lines with missense mutations in *TI1*, predicted to affect activity through alteration of (a) a conserved cysteine residue, (b) the P1' serine within the active site of the chymotrypsin inhibitory domain or (c) overall charge of the carboxy-terminal domain involved in protein dimerization, were identified. The mutants were back-crossed to the parent cultivar, Cameor, to generate mutant and wild type segregant lines, where the effects of the mutations on overall activity, isoform charge and the association properties of the encoded proteins could be examined and compared. The data show that one mutation (C77Y) leads to a significant reduction in both trypsin and chymotrypsin inhibitory activity, associated with the loss of activity of two TI1 isoforms. The second and third classes of TI1 mutations showed lesser effects on overall inhibitory activity, despite their likely impact on protein structure. The resource provides potential for providing novel germplasm with improved properties for feed and food uses.

Genetic Analysis of Leaf Development in Pea

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The Pea (*Pisum sativum*) leaf is characterized by a compound architecture. A mature leaf comprises a basal pair of laminae called stipules, one or more pairs of leaflets, and terminal structures called tendrils. Further understanding of leaf development could be provided by the molecular characterization of leaf mutants. Several leaf morphological mutants are studied in the laboratory, including *crispoid* (*crd*) mutants. These are characterized by abnormal stipules, a reduced number of leaflets and weak development of veins, suggesting that the *Crispoid* gene is involved in the formation of leaves. The gene *PsKn2*, presumed orthologous of *BP* in *Arabidopsis thaliana*, encoding a transcription factor of the Knotted-like HomeoboxI (*KNOX*I) family, and maps close to the *Crispoid* locus. Moreover, preliminary transcriptional data suggest that *PsKn2* expression is down regulated in *crd* mutants. *KNOX* genes have been shown to play an important role in the maintenance of the shoot apical meristem (SAM), and in the formation of aerial organs. *PsKn2* has been shown to be expressed at the peripheral zone of the SAM and at the base of leaf primordia, but not in the mature leaf. A mutation in *PsKn2* would thus be expected to have some of the features of *crispoid* mutants, so *PsKn2* is a candidate gene for *Crispoid.* Using genetic and molecular biology tools, we are aiming to determine whether *Crispoid* and *PsKn2* are the same gene. Understanding the role of the *Crispoid* gene and its interactions with other genes will provide a better insight into the genetics of leaf development.

Comparative cytogenetic study on two geocarpic species, *Trifolium subterraneum* (2n=16) and *T. israeliticum* (2n=12)

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Changes in chromosome number have played an important role in the evolution of the genus *Trifolium*. Along with a few species of polyploid origin there are several cases of dysploidy as evidenced by the presence of four basic chromosome numbers (x=8, 7, 6, 5). *Trifolium subterraneum* and *Trifolium israeliticum* are related species with chromosome complements 2n=16 and 2n=12, respectively. They represent an interesting case of speciation based on chromosome number reduction, however up to date attempts to demonstrate their cytogenetic affinity have never been carried out. With this study we performed a comparative cytogenetic study with the purpose of clarifying the evolutionary relationship between these species and to verify whether genomic rearrangements, other than modification of the chromosome number, are associated with the speciation process. Although karyomorphological analysis supports the hypothesis that chromosome rearrangements had a role in the reduction of the chromosome number, the physical mapping of the rDNA sequences revealed a significant remodelling of the 45S and 5S rDNA sites which greatly contributed to the differentiation of the 2n=16 and 2n=12 karyotypes. The nucleotide analysis of 5S rDNA repeats confirmed that the two species are related but constitute distinct entities. The observed genomic changes lead to the hypothesis that the 2n=12 species is the result of an evolutionary pathway which passed through intermediate forms. It cannot be excluded that the most direct ancestor of *T. israeliticum* is a species with 2n=14.

Identification of genes involved in resistance to *Didymella pinodes* in pea by deepSuperSAGE genome-wide transcriptome profiling

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Didymella pinodes, causing ascochyta blight, is one of the most important pea pathogens. Despite the devastating consequences of this disease, very little is known about the mechanisms of resistance in the host. We employed the open-architecture transcriptome profiling technique deepSuperSAGE, coupled with next-generation sequencing, to identify pea-specific genes involved in the resistance to this important disease in the resistant *Pisum sativum* ssp. *syriacum* accession P665.Two deepSuperSAGE libraries were constructed from leaf RNA of infected and control plants, yielding a total of 17,561 different UniTags. 70% of them could be assigned to known sequences from pea or other plants. 509 UniTags were significantly differentially expressed ($p < 0.05$; fold change ≥ 2, ≤ 2) in inoculated versus control plants. This study provides a detailed picture of all expressed genes and metabolic pathways differentially regulated during *D. pinodes-Pisum sativum* interaction and contribute to the identification of candidate resistance genes. Protease inhibitors, antifungal compounds, strengthening of host cell walls, detoxification of *D. pinodes* toxins and repair of cell walls could contribute to resistance. Ethylene, ABA and indole-3-acetic acid pathways were up-, while the GA pathway was down-regulated.

Localization of lodging and stem stiffness loci on a Wt10245 x Wt11238 pea map (*Pisum sativum* L.)

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Lodging is one of the constraints of field pea production. It produces favourable conditions for disease development, makes harvest difficult and increases harvest loss. The introduction of some pea traits has decreased the risk of lodging, e.g. plant height reduction or an increase in the pushing resistance of the lower part of the plant. A direct relationship exists between mechanical properties of the plant stem (stem rigidity and maximum bending stress) and lodging. The aim of the present study was an assessment of the mechanical properties of the plant stem in the mapping population and their analysis as a quantitative trait. The assessment of the mechanical properties of the plant stem could make lodging analysis easier and more reliable, provided the correlation between these stem mechanical property data and lodging scores is confirmed. A total of 104 RILs from the Wt10245 x Wt11238 mapping population were used in this analysis. Nine new SSR markers coming from Loridon's or Fondevilla's maps were added to the map. The QTLs were localized and characterized using the mixed-model-based composite interval mapping method [QTL – Network 2.0]. Lodging data came from one season and mechanical property data came from three seasons. Three QTLs were found for lodging resistance. Eleven QTLs were found for mechanical traits for a bottom, middle and a top of pea stem in 2004, three QTLs in 2010 and seven QTLs in 2011. Some loci of stem rigidity and maximum bending stress overlapped with each other. No QTL was common for both mechanical properties of the stem and lodging resistance. The suggestion that QTLs for mechanical traits are common with lodging loci was not confirmed.

Molecular cloning of complete cDNA and examination the expression level of homologue miR167 target gene *LlARF6* in *Lupinus luteus*

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The plant hormone auxin regulates a broad spectrum of developmental processes. Recent advances have been made in the understanding of auxin response pathways, primarily by the characterization of genes encoding Auxin Response Factors (ARF) in *Arabidopsis*. In addition, expression of some of *ARFs* is regulated by microRNAs (miRNAs). In *A. thaliana, ARF6* and *ARF8* are targeted by miR167 whereas *ARF10*, *ARF16*, *ARF17* are targeted by miR160. Nevertheless, little is known about possible interactions between miRNAs and auxin signaling pathway during plant development in another plant species. In this study, we identify and examine the expression level of homologue miR167 target gene *LlARF6* in *Lupinus luteus*. The identified cDNA contains miR167 complementary sequence and shows significant similarity to *ARF6* cDNAs of other plant species. Predicted amino acid sequence of LlARF6 includes all characteristic domains for ARF family transcription factors (B3 DNA-binding domain, AUX/IAA - CTD and a glutamine-rich region). Accumulation of *LlARF6* mRNA was examined in the vegetative and generative organs *L.luteus*. Our results may suggest potential role of *LlARF6* in regulation sach proceses as its homologues in other plant species.

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Transgenic approaches to overcome challenges caused by biotic and abiotic stresses in grain legumes

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Grain legumes are globally important crops especially for developing countries as important source of proteins and minerals. They are, however, affected by a number of biotic and abiotic stresses such as drought, salinity, insects, viruses, fungi and bacteria which can reduce grain yields dramatically up to 70%. Improving the legumes with all available techniques will serve the goal of food security. As durable resistance genes are scarce or absent in the respective gene pools, transgenic approaches appear to be necessary. Obviously, genetic engineering is one of the powerful tools to overcome the barriers as any promising gene from any source can be selected and transferred to obtain novel candidate lines to be included in breeding programs. Additionally, genetic engineering gives the option to transfer multiple genes responsible for combined stresses at once in a specific way by also controlling the respective expression levels. Examples will be presented i.e. the use of bacterial genes in pea for fungal and insect resistance or a potato gene to enhance drought tolerance in faba bean or combinations of different antifungal genes.

Characterisation of a double-flowered mutant in pea (*Pisum sativum*)

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Floral developmental genes such as *Unifoliata* and *Stamina-pistilloida* are known to have roles in flower and compound leaf development in pea and closely related legumes. This suggests that some elements of the floral development pathway have been co-opted for the extended leaf development programme of these legumes. Here we characterise a double-flowered mutant of pea that provides further support for this hypothesis. The mutant, which arose spontaneously in line JI 143, is delayed in flowering and has more complex flowers than wild type. In this respect it resembles the *agamous* (*ag*) mutant of *Arabidopsis thaliana*, which has petals in whorl 3 instead of stamens, sepals in whorl 4 instead of carpels and indeterminate floral meristems; flowers continue to form within flowers, so the pattern of organs (from outside to inside) is sepal, petal, petal, sepal, petal, petal, repeatedly. Studies of the double-flowered pea mutant revealed a deficit of reproductive organs, additional fused petals and floral indeterminacy. Populations segregating for the double-flowered mutation in pea showed that there were additional effects associated with the mutation including shorter internodes and simplified leaves. MADS box gene homologues of *Ag* have been isolated and mapped in pea. An approximate genetic map location of the double-flowered mutation and its relationship to pea MADS box genes has been determined.

Dehydration affected the expression of miR398 and miR408 in pea (*Pisum sativum* L.)

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MicroRNAs (miRNAs), recently recognized as important regulator of gene expression at posttranscriptional level, have been found to be involved in plant stress responses. The observation that some miRNAs are up- or down regulated by stress implies that that could play vital roles in plant resistance to abiotic and biotic stress. We investigated the effect of water stress treatment during 10 days on expression of conserved miRNAs-miR398a/b and miR408 in pea plants. This time frame could reflect the changes as closely as possible those during water stress cause visible effects under field condition. It was observed that dehydration strongly down regulates the expression of both miR398a/b and miR408 in pea roots and shoots. The down-regulation of miR398a/b and the up-regulation of potential target genes copper superoxide dismutase, *CSD1*, highlight the involvement of this miRNA in pea stress response. On the contrary, the mRNA level of cytochrome *c* oxidase subunit 5 (*COX5b*) was not changed in roots and shoots of water-stressed plants, compared to control-well hydrated plants. This suggests that *COX5b* is not target of miR398, or that its expression is regulated by some other mechanism. P_{1B}-*ATPase* expression increased during water deficit only in shoots of pea; in the roots there was no changes in expression. Our results might help to understand the possible role of investigated miRNAs and their contribution to pea capacity to cope with water deficit.

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Lucerne genomics, potential application in breeding

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Lucerne genomics is hampered by the autotetraploidy and heterozygozity of the genome. Nevertheless, next generation sequencing (NGS) technologies now offer the opportunity to get enough sequence data to assemble lucerne genome have haplotype information. We have used Illumina Hiseq technology to sequence paired-end and mate-pair libraries from one lucerne genotype. The sequences are assembled based on *M. truncatula* genome sequence. This reference genome sequence will be essential for the development of high-throughput markers: SNP or genotyping by sequencing. Lucerne genome sequence will be used to increase genetic progress in breeding programs. A first strategy could be to build, rapidly and at low cost, dense genetic maps for QTL identificationin multiple mapping populations. Positive alleles with strong effects could be selected in these populations in order to increase their frequencies in the next generation. A second strategy could be based on candidate genes known to be involved in specific trait variation. From the sequence of these genes in lucerne, association mapping could be performed in synthetic varietiesto identify and select positive alleles. With sufficient number of markers, a third strategy could be to carry out genomic selection, i.e. to predict plant phenotypes from their genotypes by using a calibration established on a training population. All these marker-assisted selection methods combined with phenotypic selection could significantly increase genetic progress that is relatively low in an autotetraploid species whose varieties are synthetic populations. Indeed they offer the opportunity to select four copies of a positive allele in a single individual instead of a single (dominant) allele with phenotypic selection.

Application of HRM analysis for genetic mapping of STS markers in *Lupinus angustifolius L.*

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High resolution melting analysis (HRM) is a powerful technique used for genotyping and mutation scanning. This method takes advantage of special saturation dyes properties that fluoresce only in the presence of double stranded DNA. After the PCR, in the presence of the dsDNA-binding fluorescent dye, amplicons are denaturated in high temperature and the fluorescence fades away. Fluorescence changes during the analysis can be applied for the detection of SNP polymorphism, small deletions or insertions using melting curves as, various genetic sequences melt at different temperature. The usability of the HRM assay has been confirmed for genotyping of various plant and animal species. The main goal of this study is to evaluate the effectiveness of HRM analysis for sequence tagged site (STS) markers genotyping in the narrow-leafed lupin (*Lupinus angustifolius L.).* The RIL mapping population 83A:476 x P27255 was tested for several legume anchor markers (Leg markers). HRM assays were conducted on LightCycler 480 and LightCycler 480 High Resolution Melting Master (Roche). STS markers, which produced specific, 200-1000 bp–long products were sequenced. Markers containing SNPs with product length greater than 300 bp were converted into smaller markers by designing new primers sets spanning fragment of the entire sequence with SNP. Next, markers were tested with the HRM technique in the parental lines of a mapping population. If the parental genotypes were easy to distinguish, markers were then analyzed in the remaining lines of mapping population and assigned to linkage groups of the narrow-leafed lupin.

Identification and characterization of *LlYUC* cDNA involved in auxin biosynthesis in *Lupinus luteus*

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Auxin is involved either directly or indirectly in every aspect of plant development and plays the integrative role in coordination of plant growth and development. Recently, the first complete auxin biosynthesis pathway that converts tryptophan (Trp) to IAA has been finally established. Initially, Trp is converted to indole-3-pyruvate (IPA) by TAA family of amino transferases and then to IAA by YUC. This two-step conversion is the main biosynthetic pathway of auxins in plants. In this study, *YUCCA* cDNA from *Lupinus luteus* was isolated. Coding sequence consists of 366 bp and encodes for 121 amino acids. Comparison between the deduced amino acid sequence of LlYUC and YUCCAs from other plant species indicates that the predicted sequence contains characteristic Flavin-binding monooxygenase-like domain, which is essential for its activity.

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Faba bean cultures *in vitro* - elimination of necrotic response with antioxidant treatments as a background for genetic transformation experiments

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Faba bean represents one of the *in vitro* recalcitrant legumes due to frequent deterioration of explant material and cultivated tissues as a result of the action of phenolic compounds. Ascorbic acid, citric acid, glutathione and active charcoal were used in a set of experiments with low-tanin faba bean cultivars aimed to achieve efficient regeneration protocol. The application of antioxidants led to decreased callogenesis citric acids treatments minimised necrotic response of explants. Citric acid, used together with ascorbic acid completely inhibited shoot growth in apex cultures. Glutathion evoked hyperhydricity of explants. Active charcoal induced rooting on media which are commonly used for shoot proliferation. In subsequent testing, the effects of combination with transformation co-cultivation compounds acetosyringone and L-cysteine was studied. Combination of acetosyringone with antioxidants did not negatively influenced shoot proliferation, except of variant with ascorbic acid. Our experimental findings recognized citric acid, L-cysteine and L-acetosyringone as the helpful agents. Citric acid worked as the best and universal antioxidant in faba bean *in vitro* cultures utilizable also for further genetic transformation experiments.

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Quantitative trait loci for stem parameters in the field pea (*Pisum sativum* L.)

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Lodging is one of the most limiting factors in attaining a stable and high yield of field pea. Our previous research suggests that stem parameters (stem flexural rigidity- stiffness, maximum bending stress- endurance, and diameter and stem wall thickness) are correlated with lodging susceptibility. Stem parameters, lodging susceptibility and plant height were tested in a Carneval x MP1401 RIL population. Three stem heights were analyzed: lower (2nd-4th internode), middle (9th-10th internode) and upper (below the first generative node). Lodging was estimated in 3 periods (before flowering, after flowering and before harvesting) andon a 9-point scale (1-susceptible, 9- not susceptible). Lodging was absent in the first two periods. In the third period the average lodging value in the population was 7.1. The genetic map used for QTL mapping was published by Tar'an et al. (2003) and rebuilt using JoinMap 2.0 software. It consists of 203 mainly AFLP markers and 12 newly-found markers (7 STS, 4 SSR and 1 isozymic). QTL analysis was performed with QTLNetwork 2.1 software. Data from two years were analyzed. Seven loci were found: endurance (group I), stiffness (D) and stem diameter (VIc) for the bottom of the stem, stem wall thickness (B) and stiffness (I) for the middle of the stem, stem thickness (IIIb) and endurance (III) for the top of the stem. Loci for lodging and plant height were not found, but lodging was negatively correlated with stem diameter and endurance in the middle of the stem height.

Identification and characterization of *LlACS* cDNA, the gene involved in ethylene biosynthesis in *Lupinus luteus*

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Gaseous ethylene (ET) is one of the phytohormones that play an important role during plant growth and development, e.g. fruit ripening, pathogen defenses, flower induction. ET is derived from S-adenosylmethionine (SAM) which is converted to 1-aminocyclopropane-1-carboxylic acid (ACC) by 1-aminocyclopropane-1-carboxylate synthase (ACS). Many of ACSs are tightly regulated at both transcriptional and post-translational levels, including phosphorylation and proteasome-mediated degradation. The regulation of enzymatic activity is thought to be crucial for ethylene synthesis. A small gene family of *ACSs* encoding different isoforms of the enzyme has been identified in many species, e.g. *Arabidopsis thaliana*, *Ipomoea nil*, *Glycine max*. In this study, cDNA of 1-aminocyclopropane-1-carboxylate synthase (*LlACS*) from *Lupinus luteus* was isolated. The full-length coding sequence of the gene consists of 1422 bp and encodes for 474 amino acids. Based on comparison between the deduced amino acid sequence of LlACS and ACSs sequences derived from other plant species, it was found that the LlACS sequence includes seven evolutionarily conserved regions and eleven amino acid residues characteristic for ACC synthases, which are essential for enzymatic activity. Our results suggest that the identified cDNA of *LlACS* encodes for completely functional enzyme.

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Translating *Medicago truncatula* genome sequence information for the development of PCR based markers and discovery of single nucleotide polymorphisms for genome analysis in legumes

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Compared to cereal crops, legumes are less well characterized at the genomic level and rightly referred as 'orphan crops'. Transfer of knowledge between model and crop legumes allows development of orthologous pan-taxon genomic tools to benefit research on resource poor taxa. Here, we developed 1180 Intron flanking gene specific markers by BLAST aligning common bean (*Phaseolus vulgaris* L.) ESTs with complete genome sequence of *Medicago truncatula.* Primers were designed from conserved exon borders in *Medicago* with near-perfect conservation (0-1 mismatch) to maximize (intronic) polymorphism discovery rates within a taxon. A random 384 PCR primer pairs representing loci from 8 chromosomes of *Medicago* were tested on members of legume family. The single copy amplification rates of 86.2% (*Medicago truncatula*), 69.5% (Pigeon pea), 83.6% (Cowpea), 82.0% (Horsegram), 80.9% (Chickpea), 69.3% (Urdbean), 82.7% (Common bean), 77.3% (Field bean), 46.6% (Groundnut) and 75.2% (Soybean) signifies the success of cross taxon primers and suggested their potential use in comparative legume genomics. Genetic diversity was assessed in 27 cowpea genotypes using 236 intron flanking markers which revealed 131 polymorphic markers with PIC values ranging from 0.054 to 0.59. The PCR products of different varieties of pigeon pea, cowpea and chickpea were sequenced and aligned using ClustalW2 to find putative SNPs. Potential SNPs detected were converted to size variation for genotyping in pigeon pea, cowpea and chickpea. Integration of these newly developed markers into genetic maps in resource poor legumes will not only aid in the map saturation but also in designing successful marker assisted selection programs.

QTL detection for partial resistance against *Didymella pinodes* in two connected mapping populations of the model plant *Medicago truncatula*

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Ascochyta blight incited by *Didymella pinodes* (formerly *Mycosphaerella pinodes*) is one of the most important fungal diseases of pea worldwide. In this study searched for Quantitative Trait Loci (QTL) regions associated with quantitative resistance to *D. pinodes* in the model legume *Medicago truncatula*. The analysis was carried out using two connected mapping populations at F_8 derived from two crosses (J6 x F83005.5 and J6 x DZA315.16), being J6 moderately susceptible and both F83005.5 and DZA315.16 resistant to *D. pinodes* infection. Parental lines and RILs population were macroscopically evaluated in terms of relative Disease Severity (rDS). In order to provide more effective detection and evaluation of the effects of the QTLs and their stability and fine mapping of candidate genes annotated on the *M. truncatula* sequence, a combined genetic map using both population was developed. The QTL analysis revealed a QTL in the LG2 explaining up to 13% of the total phenotypic variation for rDS to *D. pinodes*. Two SSR markers, MTE80 and mtic890 (3 cM apart) were the most significantly associated. The markers are located in BAC AC119409 and BAC AC125474, respectively. These two BACs are overlapping on *M. truncatula* chromosome 2. The integration of QTL analysis and genomics in *M. truncatula* will facilitate the identification of candidate genes related with host resistance.

The role of signal molecules in defence response of pea (*Pisum sativum* L.) to pea aphid (*Acyrthosiphon pisum* Harris) infestation

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Pea aphid (*Acyrthosiphon pisum* Harris) is a destructive pest of leguminous plants worldwide. Damages of pea seedlings leaves (*Pisum sativum* L.cv. Cysterski) by pea aphid *A. pisum* were dependent on the intensity and duration of infestation. It has been revealed that in pea seedlings leaves in responses to *A. pisum* infestation were induced accumulation of phytohormones that is salicylic acid (SA), jasmonic acid (JA), ethylene (ET), and production of reactive oxygen/nitrogen species (ROS/RNS) such as hydrogen peroxide (H_2O_2) and nitric oxide (NO). Following aphid infestation, these signal molecules were induced at different points of time. Strong generation of H_2O_2 and JA were observed in pea seedlings leaves at 24 hpi. In turn, after the induction of ET and NO at 48 hpi in aphid-infested leaves the generation of SA occurred (strongly increased from 72 hpi to 96 hpi). Then, JA and ET were again induced and reached the second maximum levels at 96 hpi. Therefore, at the point of 96 hpi there was the combined action of three phytohormones SA, JA and ET in the defense mechanism of pea leaves. In parallel, the increase in activity of enzymes phenylalanine ammonia-lyase (PAL) and benzoic acid 2-hydroxylase (BA2H) in the SA synthesis pathway, lipoxygenase (LOX) engaged with the biosynthesis of JA was similar to the enhanced alteration of these signal molecules. The antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) are important elements of the regulation of H_2O_2 metabolism in responses to oxidative stress. The increase in activity the antioxidant enzymes were probably connected with the defensive role of H_2O_2 and reduction of oxidative stress in pea leaves. Furthermore, the accumulation of superoxide anion ($O_2^{\cdot-}$) and semiquinone radicals was involved in the defense responses of pea leaves to pea aphid. In pea cells, the increase generation of $O_2^{\cdot-}$ played a defensive role to protect against aphid herbivory, whereas semiquinones may contribute to build-up of a barrier or activate other defense strategies. The level of these radicals increased progressively in response to the attack of pea aphids. These aspects contribute to the novel knowledge concerning the identification of regulatory mechanisms during the plant-aphid interactions.

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Comparative analysis of *GA2ox* genes encoding enzymes involved in gibberellin deactivation in different species of lupines

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The crucial moment to achieve high productivity of legumes is correctly running process of flower growth and development. In different species of lupines this stage is associated with undesirable occurrence, which is the falling of flowers. In *Arabidopsis thaliana* or cereals is known that important role in flowering play a gibberellins. Therefore, it was decided to identify the genes which encode the enzymes responsible for the phytohormone deactivation in such lupine species as *Lupinus albus* (butan, boros), *Lupinus luteus* (taper, mister) and *Lupinus angustifolius* (kadryl, sonet). Using PCR method and degenerate primers six fragments of *GA2ox* genes (492-498 nt) were amplified. These sequences were subjected to bioinformatic analysis and in this manner obtained protein fragments (164-166 aa). The presence of characteristic domains determined using BlastP and ExPASy programs indicates with a high probability that they are part of the functional enzyme proteins. Further analysis showed a high degree of identity of nucleotide and amino acid sequences (98-99%) between the varieties of the same species of lupine. In turn interspecies comparison of lupines showed a significant degree of similarity between sequences from *L. albus* and *L. luteus* (84%) and less in the case of *L. angustifolius* (54-56%). All known sequences also exhibit a relatively high degree of similarity to other plant species, which indicates their conservatism during the evolution. The presented results are just an introduction to the extensive research that in the near future will determine the precise mechanism of flowering in three species of lupines.

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Innovative imaging techniques for above and belowground legume development

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A precise and quantitative description of development processes in plants is a requirement for both phenotyping and plant physiology. While this can be done straightforward for some traits, others, like belowground development and pod filling dynamics,are difficult to study with destructive techniques and can greatly benefit from advanced non-invasive methods. The dynamic development of roots and nodules in the soil for example is a critical process for the growth and yield of legume plants and is sensitive to stress. However opacity of the soil and vulnerability to excavation hinder detailed investigation. Magnetic Resonance Imaging (MRI) can be used to study root and nodule development in the soil. Additional Positron Emission Tomography (PET) with ^{11}C tracer can quantify nodule functionality by measuring carbon import. We demonstrate the use of MRI to study both root and nodule development in soil-grown common bean. Pod filling is another dynamic and stress-sensitive process critical for yield. For seed yield under drought, for example, accelerated partitioning of photosynthesis towards pods is known to be a chief trait. We demonstrate the use of portable NMR (Nuclear Magnetic Resonance) sensors to non-invasively monitor the dynamics of seed filling in terms of water- and dry matter content in an online fully automated fashion. Short term effects of abiotic and biotic interactions can be revealed alsoby combined MRI/PET showing carbon partitioning within the plant body in 3D. The potential and challenges of these techniques will be discussed along with first results on legume development.

Development of genomic resources for improvement of food legumes

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Among food legumes, chickpea, pigeon pea, lentil, peas, mungbean and common bean are assuming significance in terms of production at global level. Efforts have been made to develop genomic resources for improvement of these food legumes for their effective use in marker assisted breeding for important traits. Different types of molecular markers such as RFLPs, AFLPs, SSRs, CAPS, RGAs and ESTs have been developed for identification and mapping of genes and QTLs for many agriculturally important traits and occasionally for germplasm screening, fingerprinting, and marker-assisted breeding. A large number of microsatellites which are known to be abundant and uniformly distributed in the chickpea genome have been used to develop a genotyping kit, analyze genetic relationships among *Cicer* species and assess levels of cross-transferability. Further, these markers have also been applied for the construction of intraspecific and interspecific genetic linkage maps and for mapping genes of agronomic importance such as disease resistance and yield related traits, thereby demonstrating that SSRs are ideal tools for chickpea. Additionally, in chickpea only about 800 STMS markers have also been reported and only 30-40% is expected to be polymorphic. Similar has been the case for the ESTs, however, at present about 30,000 ESTs are available in public domain which can be used in chickpea improvement. The genome sequence of pigeon pea developed by two separate groups in recent has provided sufficient number of practical markers for improvement of this crop. Total 47,004 protein coding genes and 12,511 transposable elements related genes were postulated by one group while other group predicted 48,680 genes on 11 chromosomes of pigeon pea. At least 25 mapping populations of pigeon pea for a number of biotic and abiotic stresses are being currently developed. In terms of genomic resources, lentil is relatively under developed with limited available data. At present lentil specific 9,513 EST sequences are available in the public domain. There is consequently a pressing need for significant efforts to either develop markers capable of cross-species transfer, in order to enrich existing genetic maps, or generate more informative species-specific genetic and genomic tools which can enable the identification of orthologous genes through genome synteny analysis in lentils. Comparatively, genomic resources for common bean are mostly available. Eleven BAC libraries and 83,000 ESTs and over 25 linkage maps, mostly low density (markers on average every 10 cM), have been developed for common bean. To maximize molecular polymorphism, the majority of mapping populations were derived from crosses between domesticated parents belonging to the Andean vs. Middle American gene pools. The development of genomic resources in Mungbean has been comparatively slow as only eight linkage maps are available and gene for bruchids tolerance and powdery mildew were mapped on chromosomes. Relatively very few markers are available in public domain, however, markers from other *Vigna* species like azukibean and blackgram showed polymorphism for Mungbean germplasm. This paper describes the current status of availability of genomic resources and focused the need of development of more practical markers for genomic enabled improvement in food legumes.

Exploiting the European *Medicago truncatula Tnt1* insertion mutant collection

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An insertion mutant collection based on the use of Tnt1 and MERE1 retroelements was developed in the *M. truncatula* model legume (Tadege et al., 2009; Rakocevic et al., 2009) during the EU GLIP project (www.eugrainlegumes.org) in parallel to another at the Noble Foundation (http://bioinfo4.noble.org/mutant/). The use of such mutant collections is essential to reveal gene functions and to uncover genetic interactions. In the case of the GLIP collection however, only the construction of the mutant lines was financed, therefore only a limited number of mutants were characterized despite the value of such characterized collections for the community. Recently, a bilateral Hungarian-French collaborative project was launched to further develop this specific genomic tool in *M. truncatula* and to facilitate the molecular characterization of new loci involved in nodule and root development. One part of this project deals with the isolation and description of symbiotic (Nod$^-$, Fix$^-$ and Nod^{+++}) mutants of this collection through large symbiotic screens. The identified lines will subsequently be confirmed for their symbiotic character, and the responsible mutated gene will be defined. Another important aim of the project is to sequence the insertion sites of the Tnt1 copies and also those of the endogenous MERE1 active retrotransposon in at least 2000 mutagenized lines and contribute to the *Medicago* flanking sequence tag (FST) database to advance further reverse genetics screens. To achieve this goal, the development of a high throughput sequencing method has been initiated.

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Strategies to identify candidate genes controlling Ascochyta blight in different legume crops

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Until now complete resistance to Ascochyta blight has not been reported neither in chickpea nor in faba bean. However, several QTLs have been located in chickpea LGs 2, 3, 4, 6 and 8. The most significant ones are those in LGs 2 (QTL_{AR3}) and 4 (QTL_{AR1} and QTL_{AR2}). Attempts to saturate $QTL1_{AR1}$ have been performed by developing BAC clones or by identifying candidate genes from the model *Medicago truncatula*. In faba bean at least two QTLs (*Af1* and *Af2*), assigned to chromosomes III and II were validated across environments and generations. In a second cross, six QTLs (*Af3* to *Af8*), were detected. *Af1* and *Af3* were both assigned to chromosome III and might correspond to the same genomic region. In this study, we are exploiting the synteny with the model species *M. truncatula* and chickpea, to establish a clear homology of QTL_{AR1} and *Af1* in both crops based on ESTs mapping. Moreover, possible regulators revealed by SuperSAGE analysis and associated with the ascochyta-faba bean and ascochyta-lentil interactions are being mapped in the corresponding maps in order to identify potential targets for molecular breeding. Finally, we will take profit from the recent chickpea whole genome sequence, to perform wide genome comparative mapping studies with the less-studied legume crops, in order to identify orthologous genes underlying not only blight resistance but other agronomic important traits. Our aim is to progress from basic mapping of QTLs to fine mapping or gene identification, thus allowing development of efficient markers for MAS in breeding.

Double haploids in pigeon pea: Interolog mapping of putative candidate genes

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Double haploids (DH) are promising tools for developing pure breeding lines with complete homozygosity in a single generation. Crop plants such as pigeon pea (*Cajanus cajan* (L.*)* Millsp.), which is a self-pollinated crop with a narrow genetic base, greatly benefit from DH technology by accelerating cultivar development. The key to successful production of DH is in the identification of gene networks, the expressed proteins and interacting partners. Independent studies in model crops for DH systems such as wheat, maize, tobacco, barley and rapeseed revealed genes responsible for induction of haploidy under stress conditions. With this background, an *in silico* approach was employed to study the genes related to induction of haploidy in pigeonpea. Interolog based protein interaction analyses was done to identify putative candidates for these processes. Functional annotation of all proteins when searched against *Arabidopsis thaliana* protein sequences showed few plausible proteinswhich could serve as markers and also predict the androgenic and/or DH competence of the crops of interest.

An optimized RNA Extraction Protocol for leaves, pods and seeds from *Phaseolus vulgaris*

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Phaseolus vulgaris is a major staple food crop and the most important legume in human diet, being a source of protein, fiber, complex carbohydrates, vitamins and minerals. Grain filling (GF) is the seed maturation phase that plays a major role in the seed storage reserve composition. Understanding GF and how it can be affected by water deficit (WD) is a topic of great agronomical and economical relevance, especially for the improvement of selecting and breeding of common bean lines with improved tolerance to this abiotic stress. We aim to use a transcriptomic approach to obtain a global insight into what genes are being expressed in seeds that have a major role in GF under WD and what metabolic pathways are activated or repressed in this condition. Additionally, we want to explore the role that surrounding organs, such as leaves and pods might have in this biological process.Gene expression analysis requires a large quantity of high quality RNA but routine RNA extraction protocols reveal to fail when applied to pod and seed tissues collected from common bean. Here we present reliable RNA extraction protocols that allow recovering high quantity and quality RNA suitable for other downstream applications like Next Generation Sequencing or Quantitative PCR.

In silico identification and annotation of some stress responsive genes in peanut ESTs

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Peanut (*Arachis hypogea* L.) is an internationally important crop for human consumption as a good source of protein and vegetable oil. Peanut is widely cultivated around the world in tropical, sub-tropical and warm temperate climate. Because of its huge genome size (2.8 Gb) and un-sequenced genome, studies on genomics and genetic modification of peanut are less as compared to other model crops. As peanut can be cultivated in arid and semi-arid regions, and drought/dehydration is the most frequent stress that reduces the yield. Therefore, study on drought stress responsive genes and its regulation is very much important. Here we report about the identification and annotation of some stress responsive candidate genes using peanut ESTs. The selection of genes was based on the publically available expression data. Due to good expression data and lack of available literature in peanut some of the stress responsive genes were screened. Individual EST of the said group were further searched in peanut ESTs (1, 78,490 whole EST sequences) using computational approaches. Various tools like Vec-Screen, Repeat Masker, EST-Trimmer, DNA Baser and WISE2 are being used for stress responsive gene identification and annotation. Research progress made towards contigs assembly, determination of biological function of genes, and prediction of domain as well as 3D structure for related proteins will be presented.

Application of a cDNA-AFLP approach to study pea resistance to *Didymella pinodes* in *Pisum fulvum* at molecular level

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Ascochyta blight is one of the most important fungal diseases of pea worldwide. This disease is caused by a complex of fungal pathogens including *Didymella pinodes, Phoma medicaginis* and *Ascochyta pisi*. Among these, *D. pinodes* is the most predominant and damaging. So far, no complete resistance to this pathogen has been described in pea. The highest levels of incomplete resistance have been identified in the wild relative of pea *Pisum fulvum*. However, the genes involved in the defence reaction to *D. pinodes* in this species are still unknown. Thus, a cDNA-AFLP approach has been undertaken to compare gene expression pattern between the susceptible *P. sativum* cv. Messire and the highly resistant *P. fulvum* P651 accessions. After digestion of RNA samples with *Eco*RI and *Mse*I restriction enzymes, the specific amplification using AFLP primer with 1 and 3 selective nucleotides respectively allowed to detect a mean of 52 markers per primer combination. Interestingly, 48% of all markers detected were differentially expressed, varying mainly between genotype indicating a high degree of polymorphism between these accessions. In addition, 20% of the markers were differentially expressed between treatments. Identification and characterisation of these gene fragments is currently underway. This will allow identifying defensive genes involved specifically in the pathosystem pea-*D. pinodes* and provide new molecular markers that will be very valuable for future mapping projects.

Identification and Characterisation of *mlo* gene family members in the model legume *M. truncatula*

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In barley, tomato and pea mutation in the *mlo* gene provides a broad-spectrum and durable resistance to powdery mildew. This gene encodes a membrane protein characterised by the presence of 7 trans-membrane domains and belong to a large gene family. While only 3 *mlo* genes were identified in barley, 15 and 17 *mlo* gene members have been described in *Arabidopsis thaliana* and *Vitis vinifera* respectively that have been classified in 6 different groups. The role of the additional *mlo* family members is still unknown although it has been suggested that they might be involved in resistance to other stresses. In order to identify the different *mlo* members in legumes and further clarify their role, a bioinformatic analysis, using several BLAST tools and prediction platforms has been initiated in the model legume *M. truncatula* for which large genomic and EST sequences is available. To date 14 putative *mlo* members from all different *mlo* subfamily have been identified within *M. truncatula* genome. Characterisation of these genes in *M. truncatula* may improve our understanding of their role in plants and contribute to the transfer of the highly valuable powdery mildew and/or other pathogens resistance traits to other legume crops.

Marker development for anthracnose resistance in narrow-leafed lupin *via* transcriptome profiling

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Anthracnose, caused by the fungus *Colletotrichum lupini*, represents the most important disease in lupin cultivation worldwide. To obtain high and stable yields it is necessary to plant resistant cultivars as there are no possibilities to overcome the fungus *via* pesticides. In a screening of plant genetic resources of *Lupinus angustifolius* we obtained a completely resistant breeding line, carrying a dominant resistance gene as revealed by genetic analyses of F_2 populations. Those F_2 populations are the basis for the development of molecular markers. Three approaches shall be applied to genetically map the resistance gene and to generate molecular markers suited as selection tools; (I) use of anchor markers already mapped in *L. angustifolius*, (II) use of sequence information drawn from model genomes and (III) use of sequence information of differentially expressed *L. angustifolius* cDNA. cDNA sequencing was done with the resistant and susceptible parents of the F_2 mapping population, both inoculated with the fungus, resulting in 7 477 070 sequence tags with a mean sequence length of 84 bp assembled to 577 contigs with a mean contig size of 486 bp. Finally informative SNPs were identified. Part of the SNP sequences is annotated to SwissProt which enables sequence filtering by their function concerning putative resistance-mediating proteins. Based on this information, 116 primer pairs have been developed so far. 73 % of these proved to be polymorphic between the parents, which could be demonstrated by high-resolution melt analysis of amplicons. To-date, 22 SNPs has been integrated in the genetic map, which consists of 20 linkage groups and 4 single clusters with 369 loci and a genetic length of 1887.2 cM. Linkage analysis suggests the resistance to be located on linkage group NLL-11. Additional efforts are necessary to find markers more closely linked to the resistance locus *Lanr*^{Bo}.

Molecular Analysis of Double Synthetics of Peanut

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Cultivated groundnut is an important source of oil and protein and is grown in many countries of the world. Due to evolutionary bottlenecks the crop has narrow genetic base. So ICRISAT has taken lead to develop synthetic groundnut by combining A and B genome species. Most recently crosses were made between two synthetics which led to the development of double synthetics. Molecular diversity check of these double synthetics was done by SSR analysis. The study included double synthetics, synthetics and the wild species parents which were used to develop synthetics. The material was analyzed using fluorescently labeled SSR (f-SSR) primers in PCR. The amplified product of PCR was separated on ABI 3700 DNA analyzer. The SSR maker data was further analyzed using Darwin software from which dissimilarity among 48 genotypes was calculated and a dendrogram was developed. The results indicate considerable genetic variation between the double synthetics and the parental synthetics.

Drought resistance studies of common bean (*Phaseolus vulgaris* L.): a proteomic approach

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Drought is one of the main abiotic stresses limiting common bean production worldwide; it can cause yield losses up to 80 % in some regions. The response of common bean to drought has not been widely studied, so an improved understanding of stress response mechanisms could help in developing drought resistant lines, consequently contributing to high productivity of this important food legume. A proteomic approach was used to identify drought-responsive proteins in leaves of two cultivars differing in their response to drought, Tiber and more sensitive Starozagorski čern. Total proteins from leaves of control and stressed plants were extracted and separated by 2D-DIGE. Protein spots of interest for further analysis were selected based on the analysis of variance from Progenesis SameSpot software and significant testing by PLS using the Jack-knife uncertainty test. Fifty-eight proteins whose abundance changed significantly between control and drought stressed plants were identified by LC-MS/MS in Tiber and 64 in Starozagorski čern. In order to determine which types of proteins are involved in drought stress response, the identified proteins were categorized into groups based on their putative biological functions. The majority of identified proteins were classified into functional categories that include energy metabolism, photosynthesis, ATP interconversion, protein synthesis and proteolysis, stress and defence related proteins. Interactions between identified proteins, demonstrated by bioinformatics analysis enabled a more complete insight into biological pathways and molecular functions affected by drought stress. The research provided the basic insight needed to further investigate the molecular regulatory mechanism of drought response in common bean.

Contained and field experiments with GM peas in Agritec

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Genetic transformation of pea (*Pisum sativum* L.) was focused on the development of increased resistance to viral and fungal pathogens and insects. For resistance to Pea Seed-borne and Pea Enation Mosaic Viruses, constructs containing sequences of respective coat proteins were used to induce pathogen-derived resistance. Starting with T1 generation, mechanical inoculation of plants with highly aggressive isolates was carried out. Plants were inspected visually (typical symptoms of virus infection) and with PCR/RT qPCR for virus presence in plant tissues. While coat protein mediated resistance (CPMR) induced resistance to PEMV expressed in T1 generation was not stable, the post-transcriptional gene silencing (PTGC) induced resistance to PSbMV was stably inherited to T3, T4 generation showing "plant recovery phenomenon". Since 2010, transgenic peas were officially released into environment for multiplication and tests of agronomic performance. Preliminary feeding tests on rats did not show any negative effect of feed mixtures enriched with transgenic pea seed meal on the growth and health conditions of experimental animals. Further, construct with *gmSPI*-2 protease inhibitor was used for the development of fungus and insect resistance. Pea transgenic lines that generated increased mortality of *Bruchus pisorum* larvae were achieved.

Acknowledgements

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Tagging Genomic Regions Controlling YMV Resistance with SSR markers in Mothbean [*Vigna aconitifolia* (Jaq.)]

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Moth bean is a drought tolerant food legume crop grown in arid & semi-arid regions of India and is an important source of dietary protein. It can be grown in low rainfall areas (200-300mm) and withstand high temperature (35-40°C). Though it has the yield potential of 1500 kg/ha in favourable environment, the average productivity in India as low as 300-400kg/ha. Among the biotic stresses that affects crop yield diseases play a very important role in moth bean. Yellow Mosaic Virus (YMV) transmitted by whitefly (*Bemisia tabaci)* is most serious disease as it adversely affects the grain and fodder yield. Identification and validation of DNA markers linked to YMV resistance gene was undertaken. Parental polymorphism using 196 legume specific SSR primers combinations were carried out with resistant (MH-43) and susceptible (GMO-25) parents to identify polymorphic markers. Only 5 SSR primers were polymorphic between the parents GMO-25 and MH-43 out of 196 primers. Bulk Segregant Analysis in F_2 of the cross GMO-25(S) x MH-43 (R) using five polymorphic SSR markers, two were found polymorphic indicating putative linkage with YMV resistance gene. Linkage analysis of the 114 F_2 individuals using putatively linked SSR markers from bulk segregant analysis revealed only one PVSSR-101 marker, out of two putatively linked SSR markers with genetic distance of 20.45cM linked to resistant gene for Yellow mosaic virus disease. The PVSSR-101 marker can be used for identification and validation of homozygotes for the *YMV mb* resistance gene plants in early segregating population, especially during back cross breeding.

Morphological and Microsatellite markers based Genetic Diversity in Cowpea [*Vigna unguiculata* (L.) Walp]

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Morphological and molecular genetic diversity analysis on 60 selected cowpea genotypes revealed relatively low magnitude of genetic diversity at both molecular and morphological levels. Genetic similarity coefficient for all the 60 accessions ranged from 82 to 100 for morphological traits. Phenotypic based UPGMA clustering pattern separated variability among these accessions based on yield and its important attributing traits and grouped all the genotypes into seven major clusters at 92 per cent similarity coefficient. Cluster I, V and VI were solitary in nature, while cluster VII had maximum genotypes (33). Molecular analysis of the same genotypes with 42 SSR primers revealed 23 primer pairs were polymorphic and 11 were found monomorphic out of 34 primers amplified. The polymorphic primers produced allele's ranging between 2 to 5 with an average value of 2.30. The Polymorphism Information Content ranged from 0.12 (primer CP 169) to 0.67 (primers CP 573). Based on the PIC values and number of alleles amplified, the primer CP 573 was found to be more informative in the present set of genotypes. The genetic similarity coefficient for all 60 accessions ranged from 57 to 96. UPGMA based dendrogram was obtained from the binary data deduced from the DNA profiles of the samples analyzed. Seven major clusters were formed on the basis of Jaccard's similarity coefficient at 65 per cent. Cluster V had maximum 28 genotypes, while the cluster II was solitary in nature and indicates enormous variability in genetic constitution. A comparative study of the dendrograms based on morphological and molecular analysis revealed that SSR markers exhibiting higher range of polymorphism produced more informative bands and had more discriminating power (57-100%) than morphological diversity analysis (82-100%).

Cross-species transferability of microsatellite markers generated from cowpea enhances marker repertoire and genetic diversity study in *Vigna* genus

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Genus *Vigna* of family Leguminosae include several agriculturally important species *viz.*, *V. unguiculata* (Cowpea), *V. radiata* (Mungbean), *V. mungo* (Urdbean), *V. aconitifolia* (Mothbean), *V. umbellata* (Ricebean) and *V. angularis* (Adzukibean). Except Adzuki bean, other five species are important pulse crops in India and other parts of tropical Asia. The comparative genome analysis to assess synteny facilitates use of genomic resources between different species which is cost effective and can speed up gene identification in species which are less studied at the genomic level. To study the conservation of microsatellite regions, a set of 168 cowpea specific microsatellite markers were tested for their transferability across five species of *Vigna* and horsegram. Two genotypes were chosen based on their utility in current breeding programs from each species. In cowpea, 108 primers amplified PCR products and 47 were polymorphic between parents of a mapping population being developed to tag genes for resistance to bacterial leaf blight. Considerable transferability was observed with markers in Horsegram (39.9%), Ricebean (36.4%) and Mothbean (28.6%). However, amplification rate was low in Urdbean (9.5%) and Mungbean (9.5%). The number of polymorphic primers varied from 9 (Urdbean) to 21 (Horsegram). From the results, it is concluded that a good number of highly polymorphic markers were identified to evaluate the genetic relationship among species of *Vigna*. It was also possible to identify a comprehensive set of SSR markers as an important genomic resource for diversity analysis and genetic mapping to test relationships of resistance to common diseases of the members of the genus *Vigna*.

Establishment of embryonic tip regeneration system and Transformation of iron uptake genes in Azuki Bean (*Vigna angularis* Ohwi & Ohashi)

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The embryonic tip regenerate system of azuki bean was firstly created. The best condition for embryonic tip regeneration was MS medium with 0.2mg/L Indole butytic acid (IBA) and 0.2mg/L 6BA. The regeneration rate was nearly 109.7% under this condition. It needs 40 days to be completed from inducing to obtain 3-5cm long shoots. The regeneration system of embryonic tip has higher efficiency, time consuming and higher tolerance to *agrobacterium tumefaciens* than hypocotyl's and epicotyl's. 100mg/ L Kanamycin and 5 mg/L Hygromycin B was suitable for screen transformation plants in azuki bean. Embryonic tips of iron-deficiency sensitive Jingnong 5 variety were inoculated with *Agrobacterium tumefaciens* EHA105 harboring pCAMBIA1200-35S::*AtbHLH39*GUS and pBI121-35S::*FIT*, respectively. *AtbHLH39* and *FIT* are important iron uptake regulating genes from *Arabidopsis* encoding bHLH protein.The frequency of GUS gene transient expression was calculated to determine the suitable condition of *agrobacterium tumefaciens* to inoculate embryonic tips. The infected method was standing and co-culture at 28ºC in dark. The frequency of GUS gene transient expression was 48.78% under this condition. When the co-culture time was 72 hours, the frequency of GUS gene transient expression was 90.47%. Nine positive *FIT* transgenic plants were obtained and identified by PCR and RT-PCR amplification with specific primer pairs of *FIT* gene. The average transferred rate of *FIT* gene was 6.47%.

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Molecular cloning and characterization of *LlACO* gene from *Lupinus luteus*

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ACO encodes for 1-aminocyclopropane-1-carboxylate oxidase, the enzyme that catalyzes the final step of ethylene biosynthesis: oxygen-dependent conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene (ET). ACO belongs to a family of mononuclear enzymes that are characterized by a 2-histidine-1-carboxylic acid iron-binding motif. In this work, we isolated for the first time cDNA of *ACO* from leaves of *Lupinus luteus* (*LlACO*) and determined its sequence. The complete coding sequence consists of 951 bp and encodes for 317 amino acids. Comparison between the deduced amino acid sequence of LlACO and ACO from other plant species revealed that the predicted sequence contains all of characteristic motifs found in ACOs, which are essential for their activity: conservative non-heme dioxygenase DIOX_N region, and a C-terminally located 2OG-FeII_Oxy region. The *LlACO* gene encodes for a protein which is similar to that of ACC oxidases identified in other plant species. Our results indicate that identified cDNA of *LlACO* encodes most likely an active protein.

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Mapping of single-locus and epistatic QTLs for seed morpho-agronomic traits from an adapted x exotic nuña bean population

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Common bean (*Phaseolus vulgaris* L.) is a widely grown and important economic grain legume. Seed quality is a critical attribute in determining commercial acceptability of common bean. Thereby, to obtain insights into the genetics of seed quality, a population of 185 recombinant inbred lines (RILs) derived from an Andean intra-gene pool cross, involving PMB0225 (dry bean) and PHA1037 (nuña bean) parents, was evaluated for seven seed traits under six different environments (long-day and short-day natural photoperiod conditions). Multi-environment QTL analyses performed detected a total of 46 QTLs, which were mapped throughout all linkage groups: 21 had only additive effects, 17 had only epistatic effects and eight had both additive and epistatic effects. For seed dimension and weight traits, 19 single-locus QTLs and 13 epistatic QTLs involved in eight epistatic interactions were detected; while for seed color and brightness, 10 single-locus QTLs and 12 epistatic QTLs involved in seven epistatic interactions were identified. Interactions between QTLs and environment were also detected, but despite this fact, QTLs with differential effect on long-day and short-day environments were not found. It may be concluded that i) the genetics of seed quality is complex in nature, where not only additive effects but also epistasis are important components of the genetic variance in the Andean common bean background, and ii) the QTLs here reported will play a significant role in enhancing marker assisted selection efficiency for breeding nuña beans. The work was supported by the PET2008-0167 and AGL2011-25562 projects of the Ministerio de Economía y Competitividad from Spain.

Glycinin and β-conglycinin protein fractions and its subunits in soybean varieties

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The ratio between glycinin and β-conglycinin protein fractions, as well as the concentration of its subunits greatly affects proteins quality, as well as the functional properties of foods made from soybeans. In this study, the concentration of major storage proteins and their respective subunits in seven soybean varieties were analyzed. In addition, the correlation between activity and concentration of bioactive proteins, such as lipoxygenase and proteinase inhibitors was determined. Glycinin and β-conglycinin comprise about 75% of the storage proteins and as such account for both quantity and quality of the kernel proteins. The 11S concentration of the soybean varieties ranged from 50.3 to 60.3% and those of 7S varied from 17.8 to 23.1% of total extractable proteins, while the ratio of 11S/7S proteins varied from 2.4 to 3.3 among the varieties. In the analyzed soybean genotypes, 7S proteins consisted of 30.1 to 37.0% of ά-subunit, 37.1 to 46.6% of α-subunit and 25.5 to 29.9% of β-subunit. In addition, the concentration of total acidic subunits (A) of the 11S proteins was significantly higher than the total basic subunits (B). According to our results, the genotype Lidija which has the lowest concentration of α-subunit and the second lowest concentration of ά and β- subunits, as well as the best 11S/7S protein ratio, could be used as a parent to improve the soybean protein quality. Also, Lana KTI-lacking variety with the highest concentration of total 11S fraction, which is a superior fraction considering its content of cysteine and methionine, could be a very good source of qualitative proteins for food production.

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Secondary metabolite profiles of seeds, roots and aerial parts of lentil (*Lens culinaris*)

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Although lentil (*Lens culinaris*) is an important crop, its phytochemical characterization remains incomplete. While there are a number of publications on secondary metabolites of lentil seeds, hardly any published data can be found on lentil roots and green parts of this plant. The aim of present study was to compare profiles of phenolic compounds and saponins in leaves, stems, roots and seeds of lentil cultivar Tina. Lentil seeds, as well as leaves, roots, and stems of 3-week plants were extracted with 80% methanol. Phenolic compounds and saponins were isolated by SPE. Samples were analyzed using UPLC-ESI-MS/MS, and identification of components was carried out on the basis of their UV and MS-spectra. Our experiments demonstrated that the phenolic profiles of individual lentil organs were different. Moreover, while lentil leaves, stems, and (in minor degree) roots contained numerous flavonoids, the phenolic composition of seeds was much less complex. As regards saponins, all analyzed samples contained soyasaponin I, soyasaponin VI, and traces of dehydrosoyasaponin I.

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Session 7

Responses to biotic and abiotic stresses in legumes

KEYNOTE LECTURE

Breeding for stress resistance in legumes

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Susceptibility to biotic and abiotic stresses is the common situation for most legume crops, representing a major limitation for legume cultivation. Relative prevalence and importance of the various diseases and stresses in each crop varies with the agroecological conditions, but the sad fact is that in each situation we face challenging problems to which little resistance and alternative control methods are available. Resistance breeding is a priority in most programs yielding significant achievements. However, progressis still slow due to the still relatively low investment on biotechnology on legume crops, but also, and mainly because of the insufficient knowledge on the plant/stress interactions and in most cases on the biology of the causal agents, with poor understanding on host specialization, existence of races and at times even on the identity of the causal agents. A number of cases studied will be presented in this talk, with a special focus on those that are specific of the Mediterranean Basin. Some results achieved so far on identification and characterization of resistance against major fungal diseases, insect pests, parasitic weeds and drought will be presented and discussed.

Study of drought resistance of chickpea and lentil in Azerbaijan condition

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The local gene pool and introduced from ICARDA material of chickpea and lentil were studied in moderately and highly continental zones (South Mugan, Mountainous Shirvan and Absheron) of Azerbaijan. Physiological parameters such as the level of quantitative variability of chlorophyll, the degree of depression, photosynthetic rate, water conductivity of stomata, CO_2 saturation level in the intercellular spaces, the rate of transpiration, protein and tryptophan content, as well as structural elements of yield were determined. Determined biological and economic parameters of chickpea and lentil showed the availability of polymorphism for drought resistance. In some chickpea genotypes chlorophyll content decreased up to 10.5%, while in others it increased up to 32.1%. It was revealed that the samples with high intensity of photosynthesis and the lowest rate of transpiration, water conductivity of stomata and CO_2 concentration in the intercellular spaces were more drought resistant. These forms phenotypically differ from others mainly for dark green pigmentation. As a result of the analysis of the above features, as well as seed production we identified the valuable genetic materials of both lentil and chickpea. These include: local forms of lentils Kx-26 (Jalilabad), Kx-78-01 (Yardimli), Kx-116/1 (Masalli), from introduced material with red seed Flip-2003-52, F.2010-62, F.2010 -65, F.2011-18 and others, a total of 24 samples. Drought resistant forms of chickpea include: local Kx-9, 11, 258 (Jalilabad), Kx-36 (Agstafa), Kx-97-01 (Yardimli) Kx-266 (Masalli) and introduced Flip 03-34/1, F .06-76, F.05-74, F.08-46, F.00-19/1, F.06-143, F.99-25 and others, a total of 46 samples. The selected materials of chickpea and lentil are in depth study on molecular level. Environmental testing of selected accessions of chickpea (24 samples) in the Nakhchivan Autonomous Republic and two varieties in the Kazakhstan Institute of Agriculture and Plant Industry also showed their prospects on a number of economically valuable traits.

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Effects of root rot pathogens on two pea (*Pisum sativum L.*) varieties in controlled conditions

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Organic farming is highly dependent on legumes to build soil fertility. However, declining trend is present inlegume, especially spring pea production in Germany. The reasons are beside low yield, foot and root diseases. Therefore, winter pea should be considered as possible alternative to spring peas. Climate change scenarios predict an increase in winter precipitation and temperatures which are expected to favor winter over spring pea. Winter peas, especially in mix cropping with cereal, provides good weed suppression, prevents soil erosion and better uses winter moisture.In addition, winter peais a good pre-cropand can be used in two-crop systems (winter pea followed by maize). The hope is also, that winter peas are less susceptible to root rot pathogens. However, little data are available. Therefore, study was carried out to evaluate the susceptibility of the winter pea variety EFB33to different isolates of *Fusarium avenaceum*, *F. solani* f. sp*pisi*, *M. pinodes* and *P. medicaginis* under controlled conditions compared to the spring variety Santana. Three weeks after sowing and inoculation disease symptoms were assessed and plant growth parameters measured. All of the tested pathogens resulted in disease. Reductions in fresh weight per unit of external tissue damage on Santana were significantly different among pathogens with 12.5% for *F. avenaceum,* 8.8% for *P. medicaginis,* 8.4% for *M. pinodes* and 3.8% for *F. solani*, respectively. Overall, EFB33 was less susceptible and the highest reduction of fresh weight caused *F. avenaceum,* 15.8%. No significant difference was observed among other pathogens.

Chilling tolerance in soybeans – pod setting and indirect selection criteria

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Soybeans have relatively high demands in temperature and water supply. Especially during flowering and early pod setting low temperatures may affect yield as well as quality of grains negatively. Therefore – with respect to extending soybean cultivation to the north, chilling tolerance is an important trait besides earliness. To compare chilling tolerance of cultivars and breeding lines, a test under controlled conditions in a growth chamber was used. Plants were subjected to a 3 weeks-period of cool temperatures (15 °C day/10 °C night) compared to a control (25 °C day/20 °C night) starting at the beginning of flowering. At the end of the stress period, free proline, soluble sugars, chlorophyll content (SPAD) and dry matter content of leaves as well as pod setting per node were determined. Plants were cultivated till harvest and pod setting, grain number and yield were recorded. Results show a clear variability in chilling tolerance as well as compensation behavior within the 35 early soybean cultivars investigated. Immediately after the stress period, pod setting was reduced by the stress treatment compared to the control by 21.8% – 91.2%. During further cultivation, some genotypes showed compensation behavior partly resulting in higher pod numbers and yield in stressed plants. This was related to a longer time till ripening in some genotypes, the delay in ripening ranging from 0 to 49 days. Really sensitive genotypes could not compensate for the reduced pod setting. Relative yields (y_{stress}*100/$y_{control}$) ranged from 12% to 180%. A chilling event during flowering time in the field in 2011 was used to validate tolerance to cool temperatures determined under climate chamber conditions.

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Phenotyping for drought stress tolerance in faba beans – from the lab to the field

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Faba beans are well adapted to the cool climatic conditions in Northern Europe. They have a high yield potential, but yield stability is often low. One reason for this is drought stress. Winter faba beans with a developmental advantage of about 2 - 4 weeks compared to summer types can use winter precipitation more effective. However, little is known about the variability with regard to physiological differences in their drought stress response. To get information on this, 200 (winter) faba bean inbred lines are phenotyped under highly controlled conditions in single leaf/leaf disc tests regarding physiological traits related to drought tolerance. First results of the lab tests show a significant influence of drought stress on relative leaf water content (86.8 – 91.2% in control vs. 11.6 – 56.1% in stress treatment), content of free proline (0.3 – 6.2 µmol/g DM vs. 167.2– 614.8 µmol/g DM) and soluble sugars (139.6 – 1302.1 µmol/g DM vs. 612.1 – 1746.9 µmol/g DM), membrane stability index (74.5 – 85.5 % vs. 13,.1 – 76.5 %) and chlorophyll content (23.9 – 49.3 SPAD units vs. 21.4 – 45.3 SPAD units) of the lines as well as a significant interaction between lines and the stress factor for all traits. To relate these results to field conditions, rain out-shelter trials are carried out with a subset of 44 out of these 200 inbred lines for determining yield and yield parameters under control vs. stress conditions. As a trait related to water use efficiency ^{13}C discrimination is regarded. The first rain out-shelter trial in 2012 revealed differences in yield response between genotypes as well as control and stress conditions (7.1 – 37 g/plant in control vs. 6.2 – 18.2 g/plant in stress treatment). Seed number per plant was more influenced by the drought stress than thousand kernel weight.

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Inhibitory activity of secondary metabolites from BTH- and BABA-treated pea against *Uromyces pisi* infection

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Pea rust (*Uromyces pisi*) is a widespread disease of pea (*Pisum sativum*) worldwide. We previously reported induction of systemic acquired resistance to rust infection in pea by exogenous applications of BTH and BABA based on a reduction of appressorium formation, as well as growth of infection hyphae and haustorium formation. This was related to activation of the phenylpropanoid pathway increasing levels of total phenolic compounds. In this work we attempted to discern by high performance liquid chromatography the particular phenolic compounds involved in reducing rust infection in pea following BABA and BTH induction of resistance. For the experiment we used cv. Messire, susceptible, and PI347321 characterized with partial resistance to *U. pisi* no associated with host cell death. Following SAR inducer application we observed an increase in the amount of total but also in the excreted fraction of coumarins, particularly scopoletin, medicarpin and pisatin although the nature and proportion of the particular coumarins differed between treatments. Interestingly BTH treatment was more actively related with phenolic accumulation than BABA suggesting differing pathways for the induction of resistance. In addition, exogenous application of scopoletin, medicarpin and pisatin to the leaves lead to a reduction of the different fungal growth stages. Thus, the data suggested that BTH and BABA had an effect on the production and secretion of particular phytoalexins and other unknown fungitoxic compounds to the leaf surface that hampered pre and post penetration fungal stages. Further studies are being carried out to determine the nature of the unknown phenolic compounds induced by BABA and BTH.

Reducing biotic stresses in legumes through intercropping with durum wheat

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Intercropping - growing simultaneously two or more species in a same field - is known as an agricultural practice which can improve yield compared to sole cropping. However its efficiency in significantly reducing biotic factors has been controversial while they can strongly affect both yield and grain quality in low input systems. The aim of our study was to evaluate the assumption that intercropping legumes with durum wheat can reduce legume pests (green aphids, pea leaf weevils and pea broad bean beetles), weeds and diseases (legume ascochyta and rust, wheat mildew and brown rust). Field experiments have been carried out in SW France since 2006. Durum wheat, winter pea and faba bean were evaluated as sole crop (SC) and intercrop (IC) with or whithout fertiliser-N supply or chemical pest management. Our results further document the contrasting effects of IC against various pests due to interactions between plant architecture, disease dispersion, insect behaviour and farming practices: i) weeds were always reduced in IC compared to legume SC but not compared to wheat SC, ii) pea aphids were significantly reduced in IC while weevils were not or slightly affected, iii) diseases were mostly reduced in IC or at the same level as in SC and rarely increased. Durum wheat-winter pea intercropping thus seems efficient in reducing some diseases and pests through environmental modifications (resources dilution, physical barrier, microclimate shift) resulting from the association of complementary functional groups of species. IC could therefore be a way to reduce the use of pesticides.

Use of molecular markers for detection of ascochyta blight and fusarium wilt resistance in Tunisian chickpea breeding lines

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Twenty three chickpea cultivars, mainly advanced lines and some Tunisian varieties released by the national chickpea breeding program, have been studied for their resistance to the two most damaging diseases: ascochyta blight (AB) and fusarium wilt (FW). The field evaluation for AB was conducted in two field locations and under controlled conditions. Two co-dominant markers associated with AB resistance were also used in this study; the CaETR marker tightly linked to QTL_{AR1} in combination with the SCAR $SCY17_{590}$ marker linked to another QTL_{AR2}. These two markers contribute efficiently in the selection of new chickpea varieties with the better combination of alleles to ensure a durable resistance for AB and are very useful in the chickpea breeding program. The same genotypes were also subject to FW field evaluation during two cropping season and under controlled conditions. Four STMS markers associated with FW resistance were also used, three of them (TA27, TA59 and TA96) were found tightly linked to the second gene controlling the resistance to race 0 of FW. While the fourth STMS used in this study (TR59) was closely linked to the first gene of resistance. The dendrogram obtained from the molecular profiles with these 4 STMS markers does not classify the studied genotypes according to their behavior toward FW. In fact, these markers are described to have extensive polymorphism. Thus, resistance allele prediction with STMS is difficult. The development of new stable marker as diagnostic or SCAR markers is on-going for genotyping correctly susceptible and resistant chickpea lines to FW which could be very interesting in assisting chickpea breeding program.

Evaluation of *Cicer* species for resistance to leaf miner (*Liriomyza cicerina* Rond.)

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In Mediterranean region including Turkey, damage of leaf miner (*Liriomyza cicerina* Rond.) is estimated as about 40% yield loses in the cultivated chickpea (*Cicer arietinum* L.). The most economic method for leaf miner control is to use resistant varieties (Singh ve Weigand, 1994). In this study, 130 genotypes of wild (*Cicer reticulatum, Cicer echinospermum*) and the cultivated chickpea were evaluated for resistance to leaf miner using 1-9 scale (1-4 = resistant and 5-9= susceptible) for two years in 2011-12 and 2012-13 growing seasons. Damage of leaf miner decreased from bigger leaflets to smaller ones and from simple and/or normal leaves to multipinnate ones. It is assumed that multipinnate genotypes have structural resistance by having the scores of 2-4 on 1-9 scale. Also, it was observed that wild genotypes were more resistant than the cultivated ones. According the obtained results, it was concluded that AWC 611 having multipinnate leaf, a mutant derived from *C. reticulatum* Ladiz., was the best resistant sources for leaf miner and can be used in breeding programs.

Incorporation of resistance to angular leaf spot and bean common mosaic necrosis virus diseases in adapted common bean (*Phaseolus vulgaris* L.) genotype in Tanzania

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Angular leaf spot (ALS) caused by the fungus *Phaeosariopsis griseola* and Bean common mosaic and necrosis virus (BCMNV/BCMV) are important diseases of common bean in Tanzania that can cause severe yield reduction when uncontrolled. This study was conducted to incorporate resistant genes for ALS and BCMNV/BCMV diseases into adapted, market class and farmers and consumers preferred bean genotype using Marker assisted selection. The parents Mexico 54 and UBR(25)95 donor of *Phg-2* and *I/bc-3* genes for ALS and BCMV/BCMNV respectively were used and the recipient being Kablanketi. In selection, SCAR markers SNO2, ROC11 and SW13 linked to *Phg-2*, *bc-3* and *I gene* respectively were used. A parallel backcrossing (modified double cross) procedure was used. The F_1, F_2 and backcrosses from single crosses were characterized. The Chi square values for ALS were 0.081 ($P<0.776$) and 0.017 ($P<0.896$) and for BCMNV were 1.609 ($P<0.205$) and 1.2 ($P<0.273$) for molecular and phenotypic screening respectively. The resistance to ALS and BCMNV was found to be monogenic and the genes involved are dominant and recessive respectively. The heritability of ALS was found to be high (0.772) implying that selection for ALS can be done early in segregating populations. High correlation values, r = 0.741 and 0.624 for ALS and BCMNV were obtained between phenotypic and molecular data, indicating high reliability for markers. In selection it was possible to select lines with multiple disease resistances. This work signifies the use of MAS for multiple gene screening.

Saturation of a Quantitative Trait Loci controlling resistance to *Orobanche crenata* in pea

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Crenate broomrape (*Orobanche crenata*) is a root parasitic weed that represents a major constraint for pea cultivation in the Mediterranean Basin and Middle East. In a previous study we identified several Quantitative Trait Loci (QTLs) associated with resistance to *O. crenata* in the pea RIL population P665 x Messire. A QTL located in LGVII was associated with a lower number of *O. crenata* tubercles per root length and explained a high proportion of the phenotypic variation of the trait (33%). In this study this genomic region was saturated with new markers by comparative mapping with *Medicago truncatula*. Nine molecular markers previously mapped in *P. sativum* LGVII and in *M. truncatula* were analysed in P665 x Messire. Eight of them could be included in the map, being *dFPA2*maker located into de QTL. In addition, a SCAR marker (*SCARK6*) was developed from a RAPD marker tightly linked to the QTL. The inclusion in our map of these markers and the *M. truncatula* EST *DNABP* allowed the comparison with *M. truncatula*. The pea region containing the QTL was located in *M. truncatula* chromosome 4. Thirty eight *M. truncatula* genes, expected to be located into the QTL region, were selected. Four of them could be mapped in LGVII in P665 x Messire genetic map. Two genes (*Medtr4g068580.1* and *Medtr4g068270.1*) co-localized with the QTL.

Studies on Water-Logging Tolerance in Pigeon pea: Future Prospectus

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Temporary water-logging during rainy season is a major yield reducer in pigeonpea. Since agronomic options are expensive some permanent genetic solutions are necessary to overcome this constraint. To identify tolerant cultivars, 49 genotypes were screened at three critical stages of crop growth. These genotypes were selected on the basis of initial screening in previous year. Screening at seed stage revealed that dark seed genotypes, long duration lines and hybrids exhibited greater tolerance to eight days of continuous water logging as compared to white or brown seeded lines. At early seedling stage, water-logging resulted in chlorosis, wilting of plants, reduction in leaf area and height in susceptible lines. While screening at late seedling stage in field conditions showed drastic effect of submergence on plant survival, there was severe phytophthora infestation in field screening and very few genotypes survive submergence at this stage. Genotypes ICP 5028, ICPH 2740, MAL 15, ICPH 2431, ICPL 20237, ICPL 87119 and ICPL 332 survived water-logging stress at all the three stages. Some anatomical and morphological traits were found to be related to Water-logging resistance. These include development of lenticles and adventitious roots just above the water level along with formation of arenchyma cells in root cortex. Linking tolerance with these adaptation traits may help in selecting resistance genotypes. Crosses have been made to study the inheritance of resistance and to develop populations for identifying molecular markers. This will enable in pyramiding the desirable traits to improve submergence tolerance through marker assisted selection.

Accumulation of nickel in red clover

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Red clover (*Trifoilum pratense* L.) is considered as one of the most important legumes, and today is the second most important, after alfalfa. Production of high-quality feed from red clover may be affected, among other things, the increased concentration of heavy metals in some agricultural areas, primarily due to anthropogenic influences. The aim of this study was to determine the level of nickel in red clover grown on soils with different content of nickel, in order to obtain information on safety of these nutrients. The examination was conducted on four types of soil: chernozem, vertisol, eutric cambisol and humofluvisol. Sampling of soil and plant material was carried out during May 2011, in the second year of red clover production. The total content of nickel in soil samples, at a depth 0.30 cm, was in the range 3.36-129.67 mg/kg. Maximum permitted level of nickel in soil is 50 mg/kg. The content of nickel in red clover was in the range 0,41- 6,87 mg/kg, which is below the critical and toxic concentrations to plants. It was concluded that the accumulation of heavy metals in plants did not depend only on the total content in soil, but also the affinity of the plant, and individual and interactive effects of various soil properties. It is necessary to further control of nickel in contaminated area, in order to prevent his entry into the food chain and provide safety food.

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The crude protein content in alfalfa grown on different soil types

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Alfalfa (*Medicago sativa* L.) is the most important forage legume. Crude protein content is one indicator of the its quality, which, among other things, is affected by the variability of soil properties. The aim of this study was to determine the effect of different soil types on the content of crude protein in alfalfa. The examination was conducted on six types of soil: chernozem, vertisol, eutric cambisol, humofluvisol, fluvisol and humogley (according to domestic soil classification). Sampling of plant material was carried out during May 2011., in the second year of alfalfa production. On the basis of these results it can be concluded that there are statistically significant differences in the content of crude protein between alfalfa plants, grown on different soil types. The highest protein content has alfalfa grown on chernozem (26.28%) and lowest on the eutric cambisols (13.98%). Soils, on which the protein content in alfalfa was higher, are characterized by good physical and chemical properties, a higher content of total nitrogen, primarily nitrogen fixation increased, as have the favorable conditions for microbial activity. This is primarily related to the pH value, because the greatest number of *Rhizobium* is in neutral and slightly alkaline soils. The expected results should be a guideline for the production practice, because the modeling of feed production, in different production conditions, raises the possibility of more intensive crop and livestock production.

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Physiological, biochemical and molecular variations for cold tolerance in desi and kabuli chickpea (*Cicer arietinum* L.) genotypes

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The present investigation was carried out to assess the variations in physiological, biochemical and molecular traits in desi (PBG1, PBG 5, GL28008 and GNG1861) and kabuli (L552, GLK 26162) chickpea cultivars contributing towards cold stress tolerance in them at various growth stages and at temperature maintained 5^0C and 10^0C during crop development. The higher biomass accumulation in kabuli chickpea cultivars at low temperature was due to profuse vegetative growth in them as indicated by increased plant height, leaf area and leaf area index in them. Physiological parameters such as leaf water content and respiratory activity was found to be higher in kabuli genotypes at all the growth stages whereas photosynthetic efficiency in terms of total chlorophyll content and photosynthetic assimilation rate was recorded to be higher in desi genotypes. Pollen viability was found to be maximum in L552 (81.8%) followed by GLK26162 (76.9%) and GNG1861 (73.8%). The higher status of antioxidative enzymes (catalase and peroxidase) along with proline content in GNG1861 and GLK 26162 at lower temperature might be responsible for their tolerance behaviour towards cold stress tolerance. The highest yield was recorded in GNG1861 (3185 Kg/ha) followed by L552 (3055 Kg/ha). GLK 26162 showed distinct physiological and biochemical characters which render it a tolerant in kabuli category with 81.3% filled pods and highest 100 seed weight of 31.6 g. All these cultivars were characterized for the molecular diversity using nineteen RAPD primers. A dendrogram constructed on the basis of UPGMA clustering method revealed grouping of these genotypes in two major clusters except for kabuli genotype GLK 26162. Cluster-I constituted four genotypes grouped into two sub clusters- A and B. Highly tolerant desi genotype GNG1861 with 76.4% filled pods and moderately tolerant kabuli genotype L552 with 82.5% filled pods were found to be closely related in sub cluster B having maximum Jaccard's similarity coefficient. The most sensitive genotype PBG 5 having minimum filled pods (65.2%) was found alone in the cluster – II.

Mitigating the impact of climate change through stable multi-disease resistant chickpea genotypes

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Chickpea is the third most important food grain legume, sown as winter crop in most parts of the world but with varied temperature and other climatic conditions. Hence, experiences there major constraints in cultivation, Ascochyta blight (AB) (*Ascochyta rabiei* (Pass.) Lab.) in cool wet geo–agricultural zones, Fusarium wilt (FW) (*Fusarium oxysporum* f.sp. *ciceris* Synd. & Hans.) and dry root rot (DRR) (*Rhizoctonia bataticola* (Butler) Taub) in warm dry conditions. Host resistance is the most economical solution for sustainable grain yield, but cultivars soon age to disease resistance, inviting a need for novel genotypes with high level resistance suitably with stable multiple disease resistance with consistent pattern over the years to overcome the impact of climate change in resistance break down. Sixty genotypes were screened consecutively for five years from 2007-08 to 2011-12 against FW and DRR by sick-plot technique and AB using controlled atmosphere field screening technique. Disease reactions were recorded on 1-9 rating scale for AB and percentage of plants killed for FW and DRR. Resistance was defined as reaction types of ≥ 3 for AB and $\geq 5\%$ plant killed for FW and DRR. The genotypes viz. GL 26115, GL827085, GL27111, GL27133, GL27163, GL28211, GL28149, GL28286, GL28295, GL28297, GL29328, GLK24096, GG1362, GG1390, GG1403, FLIP02-276, FLIP03-114, ICCV98815 with consistent disease response can be used as donors in chickpea improvement programme or directly as cultivars. The total chlorophyll content of the 60 genotypes has been correlated with these diseases and tried to work out the significant relation if any.

Investigating the role of solanapyrone toxins in Ascochyta blight using toxin-deficient mutants of *Ascochyta rabiei*

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Ascochyta rabiei, the causal agent of Ascochyta blight of chickpea, produces solanapyrone toxins (solanapyrone A, B and C). However, very little is known about the genetics of toxin production and the role of the toxins in pathogenesis. Generating mutants deficient in the toxin biosynthesis would provide information on the role of the toxins during infection processes. Partial genomic sequences of the solanapyrone biosynthesis gene cluster in *A.rabiei* were identified, based on the homologous *sol* gene cluster (*sol*1 - *sol*6) in *Alternaria solani*, which also produces the same toxins. The *sol*5 and *sol*4 genes which encode solanapyrone synthase and a fungal specific transcription factor, respectively, were targeted to generate toxin-deficient mutants. Deletion of either the *sol*4 or the *sol*5 gene abolished production of solanopayrone toxins. The *sol*5-deletion mutants produced no solanapyrone toxxins and accumulated the precursor prosolanapyrone II, whereas the*sol4*-deletion mutants did not produce either solanapyrone toxins or the prosolanapyrone II. Solanopyrone toxins, but not the prosolanopyrone II precursor, showed toxicity to chickpea. Both the mutants showed normal growth patterns and conidiation in culture. Virulence of the toxin-deficient mutants was examined in pathogenicity assay using two-week old chickpea seedlings. Both *sol*4 and *sol*5 mutants showed no reduction in virulence, producing disease similar to the disease levels produced by wild-type strain. These findings indicate that *sol*4 gene is a positive regulator for solanapyrone biosynthesis, and that solanapyrone is not essential for the chickpea seedling infection.

The chemical communication between *Bruchus rufimanus* and its host-plant *Vicia faba*

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The Broad Bean Weevil (BBW) (*Bruchus rufimanus* Boheman) is a serious pest of the broad bean (*Vicia faba* Linnaeus) in Europe damaging the beans and decreasing the commercial value. Little is known on the biology and ecology of the pest. The BBW host-plant selection and colonisation was studied in France under field and laboratory conditions. In the field we observed that the BBW males arrived first during the flowering stage closely followed by the females. The colonizers were sexually mature and active BBW. Under laboratory condition, in a wind tunnel, the flowering stage of the bean revealed to significantly more attractive to females than the leave stage. The volatiles from bean plants headspace were collected at leaf, flowering stage of the bean plants to characterize the chemical signal emitted and perceived by the colonising BBW.

Development of genotypes tolerant to heat stress and for photoperiod insensitivity in Faba bean

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Faba bean was originated from the Middle East with the beginning to agriculture and distributed worldwide making specific adaptation to different ecosystems with different temperatures. It is cultivated worldwide in environments with temperatures varied from -15 to +37 degrees C during cropping seasons. Due to climate change and temperature increases, the crop is frequently exposed to higher temperature causing more heat and drought stresses. A set of 200 genotypes representing different geographical regions were evaluated in ICARDA-Terbol (Lebanon) station during the summer season for four consecutive years (2008-2011) under maximum temperature varied from 37 to 42 degrees Celsius. Recurrent selection technique was applied to identify single plants adapted to temperature above 37 during flowering and podding set with photoperiod insensitivity. 470 single plant progenies were retested in augmented design with repetitive checks in summer 2012 in Terbol-Lebanon. The lines were scored from 1 (healthy genotypes) to 9 (plants were killed). Data on days to flowering varied from 45 days to 60 days, days to maturity from 119 to 130 days and 2 genotypes with high dormancy were germinated after 60 days of planting. Our results indicate that 90 selected lines reached the maturity with good pod filling with score from 1 to 3. The genotypes identified will be multiplied and send to NARS for testing their adaptability to different heat prone areas.

Salt content of the medium and *in vitro* shoot multiplication in pea

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In vitro technologies are integral part of using biotechnological methods in pea (*Pisum sativum* L.) breeding. In our experiments the effect of different salt contents were tested on shoot multiplication, number of nodes, callus development and shoot length (mm) of eight cultivars ('Baccara', 'Celica', 'Erbi', 'Graphis', 'Hanka', 'Hunor', 'Izabel', 'Janus'). Micro and macro elements of Gamborg and Murashige-Skoog media were used in four combinations (Gamborg, MS, MS macro + Gamborg micro, Gamborg macro + MS micro) and S media (S macro + Heller micro) was also tested. 0.3 mg l^{-1} naphthalane-acetic acid, 0.5 mg l^{-1} benzyladenine were also added to media. Nodal segments of shoots were placed on medium and cultured for 4 weeks at 22 °C, and 16h photoperiod. Slight differences in the multiplication rate could be detected between media and varieties. The longest shoots developed on S – Heller media in 'Janus', while mostly MS medium resulted in the longest shoot in other varieties. Low rate of callus development was detected on MS macro–Gamborg micro medium. Number of shoots was the highest on MS medium in five varieties.

Salt stress response in three pea species (*Pisum arvense, P. sativum and P. fulvum*) *in vitro* cultures

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Salinity is one of the major abiotic factors limiting plants productivity. Processes, such as seed germination, seedling growth and vigor, vegetative growth, flowering, and fruit set, are adversely affected by high saltconcentrations, ultimately causing diminished economic yield and also quality of products. Shoots of micropropagated *Pisum fulvum, P. sativum* and *P. arvense* were treated with 75, 120, 150 and 200 mMNaClin order to study pea tissues capacity to tolerate salt stress and their antioxidant capacity. We used the phenol content and DPPH antioxidant assay as oxidative damage indicators. Results showed that salt stress caused slower plant growth, leading to an increase in lipid peroxidation and a decrease in total chlorophyll in pea tissues. Further, results showed that *P. fulvum* is to be considered as potentially less sensitive to high NaCl concentrations in comparison to *P. sativum* and *P. arvense* and could represents a gene pool which could be used for improving the characteristics of field pea.

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Induced inaccessibility in pea to powdery mildew: insights gained from the use of inhibitors

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The term induced inaccessibility has been adopted to describe situations where early failed attacks by biotrophic pathogens to plant cells increase resistance to later attacks in that and neighboring cells. This kind of resistance widely relay in penetration resistance events however the particular cellular and molecular bases remain unknown. In the present study, we investigated the role of *de novo* protein formation and cytoskeleton assembly on induced inaccessibility of pea (*Pisum sativum*) cells. For this aim, we sequentially inoculated pea leaves of the susceptible cv. Mesire, with an inappropriate powdery mildew species (*Blumeria graminis*f. sp. *avenae*), which causes a non-host interaction and then with pea powdery mildew (*Erysiphe pisi*). The epidermal pea cells in which the inappropriate powdery mildew failed to penetrate and their neighboring cells showed inaccessibility to subsequent infections by the pea powdery mildew. However, when the pea plants were treated with cytochalasin, an specific inhibitor of actin polymerization and cytoesqueleton formation, or ciclohexImide, a protein synthesis inhibitor, the inaccessibility were greatly repressed. Microscopic and proteomics approaches are being carried out to understand how this events influence the inaccessibility in pea.

Effect of endogenous signals on regulation mechanism of synthesis and accumulation of flavonoids in yellow lupine infected by *Fusarium oxysporum* and its impact on the cytoskeleton of plant cells

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Flavonoids are an important group of secondary plant metabolites with many and diverse key functions in plants. They are known as agents of defense against pathogens, with antimicrobial and antioxidant functions. Recently, they have also been shown to function as intracellular signal molecules. The first aim of the study was to examine cross-talk interactions of sucrose and infection caused by *Fusarium oxysporum* f.sp. *lupini* on expression of phenylpropanoid pathway genes and precisely the flavonoid biosynthesis pathway. Additionally, the accumulation and cytochemical localization of end products, i.e. flavonoids, were analyzed in embryo axes cells of *Lupinus luteus* L. cv. Juno. Moreover, it was checked whether the accumulation of flavonoids, especially genistein affects on the changes in the cytoskeleton of cells. The second aim of the study was to examine cross-talk of exogenous nitric oxide (NO) and sucrose in the mechanisms of synthesis and accumulation of isoflavonoids in yellow lupine embryo axes. It was verified whether the dialogue of these molecules can modulate defence response of axes to infection and development of the pathogenic fungus *F. oxysporum.* The experimental protocol used in this study was based on a model system: embryo axes from germinating yellow lupine seeds inoculated with *Fusarium oxysporum* f.sp. *lupini* and cultured in vitro on a medium with or without sucrose, analyzed to 96 h after inoculation. The obtained results show that sucrose as signal molecule stimulated phenylpropanoid metabolism. Sucrose strongly stimulated the expression of the flavonoid biosynthetic genes, i.e. phenylalanine ammonialyase (PAL), chalcone synthase (CHS), chalcone isomerase (CHI) and isoflavone synthase (IFS). The sensing of carbohydrate levels and the response to fungal pathogens may be interrelated at some level. In the early phase of infection the flavonoid biosynthesis pathway is considerably enhanced in yellow lupine embryo axes as a strong signal amplification effect of sucrose and the pathogenic fungus *F. oxysporum.* Moreover the strong accumulation of isoflavonoids in inoculated *F. oxysporum* embryo axes of yellow lupine pretreated with a nitric oxide donor with a high level of sucrose is a result of amplification of the signal coming from sucrose, the nitric oxide donor and the pathogenic fungus. Sucrose and nitric oxide act as signal molecules, which correlated with up-regulation of genes engaged in isoflavonoids production.

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Response of dry bean (*Phaseolus vulgaris* L.) genotypes to water shortage

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This research was conducted to determine optimum timing and frequency of common bean irrigation in Konya. Field trial was conducted according to "Randomized Complete Blocks Design" with three replications in Selcuk University, Agricultural Faculty, Campus-Konya trial fields during the year of 2009. Sowings were made on 15th of May and harvest was made on September. After sowing, the plots were irrigated by sprinkler for only two hours to provide the emergence. A total of 41 common dry bean genotypes were grown in field conditions with only 2 drip irrigations during the flowering (50th day after sowing) and pod filling (58th day after sowing) periods for 6 hours (from 0am to 6am) per irrigation which was provided enough water to effective root depth. Some agronomical characteristics were determined on behalf of plant response to limited water. According to the results, number of main branch per plant was significant on the level of $P<0,05$ and, all the other investigated characteristics were significant on the level of $P<0,01$. Means of the investigated characteristics were ranged as following: number of main branch/plant 3,33-7,33; number of leave/plant 16-108; plant height 45cm-162cm; number of pod/plant 12-26; number of seed/pod 3,0-5,8; first pod height 3,56cm-6,67cm; biologic yield 212kg da^{-1}-604kg da^{-1}; seed yield 114kg da^{-1}-355kg da^{-1}; harvest index 46%-90% respectively. The results implicated that all the investigated characteristics were in parallel with previous studies. It can be concluded that the timing and method of irrigation is more effective than making irrigation in a random period and excessive water.

Molecular characterization of pea powdery mildew *er1* resistance

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The powdery mildew disease affects several crops and is also one of the major threats for pea (*Pisum sativum* L.) cultivation worldwide. The gene *er1*, first described over sixty years ago, is the most used powdery mildew resistance source in pea breeding. We recently used an homology-based cloning approach to demonstrate that, similarly to other recessive powdery mildew resistance sources identifies in barley and tomato, *er1* resistance is caused by loss-of-function mutations of a homolog belonging to the *MLO* gene family, namely *PsMLO1*. Five different loss-of-function mutations in the *PsMLO1* gene sequence have been characterized in pea germplasm, and we exploited this information to develop new molecular markers suitable for *er1* assisted selection. These markers are built on polymorphisms directly responsible for the resistant phenotype, and can therefore be referred to as functional markers. Taken together, they overcome many limitations associated to previously reported *er1*-linked markers. In general, our studies suggest the possibility to use reverse genetics approaches targeting *MLO* homologs to introduce broad-spectrum resistance across crop species affected by the powdery mildew disease, among which several cultivated legumes.

Relations among leaf and stomatal characteristics and drought tolerance indices in cowpea under rainfed and irrigated conditions

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In this study, leaf characteristics, stomata size and density, mean productivity, geometric mean productivity, stress susceptibility index, stress tolerance index, tolerance, yield index and yield stability index were determined in cowpea (*Vigna unguiculata* (L.) Walp.) cvs. Karagoz-86 and Akkiz-86 under rainfed (non-watered) and irrigated (well-watered) conditions. Field trials were carried out at the experiment field of Faculty of Agriculture, Ondokuz Mayis University of Samsun (41.3°N longitude, 36.3°E altitude, 150 m above sea level), Turkey, in 2005 and 2006 years. Leaf fresh and dry weight, number of leaves, leaf and leaflet length and width, the number of stomata, stomata length and width were determined. Measurements were made on randomly selected 20 stomata by preparing slides from the clear nail polish impression on both lower and upper epidermis of the leaflets using a scaled eyepiece of microscope. Stomata were counted and measured using a 10x20 magnification. Drought indices were estimated for each genotype based on seed yield under stress and non-stress environment. Correlations among leaf characteristics, stomata size and density, and drought tolerance indices under rainfed and irrigated conditions were determined.

In silico identification and annotation of some stress responsive genes in peanut ESTs

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Peanut (*Arachis hypogea* L.) is an internationally important crop for human consumption as a good source of protein and vegetable oil. Peanut is widely cultivated around the world in tropical, sub-tropical and warm temperate climate. Because of its huge genome size (2.8 Gb) and un-sequenced genome, studies on genomics and genetic modification of peanut are less as compared to other model crops. As peanut can be cultivated in arid and semi-arid regions, and its growth is drastically affected by various stresses that reduces the yield. Therefore, study on stress responsive genes and its regulation are very much important. Here we report about the identification and annotation of some stress responsive candidate genes using peanut ESTs. The selection of genes was based on the publically available expression data. Due to good expression data and lack of available literature in peanut some of the stress responsive genes were screened. Individual EST of the said group were further searched in peanut ESTs (1, 78,490 whole EST sequences) using computational approach. Various tools like Vec-Screen, Repeat Masker, EST-Trimmer, DNA Baser and WISE2 are being used for stress responsive gene identification and annotation. Research progress made towards contigs assembly, determination of biological function of genes, and prediction of domain as well as 3D structure for related proteins will be presented.

Spatial and temporal relationships between pea aphids (*Acyrthosiphon pisum* Harris, 1776) and their natural enemies in the course of field peas (*Pisum sativum* L.) occupation

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Spatial distributions of pea aphid (*Acyrthospihon pisum*) and its natural enemies in field peas (assessed in large field trials in the Czech Republic during 2008 - 2012, project NAZV: QH82027) were influenced by the way of growing the crop. In field pea monocultures the aphids tended to aggregate into clusters significantly more (Spatial Analysis by Distance IndEces was used; SADIEShell version 1.22). Especially that was well apparent when insecticides were applied into the crop some time (mostly 14 days) before the beginning of pea aphid fly activity. There was easy to localize patch clusters (they were indicated by the values of aggregation Indices I_a and J_a, $p < 0,05$), usually more than one per field, with differently large areas and with different distances one another. In patch clusters (focuses) the infestation of plants were increasing intensively in time and the infestation extended across the whole crop from them. The distributions of aphids in field peas intercropping with spring cereals were more uniform (Indices of aggregation, $I_{a,}$ were usually smaller than unity), aphids had weaker tendency to create significant clusters and clear focuses of infestation in the crop and their population growth were less dramatic. The distributions of pea aphids and their natural enemies (especially Syrphid´s larvae and other predators and parasitoid *A. ervi*) were significantly associated only in field peas intercropped with spring cereals. Field pea growing in monocultures and also using of insecticides had clear negative effects on spatial and temporal associations (expressed by Indices of associations I_m) between distributions of pea aphids and their natural enemies in crops. Pea aphid´s predators and parasitoids were able to find their host significantly earlier (temporal view) and more effectively (spatial view) in field peas intercropping with spring cereals compared to field peas monocultures (with or without insecticides). That fact explains earlier decline of pea aphid populations in ecological mixtures compared to monocultures.

Inheritance of rust resistance in lentil (*Lens culinaris*)

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Lentil (*Lens culinaris* L. Medikus) is an important cool season grain legume of South Asia with 34 and 46% share in global production and area. In India alone, it is grown on 1.46 m ha area with 0.99 Mt production. Lentil rust caused by *Uromyces viciae-fabae* is a widespread disease in North India causing 30-60% yield losses. There are contradictory reports on number (mono or digenic) and nature (dominant or recessive) of rust resistance gene(s). The aim of the present study is to work out the genetics of rust resistance. A widely adapted rust susceptible Indian cultivar, L9-12, was crossed as female with a rust resistant advance breeding line, FLIP2004-7L, from ICARDA, Syria. The F_1, F_2 and F_6 RILs were grown at Gurdaspur (a hot-spot for rust) to screen for rust reaction under natural field conditions. Data on reaction to rust were recorded on 1-9 rating scale, where 1=highly resistant and 9=highly susceptible. The plants/progenies showing ≤ 3 score were categorized as resistant and those showing >3 were categorized as susceptible. All the F_1 plants gave susceptible reaction to rust, while out of 169 F_2 plants, 120 were susceptible and 49 were resistant and thus gave good fit to the 3:1 ratio (χ^2=1.437). These results showed that resistance to rust is controlled by a single recessive gene. These results were further corroborated with segregation pattern in RIL population derived from the same cross. Out of 137 RILs, 78 were susceptible and 59 were resistant and gave a good fit to the 1:1 ratio (χ^2=2.635). The results of the present study suggest that inheritance of rust resistance in lentil is simple and controlled by a single recessive gene, and can be easily incorporated into locally adapted high yielding elite lines through targeted hybridization program.

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Analysis of *eIF4E* gene sconfering resistance to *Pea seed-borne mosaic virus* (PSbMV) and other potyviruses in pea

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Pea seed-borne mosaic virus (PSbMV) belongs among the most frequent viral pathogens causing severe losses in field pea and other legumes. These losses might be prevented by growth of resistant varieties. Resistance to the common P1 strain of PSbMV isconferred by a single recessive gene (*eIF4E*), localized on LG VI (*sbm-1* locus), while to lentil L1 strainis localized on LGII at*sbm-2*locus. Gene-specific single nucleotide polymorphism and co-dominant amplicon length polymorphism markers were developed. From the comparison of genotype verses phenotype for selection of resistance/susceptibility in F_2 plants, there was a 26% discrepancy between the PCR and ELISA-based assays with potentially susceptible heterozygote plants missed. We used qRT-PCR to analyze expression of both resistant and sensitive *eIF4E* alleles in heterozygotes, sensitive and resistant homozygotes after PSbMV infection, together with evaluation of viral concentration. The novel *eIF4E* alleles were detected in wider pea germplasm and TILLING mutants and these were virologically tested. We have analyzed *eIF4E* allelic variation in over 2000 accesions within geographical context. Furthemore, analysis of second *sbm-2* locus with *eIF4E iso* and *eIF4G* genes as candidate targets, will be presented. It can be expected that information gain in this study might be extended to other legume species, particularly to economically important species of tribus *Fabeae* or even to subfamily *Papilionoideae* such as chickpea, lentil, faba bean or soybean where Potyviruses cause problems.

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Results of *in vitro* selection of pea (*Pisum sativum* L.) for drought-resistance

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The purpose of researches was the development of *in vitro* system for selection of drought-resistant variants of pea. Results of our experiments showed that the selective system with polyethylene glycol (PEG-6000) in concentration of 15-20% can be used for selection of osmotic-resistant callus lines. Ability of pea callus to regenerate shoots after subculturing on selective media was shown. It was established, that efficiency of shoot regeneration depended on an initial genotype and PEG concentration in selective media. There was successfully obtained rooted pea regenerants on a nutrient medium containing auxin. Generation R_2 … R_3 obtained as a result of selection on medium with PEG, has been studied in vegetative experiment with artificial drought and in field trials. Ability to keep water during fading is one of the basic parameters of drought resistance of plants. Regenerated lines, selected in rigid selective loading, have shown the superiority over an initial cultivar on water-keeping ability. Thus the share of a working adsorbing surface of root system increased and reduction of the stipules area was observed. The comparative analysis of yield components has shown, that the majority of regenerated lines have significantly exceeded an initial cultivar in many studied parameters. Regenerated lines have appeared to be long-stem and late-ripening. Number of productive nodes significantly depended on environmental conditions and reduced in 1,5-2 times in adverse year. Regenerated lines had higher number of productive nodes and pods on a plant in comparison with initial cultivars.

Trichoderma **spp. isolates growth promotion activity on soybean seeds and their antagonistic effect on pathogen *Sclerotinia sclerotiorum***

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Trichoderma species have been registered as species with important growth promotion potential as well as antagonistic effect on different pathogens such as *Sclerotinia sclerotiorum*, *S. minor*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium*, *Alternaria, Leptosphaeria*, *Pythium*, *Phytophtora* and *Verticillium* species. *Trichoderma* isolates tested in this research were isolated from different soil types from Vojvodina region (Serbia) and refined to single-spore isolates used for further research. Dual culture test was used for screening of *Trichoderma* isolates' antagonistic effect on *Sclerotinia sclerotiorum in vitro*. All isolates had high Radial Growth Inhibition factor (RGI) of pathogen (34.4 – 52.2%) and high colonization percentage (46.7 – 100%) as well. Because of their good antagonistic effect, possible growth promotion activity of *Trichoderma* isolates on soybean seeds were tested under glasshouse conditions. Soybean seeds were coated with suspensions of different *Trichoderma* isolates, while sterile distilled water was used as a control. Seed germination percentages and root and shoots length were measured and data were analyzed in Statistica using Duncan's test. There were no significant effects on shoots length among all tested isolates comparing to control. Among tested *Trichoderma* isolates, the three showed significant positive effect on both root length and germination, while two isolates exhibited no significant effect on both measured parameters. The rest of tested isolates showed significant positive effect only on root length or germination. Isolates with highest growth promoting potential and antagonistic effect will be included in further more comprehensive research.

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Phomopsis longicolla **β conidia in naturally infected soybean**

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P. longicolla is primarily known as a soybean seedborne pathogen but it can be isolated from all parts of the plant. Atypical symptoms have been frequently noted on the basal parts of soybean stems of mature plants: slightly sunken lesions with irregular shapes and sizes, bordered by a thin black margin with diffusely distributed pycnidia with α and β conidia. Initially, it was believed that the causal agent was the fungus *P. sojae*, however morphological characteristics of the isolates suggested that the causal agent was not *P. sojae*. This study was an attempt to prove that, under certain conditions, *P. longicolla* produces masses of β conidia. Beta conidia were observed for quite some time in the field, but they were believed to belong to *P. sojae*, which typically forms this type of conidia. However, after isolation from infected plant parts, the fungus formed colonies and reproductive organs which were identical to the morphological characteristics of *P. longicolla* described by Hobbs et al. (1985). In other words, we found evidence that the isolated fungus was *P. longicolla*. We obtained the same results when several monosporic isolates were separated from pycnidia that contained a high proportion of β conidia. Since morphological characteristics are not a reliable indicator for the identification of *Diaporthe/Phomopsis* species, the final confirmation was obtained by a molecular analysis. The sequencing of three different regions of DNA clearly indicated that the PDS isolates belonged to the species *P. longicolla*. Monosporic isolates, exhibited a high level of pathogenicity on soybean seeds and plants, after artificial inoculation.

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Identification of resistant sources for dry root rot in chickpea (*Cicer arietinum* L.)

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Chickpea is one of the most important pulse crops of India which contains high quality easily digestible protein (25 per cent), making it an important source of protein for the vegetarians of the country. Being the larger producer and consumer, India is still importing Chickpea as the productivity is far below its potentiality. Many biotic and abiotic stresses are limiting the productivity of Chickpea. Dry root rot caused by *Rhizoctonia bataticola* is emerging as a serious disease and causing 30-40 per cent yield loss among various diseases caused by more than 100 pathogens which attack Chickpea. Although found in all Chickpea growing areas of India, it is most severe in Central and South India, where the crop is grown under rain fed conditions. High day temperature (30°C) and dry soil conditions at flowering and podding stage rapidly increase the severity of the disease. The reaction of 520 Chickpea genotypes was evaluated under laboratory condition using Blotter Paper Technique with artificial inoculation of the disease to identify the resistant source for the disease. Among 520 genotypes, three were found to be resistant *viz.*, PG 06102, BG 2094 and IC 552137. Twenty one genotypes showed moderate resistance reaction and rest were susceptible for dry root rot in Chickpea. The identified resistant genotypes may serve as potential donors for the development of resistant varieties for dry root rot in Chickpea.

Identification of resistance sources for mosaic virus disease in cowpea [*Vigna unguiculata* (L.) Walp]

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Cowpea [*Vigna unguiculata* (L.) Walp] is one of the most important, multi season and multipurpose grain legume grown in the tropics and subtropics. This leguminous food crop is greatly affected by wide array of diseases of biological origin especially viruses which cause devastating effects and become real constraints to increase the yield of cowpea in several countries. The use of resistance gene provides an effective and economic solution for viral diseases. Thus, identification of resistance source for the disease is very important for development of resistant high yielding varieties. In the present investigation, 196 genotypes of cowpea were screened in the field condition at Zonal Agriculture Research Station, University of Agricultural Science, Bangalore to identify the resistant source for cowpea mosaic virus. The disease incidence of mosaic virus was scored after 4-5 weeks of sowing at weekly intervals. Twenty three genotypes showed highly resistance reaction. 45, 30, 20, 35 and 78 genotypes showed resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible reaction respectively. Further 196 genotypes were screened in glass house condition by sap inoculation of cowpea mosaic virus. Only two genotypes *viz.*, V 578-17 and HC 03 02 were highly resistant. 9, 12, 5, 12 and 156 genotypes showed resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible reaction respectively. The identified resistant genotypes can be used as potential donors for the development of resistant varieties for Mosaic Virus disease in Cowpea.

The biology and ecology of *Bruchus rufimanus* (bean seed beetle) – monitoring and distribution of the pest across the UK

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The bean seed beetle, *Bruchus rufimanus* (Boheman), is an economically important pest of bean crops throughout Europe and has become a serious pest in the UK. Its principal hosts are spring and winter sown field beans, *Vicia faba* var. minor and broad beans, *Vicia faba* var. major. It is suspected that its spread across Europe is a consequence of increasing average temperature, possibly due to climate change. The bean seed beetle is the primary cause of quality problems in UK produce. It is the only bruchid pest endemic in the UK, this being the most northerly latitude at which it is able to survive and reproduce. All of the damage is caused during the growing season and no further damage is sustained during storage. As a result of increasing levels of damage in produce over the last twenty years, research has focused on investigating the biology of the pest and methods of monitoring and controlling activity. Monitoring has allowed a unique opportunity to map distribution across the UK. Unlike many other pests it has been possible to map presence and intensity in all regions using data from laboratory tests carried out by one of the largest grain merchants in the UK. The importance of collecting these data is emphasised by recent movement of the pest north, possibly caused by increased average temperatures. Temperature is a critical factor in the reproductive development of the female adult beetle, oviposition starting only when temperatures reach 20°C.

The most important diseases in forage legumes in Serbia

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Lucerne (*Medicago sativa* L.), red and white clover (*Trifolium pratense* L., *T. repens* L.), field pea (*Pisum sativum* L.), common and hungarian vetch (*Vicia sativa* L., *V. pannonica* Crantz.) are the most important forage legumes in Serbia. They are used for making hay, silage, green forage and dried grain of field pea (like supplement for concentrate use). Areas under the forage legumes in the world are expressed in millions of hectares, which are constantly increasing. Forage legumes have occupied an important place in the agricultural production in Serbia over the last ten years. Diseases appearance is increasing with expansion of legumes cultivated areas. Different pathogens can be hazardous in favorable climatic conditions, and some of them can even appear in epiphytotic ratio. The most frequent diseases which were registered almost every year in Serbia at lucerne, clover, field pea and vetch are: *Pseudopeziza medicaginis* (Common leaf spot), *Uromyces striatus* (Rust of alfalfa), *Fusarium oxysporum* f.sp. *medicaginis* (Fusarium wilt), *Verticillium albo-atrum* (Verticilium wilt), *Alfalfa mosaic virus* (AMV), *Pseudopeziza trifolii* (Common leaf spot), *Erysiphe trifolii* (Powdery mildew), *Uromyces trifolii* (Rust), *Cuscuta sp.* (Dodder) *Erysiphe pisi* (Powdery mildew), *Peronospora viciae* (Downy mildew of peas), *Ascochyta pisi* (Ascochyta blight of peas), *Uromyces pisi* (Rust of pea). Good pest management considers: growing resistant varieties, usege of certified seed, good agricultural practices and chemical control of pathogens.

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Session 8

Non-food, non-feed and other alternative legume uses

KEYNOTE LECTURE

Genetic and molecular analysis of sustainable biofuel production from the legume tree Pongamia pinnata

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Pongamia trees are sourced from tropical and subtropical regions of the world (India, Indonesia, northern Australia, and other localities in that general region). They are rich with seeds about 1.5-3.0 gm dry weight after 2 to 4 years. These seeds contain 35-50% plant oil which contains mainly C18:1 oleic acid (50-55%). Pongamia oil is suitable for both biodiesel as well as aviation fuel production. Mature trees with 20,000 seeds are not uncommon. The estimated oil yield per hectare per annum is between 3 to 5 tons. Pongamia, despite its warm weather natural habitat, is very adaptable. They grow, then have a winter dormancy period of about 3-4 months, and yield good seed in central Queensland, where winter night temperatures are -5 to -6 degrees C). They also are deep-rooted and thus very drought-tolerant. Plants grow on salty soil and even next to the ocean in beach environments. Pongamia is outcrossing with a diploid chromosome number of 22. Its genome size is about 600-700 Mbp, based on comparison to soybean chromosomes. Because of the outbreeding the progeny seed are heterogeneous and variable. We have developed ISSR DNA markers to determine genetic diversity. We have initiated multi-faceted analyses of Pongamia to facilitate the domestication of elite mother trees. Deep sequencing of its genome has allowed gene isolation using orthologous soybean genes for fatty acid, nodulation and ureide metabolism. The chloroplast (152 kbp) and mitochondrial (425 kbp) genomes were sequenced, assembled and annotated. Great similarity exists to those organelle genomes from Lotus japonicus. We developed classical clonal propagation procedures by rooted cutting, tissue culture and grafting to evaluate the genetic versus the environmental influence on traits in different parent genotypes. We have Life Cycle Analysis data of Pongamia grown in plantation style for 4 years since seed germination. Above ground biomass exceeds 13 kg per tree with 4 m height trees and 10-15 cm diameter stems. Analysis of nodulation and nitrogen fixation indicated determinate development progressing to lobed nodules with greater age. Parameters of nodulation appear to be similar to those seen in other annual legumes such as soybean. We strongly believe that the use of a high seed number, high oil legume tree is optimal for sustainable production of biofuel and co-products for the future biofuel industry.

The effect of legume cover crops on soil phosphorus availability

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Legume can fix atmospheric N and sequester carbon. Some legumes are able to uptake sparingly soluble soil phosphorus. Additionally, all those effects may contribute to a beneficial increase in soil microbial activity. In this work three ground-cover treatments were imposed to an olive orchard located in NE Portugal: Natural vegetation (Nat veg); Natural vegetation fertilized with 60 kg N ha^{-1} yr^{-1} (Nat veg +N); and a mixture of self-reseeding annual legumes (Legumes). Three years after the establishment of the ground-cover treatments, soil samples were taken from the 0-10 and 10-20 cm depths. Extractable soil P was determined by the Olsen and Egner-Riehm methods, the acid phosphatase activity accordingly to Tabatabai and Bremner and soil organic carbon by the Walkley-Black method. In the 0-10 cm soil layer, the acid phosphatase activity was significantly higher in the treatments Legumes and Nat veg+N (7.81 and 7.30 μg p-Nitrophenol g^{-1}) than in the Nat veg (4.73 μg p-N g^{-1}). Considering both soil layers, there was observed a significant linear relationship between oxidizable organic C and the acid phosphatase activity (R^2=0.69). The P extracted by the Egner-Riehm method was better correlated with the acid phosphatase activity (R^2=0.42) than P extracted by the Olsen method (R^2=0.31) when all samples were taken into account. It seems that a similar effect of the legumes species on the acid phosphate activity may be achieved with the natural vegetation if fertilized with N.

OSCAR – a new European project on cover crops

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There is widespread concern over the damage caused by modern agriculture to soil structure and the ecosystem services provided. One approach to overcome this problem is conservation agriculture (CA) which aims to maintain soil structure by minimising soil disturbance, maximising soil cover and using crop rotation. However, despite recent legislation supporting minimum tillage and direct seeding CA is still practised on less than 4% of the agricultural land in Europe. This underlines the need for major improvements in the approach together with consolidation ofinformation about alternative cropping methods and their biological and economic value and performance.A new European FP7-funded project, OSCAR,is conducted by 22 partners and led by University of Kassel.OSCAR aims to Optimise Subsidiary Crop Application in Rotations.It extends existing knowledgeanddevelops novel cropping systems based on cover crops (CC), catch crops, living mulches (LM) and other subsidiary crops (SC). OSCAR aims to enhance the implementation and increase the duration of soil coverage, introduce diversity to the crop rotation and reduce the intensity of soil tillage. To maximise the potential of ecological benefits, both conventional and conservation agricultural systems are considered, encouraging a high level of innovation and offering a more immediate transfer into practical agriculture. The project encompasses the identification and selection of new SC and the development of adapted farm machinery for the various CC and LM species.OSCAR will enable a substantial number of growers in different regions to adopt CA methods and make a durable contribution to soil conservation.

Annual legumes as green manure crops

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There is a large number of annual legume species having an economic importance either on a world or local scale, such as pea (*Pisum sativum* L.), faba bean (*Vicia faba* L.), lentil (*Lens culinaris* Medik.), grass pea (*Lathyrus sativus* L.) and vetches (*Vicia* spp.). All these crops are multi-functional and are used for both human consumption and in animal feeding. Apart from these various food and feed uses, annual legumes may represent a quality green manure, playing one of the most significant roles in organic farming and sustainable agriculture. In addition to their ability to increase soil fertility by a symbiosis with nitrogen-fixation bacteria, annual legumes are able to produce a considerable amount of aboveground biomass, also rich in nitrogen. For instance, aboveground biomass nitrogen yield in grass pea may surpass 300 kg ha^{-1}, while in lentil it is higher than 100 kg ha^{-1}. If cut in fool bloom, what in most years and in temperate regions occur in May, and incorporated, the aboveground biomass of most annual legumes have a long-term and beneficial effect on the productivity of numerous succeeding crops, such as silage maize (*Zea mays* L.) or sorghum (*Sorghum bicolour* (L.) Moench). Recently, there have been launched specific breeding programmes on annual legumes for their non-food use as green manure, aimed at developing cultivars with high and quality aboveground biomass and its faster degradation when incorporated, due to a decreased proportion of lignin and cellulose.

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Reducing use of fossil energy by biological N fixation

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Biological N fixation can be increased greatly in Finnish agriculture. Intensive use of legumes in grasslands, utilization of green manure and undersown crops, and maximal growing of pulse crops can reduce use of fertilizer N by 60 % compared to current situation. It would save fossil energy more than 3000 TJ per year. The potential of biological N fixation for saving fossil energy was examined through current field area of different crops and farm types. Such restrictions like adequate crop rotation and soil type were taken account. The amount of energy in fertilizer N fabrication was calculated according to the most effective techniques in current factories. The change in energy demand of machines at farms was calculated, too. It seems obvious that increased use of legumes would improve C content and structure of soils. Decreasing production of synthetic fertilizers would diminish greenhouse gas emissions from industry, but effect of different plant materials and cultivation techniques on emissions from agriculture needs research. The study shows that use of synthetically produced fertilizer N, and thus consumption of fossil energy, is possible to be decreased markedly with help of biological N fixation. Motivation of farmers is increased not only by policy making, but also because of concern about soil fertility and desired reduction in use of expensive synthetic fertilizers.

All legumes are beautiful, but some legumes are more beautiful than others

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Apart from their prominent roles as food and feed, legumes have numerous forms of non-food uses, with green manure and biofuel as the most widely present in agriculture and industry. Most legume species are easily recognised for the colour of their flowers and it is rather natural there are legumes cultivated especially for ornamental purposes. One of the most renown decorative legume species is sweet pea (*Lathyrus odoratus* L.), an annual with great diversity of flower size and petal shape. Breeding sweet pea has a tradition long more than a century, especially in UK, resulting in developing a large number of sweet pea cultivars of diverse flower colour, size and shape and fragrance length and intensity. The largest collection of sweet pea in the world was established and is maintained by Roger Parsons, gaining a status of a UK national collection and comprising about 900 cultivars of sweet pea and around 100 taxa of other *Lathyrus* species. Garden lupin (*Lupinus polyphyllus* Lindl.) is a perennial herbaceous species native to North America. The most significant efforts in breeding garden lupin were made by George Russell from UK, developing an ideotype present in majority of the contemporary cultivars. There are other ornamental lupin species, such as Texas Bluebonnet (*Lupinus texensis* Hook.), the favourite of many Texans and one of the state flowers of Texas. Among the woody perennial legumes, grown as ornamentals, it is noteworthy to mention Japanese wisteria (*Wisteria floribunda* (Willd.) DC.), a liana species, as well as deciduous trees, such as Judas tree (*Cercis siliquastrum* L.), honey locust (*Gleditsia triacanthos* L.), golden-chaintree (*Laburnum*×*watereri* (G. Kirchn.) Dippel) and Pagoda Tree (*Styphnolobium japonicum* (L.) Schott).

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The effect of legume cover crops grown in an olive orchard on soil microbial activity

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Microbial biomass and soil respiration are major indicators of the soil biological fertility which can be affected by soil management practices. The aim of this study was the evaluation of the effect of three ground-cover treatments, imposed to an olive orchard, on soil microbial biomass and activity. The ground-cover treatments were: Natural vegetation (Nat veg); Natural vegetation fertilized with 60 kg N ha^{-1} (Nat veg +N); and a mixture of eleven self-reseeding annual legumes (Legumes) grown as a cover crop. The experiment was carried out in a rainfed olive orchard located in Mirandela, NE Portugal. Three years after the experimental set-up had been installed, random samples of soil were collected in two depths, 0-10 cm and 10-20 cm. The microbial biomass C (Cmic) was determined by the fumigation-extraction method; the basal respiration (BR) as the rate of CO_2 evolved in a 7 days soil incubation at 25 ºC; and soil organic C (Corg) by the Walkley-Black method. The Cmic:Corg ratio and the metabolic quotient (qCO_2), defined as the respiration rate per unit of biomass, were thereafter estimated. The values of Cmic and BR were significantly higher under the Legumes treatment in comparison with Nat veg +N and Nat veg. It seems that Legumes increased the C stored in the soil and promoted soil microbial activity, two soil quality factors usually related to the sustainability of the agrosystems.

Tree nutritional status and olive yield after three years of the cultivation of legume species as a cover crop in an olive orchard

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Growing self-reseeding annual legumes in olive orchards may increase the sustainability of the cropping system by increasing soil organic carbon and reducing the use of nitrogen fertilizers. In this work, results are presented on the nitrogen nutritional status of trees and olive yields three years after the introduction of different ground-cover treatments in an olive orchard located in NE Portugal. The experimental design included three ground-cover treatments: Natural vegetation (Nat veg); Natural vegetation fertilized with 60 kg N ha^{-1} yr^{-1} (Nat vet +N); and a mixture of eleven self-reseeding annual legumes (Legumes). The experiment started in October 2009. The nutritional status of olive trees was assessed by leaf analysis, collecting leaves in July 2012 and January 2013 and determining the concentration of nutrients in leaves. In November 2012, the olive yields were recorded from eight pre-tagged trees per treatment. Nitrogen concentration in leaves and olive yields were significantly different among the ground-cover treatments. In July 2012, leaf N concentrations were 10.8, 11.9 and 14.1 g kg^{-1}, respectively in the treatments Nat veg, Nat veg +N and Legumes. In January 2013, the values were 12.6, 15.6 and 15.7 g kg^{-1}. The olive yields were 2.9, 5.6 and 8.6 kg tree^{-1}, respectively in Nat veg, Nat veg +N and Legumes. Three years after the establishment of the ground-cover treatments, the olive trees of the Legumes treatment showed a better nitrogen nutritional status and a higher productivity than those managed with natural vegetation even when they were fertilized with 60 kg N ha^{-1}.

Session 9

Understanding and enhancing the legume cropping environment

KEYNOTE LECTURE

Understanding and enhancing the legume cropping environment

CA Watson[1], M Reckling[2], A Bues[2], T Kuhlman[3], K Lindström[4], D Murphy-Bokern[5], S Preißel[2], CFE Topp[1], P Zander[2], FL Stoddard[4]

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Legumes in farming systems provide a number of functions, not only the obvious ones associated with food or feed production. Grain legumes are grown as components of crop rotations, often providing a "break" from pests and diseases as well as supplying nitrogen to the following crop. They can be grown as either as sole crops or intercropped with cereals. Forage legumes can be grown as components of temporary pastures (leys) or as more permanent grassland. Apart from for seed, the majority of forage legumes are grown in mixtures with grasses, other legumes and forbs. In many cases more than one variety of a legume will be grown within the mixture to provide the desired forage characteristics. From a systems perspective it is important to understand the agronomic challenges associated with legume production and identify solutions to the factors that affect both yield and quality. These include a wide variety of issues from crop nutrition to weed control as well as issues associated with grazing management. However, legumes also have a number of impacts on both resource use efficiency and environmental impact not only during the production period but also on following crops. This paper will explore the agronomic and environmental issues associated with legume production in farming systems.

Are durum wheat-grain legume and sunflower-soybean intercropping efficient solutions to produce legume in low input systems?

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Intercropping (IC) - growing simultaneously two or more species in the same field - could increase resources use efficiency by interspecific complementarity due to species differences in aerial and root system architecture, in growth dynamics and to legume N_2 fixation. The main objective was to analyse the dynamic functioning of durum wheat-grain legume (pea or faba bean) winter IC and sunflower-soybean summer IC in order to evaluate their potential advantages for global yield, grain quality and N acquisition depending upon cultivars, species and sowing patterns. Experiments have been carried out in SW France : i) durum wheat-grain legume IC at INRA Toulouse since 2005 using a wide range of IC combinations (varieties and N treatments) and ii) sunflower-soybean IC at CETIOM and INRA in 2010-2011 with cultivars showing various precocities and differing row patterns. Our work demonstrates that IC is particularly suited to low N input systems (no or low amounts of N fertilizer) due to the complementary use of N sources for both winter and summer intercrops. Cereal-grain legumes IC grain yield was higher than the SC mean value and wheat grain protein content was improved. For sunflower-soybean IC, 2 rows of the earlier sunflower combined with 4 rows of the later soybean was the more efficient pattern. IC is also efficient to produce legume, which is sometimes difficult in SC. However, before optimising these systems, further research is still needed to analyse the effects of precocity, aerial architecture, row structure and N management.

Efficiency of weed control methods in sugarsnap peas cultivation

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Weed infestation of the plantation of sugarsnap peas is one of the main factors contributing to yield decrease and difficulties in plant harvesting. Therefore the goal of the research was to specify the influence of the selected ways of weed control on yield and on limiting the phytocenosis factors of sugarsnap peas field. Strict field research was conducted in years 2009-2011 in the south eastern region of Poland, at the Research Station in Krasne (50°03'N, 22°06'E) of Rzeszów University in Rzeszow. The variables were as follows: (I) varieties of sugarsnap peas - Sokolik i Milwa and (II) ways of weed control: control without plant protection, mechanical with harrowing (once after sowing, twice after sprouting), mechanical and chemical (three times harrowing as above + after sprouting MCPB - 2,0 l/ha, chemical (after sowing chlomazon 0,2 l/ha + linuron - 1,0 l/ha, after sprouting bentazon - 2,0 l/ha), chemical with adjuvant (after sowing as above, after sprouting bentazon - 1,5 l/ha + adjuvant - 1,5 l/ha). Used methods of weed control resulted in considerable changes in weed communities of sugarnip peas varieties. After their application the number of varieties of weeds decreased 10,5–50,1% in comparison with the control sample, the number of weeds decreased 70,9–87,5% and their air dry weight decreased 38,9–62,4%. The widest spectrum of weed control, including dominating varieties *Chenopodium album, Viola arvensis*, *Fallopia convolvulus,* on the fields with both Sokolik and Milwa varieties, were noticed on the fields where chemical and chemical with reduced dose of bentazon with adjuvant regulations were applied. No considerable differences were notices in yields of sugarsnap peas between chemical regulation and chemical regulation with adjuvant. The weakest results as far as yields and weed limitation were concerned were obtained on control fields and on fields with mechanical harrowing.

Yield stability in varietal mixtures of faba bean, *Vicia faba*

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Spatial and temporal variation in growth and N_2 fixation poses problems for the sustainability of legume cropping systems. In varietal mixtures, complementary tolerance to biotic and/or abiotic stress may lead to higher yield stability, while heterogeneity in chemical composition may be a potential disadvantage for the quality of harvested products. To investigate the potential benefits of legume varietal mixtures, three varieties of faba bean, *Vicia faba*, were cultivated in field as single varieties and in two- and three-varietal mixtures. The field experiments were replicated at three locations during two years. Harvest of whole-crop forage was performed in parts of the experimental plots at pod-fill, and grain harvest was performed at full maturity. Samples from both harvest occasions were used for analyses of product quality and N_2 fixation. Occurrence of the fungal pathogen *Botrytis fabae*, causing chocolate spot disease, was estimated by repeated visual grading in the field plots and complemented by DNA-based quantification of *B. fabae* infections in sampled leaves. Several of the varietal mixtures had as high grain yields as the highest-yielding single varieties, and spatial variations in yield were often lower in varietal mixtures. One of the highest-yielding single varieties also showed high occurrence of chocolate spot disease. Combining this apparently disease-sensitive variety with other varieties reduced the disease occurrence without compromising the high yield level. This holistic study highlights the benefits that cropping systems diversification can provide as a strategy to prevent disease outbreaks and improve yield stability in legumes.

Lucerne and clover in pure stand or in association with ryegrasses under intensive conditions in Flanders

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Monocultures of red (*Trifolium pratense* L) and white clover (*Trifolium repens* L.), lucerne, perennial (*Lolium perenne*L.) and Italian ryegrass (*Lolium multiflorum* Lam.) and grass/legume mixtures were compared at 3 levels of chemical N fertilisation: 0, 105 and 265 kg N ha^{-1} in order to investigate the effect on botanical composition, dry matter yield, forage quality and nitrate residue in the soil at the end of the growing season. From agricultural point of view, mixtures of ryegrass with clover or lucerne, fertilised with 105 N ha^{-1} were the best in the 4-year period (2004-2007). The levels of nitrate residue in the soil were very acceptable for all the treatments and were the highest for the legume monocultures and the lowest for the grasses.

Developing schemes for intercropping annual legumes

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We established four main principles for mutual intercropping annual legumes: same time of sowing; similar growing habit; similar cutting time; and one component has good standing ability (supporting crop) and another one poor (supported crop). There are three main intercropping groups: (1) 'tall' cool season legumes; (2) 'short' cool season legumes; (3) warm-season annual forage legumes. In the 'tall' cool season group, faba bean and white lupin are supporting crop, while the supported crops are normal-leafed pea common vetch, Hungarian vetch, hairy vetch and grass pea. This may be beneficial for both by weed reduction and preservation of photosynthetically active leaves. The 'short' cool season group comprises semi-leafless pea as supporting crop and normal-leafed, bitter vetch and lentil as supported crops. If intercropped, semi-leafless pea provides improved standing ability of the whole intercrop, while normal-leafed pea, bitter vetch or lentil fill the available space within the stand and contribute to better utilisation of sunlight, at the same time reducing weeds. In the warm-season group, soybean and pigeon pea are supporting crops, while mung bean, adzuki bean, black gram, cowpea and hyacinth bean are supported crops. Soybean may bear cowpea or hyacinth bean stems and thus assist in preserving their leaves and profit from significantly decreased weed infestation.

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Alterations of soybean grain quality by different foliar fertilizers

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Nutritive value of soybean grain could be increased with foliar fertilizers by alteration of mineral components and antioxidants. The trial included application of different organic fertilizers: Zlatno inje, Bioplant Flora, Algaren BZn, Zircon, as well as phytohormone preparation Epin-extra, which were applied at the beginning of flowering (second half of June of 2012) on two soybean varieties, ZPS 015 and Nena. Application of fertilizers caused the increase of average grain yield compared to control up to 17% in Zircon treatment, and enhancement in 1000 seed weight, up to 5% in Zlatno inje treatment. All applied treatments slightly decreased an average Ca, Mn and total glutathione content compared to control, while Epin-extra increased Ca, Cu and oil content. Foliar fertilizer Bioplant Flora brought an increase of Mg, Fe and Zn content, while Algaren BZn contributed to higher level of phenolics and P in soybean grain, as compared to control. Analysed constituents showed greater variation in grain of ZPS 015, as compared to Nena. Higher amount of mineral constituents found in ZPS 015, emphasised its potential as a source of minerals in diets, while the higher contents of glutathione, phenolics and inorganic P in grain of Nena underlined its quality as a source of antioxidants.

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Nutrients status in soybean grain as a result of different intercropping and fertilization regimes

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Different cropping systems and application of diverse fertilizers in ecological production can affect soybean grain composition. From that point of view, experiment with red grain maize variety (Rumenka) and black grain soybean (Dukat) was carried out. Three treatments were applied: single crop (SC), alternating rows of both crops (AR) and alternating strips (3 rows of each species - AS). Fertilization regimes included fertilizers: Ofert (organic fertilizer), Uniker (microbiological fertilizer), urea and control (without fertilization). Both crops achieved the highest grain yield in AR and AS, in Uniker treatment. All applied fertilizer regimes increased mineral nutrient's content, compared with control. Uniker increased average K content, up to 12%, while Ofert increased average contents of Ca, Mg and Zn, up to 9, 6 and 22%, respectively. As opposed to applied organic and microbiological fertilizers, urea increased average contents of Fe, Cu, Mn and P, up to 10, 18, 16 and 4%, respectively. The positive impact of Ofert on soybean grain quality was also evident in increase of average antioxidants content: total phenolics and glutathione, up to 11 and 5%, respectively. Among all cropping systems, AR treatment induced slight increase of grain yield, together with average Ca and K contents. AS treatment showed positive effect on Mg, Zn and P accumulation, as well as increase of total phenolics and glutathione. On the other hand, SC was more effective for Fe, Cu and Mn accumulation. Preliminary results of tested cropping systems, which could enable better utilization of space and nutrients, indicated variations in soybean grain quality.

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Response of three varieties of peanut (*Arachis hypogaea*) to the inoculation with Rhizobium and the stress saline

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The performance of three peanut cultivars inoculated with two different rhizobial strains, which differ in their salt tolerance, was evaluated under saline water irrigation and compared with that of nitrogen-fertilized plants. Under non-saline conditions, higher yields were obtained using nitrogen fertilization rather than inoculation for all the varieties tested. However, under salt stress, the yield of inoculated plants became comparable to that of nitrogen-fertilized plants. Thus, nitrogen fixation might represent an economical, competitive and environmentally-friendly choice with respect to mineral nitrogen fertilization for peanut cultivation under moderate saline conditions.

Forage yield in autumn-sown intercrops of annual legumes and brassicas

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A small-plot trial has been carried out in the trial years of 2010/2011 and 2011/2012 at the Experimental Field of the Institute of Field and Vegetable Crops at Rimski Šančevi. It included eight intercrops of autumn-sown brassicas with autumn-sown annual legumes. Two brassicas, fodder kale (*Brassica oleracea* L. var. *viridis* L.) cv. Perast and rapeseed (*Brassica napus* L. var. *napus*) cv. Zorica were supporting crops for four legumes, pea (*Pisum sativum* L.) cv. NS Krmni, common vetch (*Vicia sativa* L.) cv. NS Tisa, Hungarian vetch (*Vicia pannonica* Crantz) cv. Panonka and hairy vetch (*Vicia villosa* Roth) cv. NS Viloza, acting as supported crops. All six cultivars were also sown as sole crops. The highest two-year average individual contribution in the total forage dry matter yield among brassicas was in fodder kale (4.5 t ha^{-1}) when intercropped with Hungarian vetch, while the highest individual contribution in the total forage dry matter yield among legumes was in hairy vetch (6.6 t ha^{-1}) when intercropped with rapeseed. The two-year average values of LER_{FDMY} ranged between 1.05 in the intercrop of fodder kale with common vetch and 1.14 in the intercrop of fodder kale with Hungarian vetch. The autumn-sown intercrops of brassicas with legumes have demonstrated a considerable potential for forage production.

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Influence of sprinkling irrigation on yield and quality of lupine seed and the residual effect on wheat cultivation

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The field experiments were carried out in 2011 - 2012 in Złotniki Experimental Station, near Poznań, as a completely randomized design in four replications. Investigated factors: 1. water variant: irrigated - sprinkling irrigation was applied when the water content in 0 -30 cm soil layer fell to 70 % of field capacity in the period of the greatest susceptibility of plants to water deficit, non irrigated - natural water conditions, 2. species of lupine: yellow lupine, indeterminate variety Mister and narrow-leaved lupine, indeterminate variety Baron. After harvesting lupine were sown winter wheat, which was not wintered so it was replaced by spring wheat. Yield of seed lupine in experiment depending on weather conditions. In 2012 seed yield of lupine was less than in 2011. Average, sprinkling irrigation of yellow lupine decrease of protein content in seed about 1.39% but increase seed yield about 39.1% and protein yield about 69.4%. In narrow lupine increase were observed for protein content (0.65%) and for seed yield (64%) and protein yield (66.4%). In condition with natural precipitation the yielding of spring wheat cultivation after yellow lupine and narrow lupine was similar. Sprinkling irrigation increase seed yield of wheat about 1.07 t·ha^{-1} (20.3%).

Challenges for soya bean (*Glycine max*) production in Northern Europe

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Although, pioneer researchers in the Nordic-Baltic countries tried to cultivate soya beans as early as in the 1940's, this crop never became any success. In the early 2000's scientist, seed companies and farmers in Northern Europe restarted the process to introduce soya beans as a part of the modern cropping system. In a recent Swedish field experiment study 2010-2012, we have investigated some of the factors that influence plant development and yield. It is clear that soya beans can be cropped in Sweden even at latitudes as far north as 59 ° (Stockholm region). The marketable yield is typical 2 metric tonnes per hectare. Row distance tend to influence yield in some cases, but it not clear if the investigated row distances (12,5; 25 and 50 cm) are connected to other factors such as seed cultivar or seeding date. One hypothesis is that increased row distance will give a warmer stand, hence an earlier maturity, especially if the rows are seeded in a north-south direction. Seeding date has proven to be quite flexible; from early May to early June. Some cultivars benefits from early seeding while others are more plastic, they will regardless of seeding date result in about same yield. Our experience is that selection of cultivar is the most crucial factor for success in Northern Europe. Triple-zero (000) varieties should be used. However, we experience that some of the 000-varieties on the market behave quite different in Scandinavian latitudes compared to Central Europe or Canada.

Will row distance influence yield of dry yellow peas (*Pisum sativa*) and faba beans (*Vicia faba*) in a North European cropping system?

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In Scandinavia, the most common row distance in grain, oilseed rape and legumes is 12 (also referred to as 12.5) cm. There is a strong tradition that this row distance is the most optimal for the majority of crops. This strong belief has made it difficult to introduce and use seeding technology based on other row distances such as 25 cm, a quite common row distance in grain and legume cropping in Russia, Canada and USA. Seeding systems using 25 cm are often used in dryer geographical areas or in extensive cropping where you expect lower yields (1-4 MT ha^{-1}) compared to more intensively farmed areas such as Northern Europe, with yields up to 8-10 MT ha^{-1}. Sweden is today dominated by seeding machines using 12 (12.5) cm row distance. In an ongoing project 2011-2013, we have compared two seeding systems (Väderstad Rapid; 12.5 cm and System Cameleon by Gothia Redskap; 25 cm) and their influence on yield of dry yellow peas (*Pisum sativa*) and faba beans (*Vicia faba*). Yield of faba bean was not affected by row distance neither in 2011 nor in 2012. The yield were higher in 2012 (about 4.5 MT ha^{-1}) than in 2011 (about 3 MT ha^{-1}). For yellow pea the results are less clear. The 12.5 cm system resulted in significantly higher yield in 2011 compared to the 25 cm system, while in 2012 the 25 cm system was significantly better than the 12.5 cm system. It should be noted that 2012 was a wetter and generally colder season than in 2011. We conclude that faba beans can be cropped with good results regardless of row distance. For pea, soil compaction by the heavier 12.5 cm seeding machine might be a factor that explains the lower yield in 2012.

Biological Nitrogen Fixation (BNF) by faba bean at the centre for sustainable cropping (CSC), Dundee, Scotland

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Faba beans are widely grown in East Scotland, but little is known about their capacity for BNF under these conditions. We therefore used the ^{15}N natural abundance technique to measure BNF by 5 varieties of faba beans in a rotation at the CSC under conventional (*i.e.* a "normal" dressing of N free fertiliser) and "sustainable" (compost-fertiliser) regimes. Using non-legume dicot weeds as non-fixing references, the %N derived from air (%Ndfa) at early- to mid-pod fill for all varieties combined was estimated as over 80% for the sustainable, and slighly less for the conventional fertiliser treatments in the years examined (2011, 2012). At final harvest, estimates of the total amount of grain N fixed under each regime did not differ between the sustainable and conventional split-field halves at over 180 kg N ha^{-1} yr^{-1}. However, there were significant differences between varieties in terms of BNF when grown under the two regimes. In addition, the amount of fixed N remaining in the non-grain material (*i.e.* shoots and roots) at final harvest was up to 100 kg N ha^{-1} yr^{-1}, depending on treatment and variety. It is concluded that under a zero applied N regime, faba beans may obtain a very high proportion of their N-requirements *via* BNF in East Scotland, and that it may be possible to optimise the amount of N fixed by matching faba bean varieties to particular fertiliser regimes.

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Reaction of white lupin (*Lupinus albus* L.) to the initial nitrogen feeding and foliar feeding

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White lupin occupies a small growing area in Poland, in spite of vast yielding potential. Increased relevance of this species is possible thanks to spreading the determinate types and improvement of their agrotechnology. Varieties of white lupin of determinate type are characterized by shorter growing season, earlier and more balanced ripening and different, as compared with traditional varieties, dynamics of gathering photoasimilates in seed yield. In years 2009-2011 strict field experiment was conducted at the Experimental Station of Biological and Agricultural Faculty of Rzeszów University. The goal was to specify the reaction of white lupin (Boros variety) to the initial nitrogen feeding (ammonium nitrate 34% N) and to foliar feeding (Basfoliar 6-12-6). It was noticed that nitrogen feeding with foliar feeding prolonged the growing season and positively influenced crop stand before harvesting as compared with control sample. Initial nitrogen feeding considerably increased the number of pods on a plant and mass of thousand grains, but the number of seeds in a pod and protein content were not changed. Foliar feeding did not change the elements of yield structure but caused a considerable 0.15% increase of protein in seeds. Yielding of white lupin increased considerably both after initial nitrogen feeding only and after nitrogen feeding combined with foliar feeding. The obtained difference, as compared with control sample, was respectively 0.18 $t \cdot ha^{-1}$ and 0.26 $t \cdot ha^{-1}$ seeds.

Reaction of yellow lupin (*Lupinus luteus* L.) to foliar feeding with macro- and microelements

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Lequmes are an especially valuable group of cultivated plants. Yellow lupin is one of them. It is characterized by high protein content in seeds and the lowest soil requirements among all lupin varieties cultivated in Poland. Besides yellow lupin in crop rotation is a very good forecrop and effects of its positive influence can be noticed during subsequent years after its cultivation. In years 2011-2012 a strict field experiment was carried in the Research Centre for Cultivar Testing in Dukla attached to Central Research Station of Cultivated Plants Varieties in Słupia Wielka. The goal of the experiment was to specify the reaction of yellow lupin (varieties Mister and Taper) to foliar feeding (Basfoliar 6-12-6). The research project was financed with the means of the National Science Centre. It was noticed that the application of foliar feeding considerably modified the leaf area index (LAI), but did not influence the mean tip angle (MTA). The content of chlorophyll evaluated with SPAD index in the final phase of budding was considerably higher on the sample with foliar feeding than on the control sample. Research results proved that the applied fertilizer considerably increased the number of pods on a plant. The number of seeds in a pod and mass of thousand grains were not considerably different. Applied foliar feeding positively influenced the seed yield. The obtained considerable difference, in comparison with control sample, amounted to 0.37 $t \cdot ha^{-1}$. The contents of general protein and raw fat were not considerably modified by Basfoliar 6-12-6. Considerable differences in most examined parameters between Mister variety and Taper variety were confirmed.

Grain legume and spring wheat intercropping: effect on grain yield and crude protein content

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Nowadays, in organic or low input farming systems, diversified crop rotations with legumes are essential implement in enriching the plant-soil-system with nitrogen and improving cereal grain quality. At the same time it is very important to enhance protein-rich feed for livestock. The study focused on grain yield and crude protein content. The experiment was carried out in 2007, 2008 and 2009 on a loamy *Endocalcari-Ephypogleyic Cambisol* in Dotnuva (55°24'N, 23°50'E). Grain legumes: field pea (*Pisum sativum* L.), lupin (*Lupinus angustifolius* L.), bean (*Vicia faba* L.), vetch (*Vicia sativa* L.) and spring wheat (*Triticum aestivum* L.) were sown as intercrops and sole crops and were grown organically for grain. The productivity of spring wheat in intercrops depended on the species of grain legume, however, the results varied over the experimental years due to different weather conditions. In 2007, the vetch-wheat intercrop produced a significantly higher grain yield than wheat in sole crop or in the other intercrops. In 2008, no advantages of legume and wheat intercrops were revealed. In 2009, the yield vetch-wheat intercrop was higher compared with the sole wheat and other intercrops produced a similar yield compared with sole wheat. The concentration of crude protein was higher in grain yield, when spring wheat had been grown in intercrops. Vetch-wheat intercrops accumulated significantly more crude protein in grain compared with the other intercrops.

The quantitative evaluation of biological process of nitrogen reduction by yellow lupine (*Lupinus lutes* L.) and narrow-leaved lupine (*Lupinus angustifolius* L.)

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The quantitative evaluation of biological reduction process of nitrogen by two kind of lupine (Yellow lupine and narrow-leaved lupine) in the field conditions upon three different methods of soil plough was the aim of this work. The field experiment was carried out on the sandy loamy soil in 2012 year in which for vegetation period (from April to August) mean temperature was 15.4°C and sum of rainfal 412 mm. The scheme of experiment encluded the following objects according to type of soil cultivation: A – conventional, B – reduced, C – direct seeding (no tillage). The lupines were sown in the III decade of march and nitrogen was applicated in the I decade of may by the development stage of plants at 5th leaves. The dose of nitrogen was 30 kgs per hectare in the form of $(NH_4)_2SO_4$ which contained at 37.5 at % of ^{15}N. The total yield (seeds and straw) of yellow lupine reached 11.6 $t{\cdot}ha^{-1}$ (seeds 3.12 $t{\cdot}ha^{-1}$) and blue lupine 8.07 $t{\cdot}ha^{-1}$ (seeds 2.70 $t{\cdot}ha^{-1}$). The uptake of total nitrogen in kgs per hectare reached for lupines: yellow 258.0 and once 152.8 respectively. In this total amount the nitrogen 160.4 (in seeds 115.9) and 66.6 kg in seeds 48.7 come from biological reduction of nitrogen by yellow and narrow-leaved lupine what mean 62.5 and 43.5 % of total nitrogen. The kind of soil cultivation significantly differentiated the yields of both type of lupine. This parameter influenced stronger on the yield of yellow than blue lupine. The highest yield of yellow lupine 13.4 $t{\cdot}ha^{-1}$ was stated on the reduced type of soil cultivation (B) and lowest (9.63 $t{\cdot}ha^{-1}$) on the tillage type (C) but for narrow-leaved lupine these values were as 8.83 (A – conventional) and 6.98 (C – direct seeding) $t{\cdot}ha^{-1}$ respectively. The kind of soil cultivation differentiated the total uptake of nitrogen. The highest values for this parameter were stated from objects with full soil plough for both type of lupines and the lowest from tillage objects.

Optimizing durum wheat-grain legumes intercropping through cultivar choice

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Intercropping (IC) is defined as the simultaneous growing of two or more species for a significant period of their growth.Grain legume-cereal intercropping reveals many potential advantages in productivity, stability of outputs and ecological sustainability particularly in low N-input systems. The advantages of intercrops compared to sole crops (SC) result from interactions between intercrop components greatly influenced by morphological and physiological traits among species. The aim of this work is to enhance complementary vs. competitive interactions between intercroped species by identifying the relevant characteristics of involved cultivars so that to finally infer cultivars couples adapted to intercropping adressing various production objectives. A trial was carried out in 2011-2012 at the Auzeville station of the "Institut National de la Recherche Agronomique". Two durum wheat, four pea and four fababean cultivars chosen for their contrasting traits were evaluated. All the cultivars were grown as SC, half density SC and IC in a replacement design to evaluate inter- and intra-specific interactions. Dry weight, leaf area index, nitrogen content and aerial architecture were determined throughout the growing season, and yield components at maturity. The first results show that wheat was the strongest competitor in IC. IC legume grain yield was reduced to less than half compared to SC.However, the IC yield was greater than the average SC yields. Analysis indicate that legumerelative height and precocity are the most important factors that influence the performance of the intercropping and should be used for the design of cultivar ideotype couples performing well as intercrops.

Effect of seeding rate on plant density and dry matter yield of alfalfa

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Alfalfa, as the most important perennial forage legume, is cultivated in Serbia on about 200.000 ha. In the world and in Serbia sowing rates are very variable (15-30 kg ha^{-1}). The aim of this research was to investigate effect of seeding rate on dry matter yield of alfalfa and number of plants. The trial with three replications was located at experimental field of Institute of Field and Vegateble Crops at Rimski Šančevi. In the second and third year of growth (2010-2011) influence of two different seeding rates (8 kg ha^{-1} and 16 kg ha^{-1}) on dry matter yield was tested. A two-year average of the total dry matter yield was determined from the four cuts of four cultivars. The number of plants per square metre after emergence, then in the fall of the seeding year, and again the next spring and autumn was determined. A sowing rate had no influence on the yield of dry matter of alfalfa. The same dry matter yield (22.2 t ha^{-1}) was achieved under both low and high seeding rate. The sowing rates did not affect plant density. At the end of the year of establishment number of plants per square metre was approximately the same in both seeding rates (8 kg ha^{-1} - 270 plants per m^{2} and 16 kg ha^{-1} – 273 plants per m^{2}). In the third year of growth a plant density of 88 plants per square metre was the same in both low and high seeding rates. Results obtained in our study clearly show that higher seeding rates do not produce higher yields and higher density of alfalfa plants.

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Phosphorus efficiency in faba bean and narrow-leafed lupin

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Phosphorus (P) is the second most important element for plant growth. P fertilizers are applied to agricultural soils in order to make P available to crops and meet their nutritional requirements, but this contributes to negative environmental impacts such as eutrophication. Legumes have been known to enhance the soil nutritional status by fixing nitrogen; in addition, they can mobilize P from less labile P pools, improving soil fertility and structure and thus can be a source to improve the nutrition of subsequent crops. Faba bean (cv. 'Kontu') and narrow-leafed lupin (cv. 'Haags Blaue') were grown in a crop rotation trial at Viikki, Helsinki, Finland in 2011 and 2012. Seeds were inoculated with appropriate rhizobium and plots were fertilized with 125 kg/ha of 16-7-13 fertilizer. At maturity, 50 plants per plot were selected, separated into shoots and roots, roots were washed, and all components were oven-dried at 80°C. P content of soil and plant samples was determined by inductively coupled plasma-optical emission spectrometry (ICP). Faba bean showed 50% higher root P absorption efficiency ($P<0.05$) than the lupin (98 and 67 mg P /g root), while P utilization efficiency was 50% higher in narrow-leafed lupin than in faba bean (372 and 239 g plant /g P). Furthermore, after harvest, P content of soil samples from faba bean plots was 10.9 mg/l while in those from narrow-leafed blue plots it was 14.5 mg/l. Faba bean and blue lupin showed clear differences in their P efficiency.

Effect of field pea companion crop on alfalfa pigment content

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Field pea has potential to be a suitable companion crop for alfalfa establishment. It is a fast-growing crop, harvested early and thus reduces duration of competition. Compared to other companion crops, such as small grains, it allows better light transmition through canopy to an undersown species. The aim of this study was to determine the suitability of field pea as a companion crop for alfalfa establishment. A two-factor trial was carried out at the experiment field of Institute of Field and Vegetable Crops at Rimski Šančevi from 2004 to 2009. The experiment involved two pea varieties (Jezero - semi-leafless and Javor - normal leaves with reduced leaflet size) and four pea densities (0, 30, 60 and 90 plants m^{-2}) and control variant with oat as companion crop. When the field pea had reached the harvestable stage, samples were taken for determining alfalfa chlorophyll and carotenoids content. In average, chlorophyll and carotenoids content were higher in all treatments of companion cropping than in the sole crop of alfalfa. Weather conditions had significant influence on pigment content during the experimental years. Lower alfalfa chlorophyll content in treatment with Jezero (12.08 mg g^{-1}) indicates better light condition for undersown crop, compared to treatment with Javor (14.54 mg g^{-1}). The lowest content of total chlorophyll was in pure alfalfa (11.13 mg g^{-1}) and increased proportionally with nurse crop density. The ratio of chlorophyll and carotenoids content was 3.5 in pure stand of alfalfa and in all other treatments were more then 4.

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Effect of Polish habitat conditions on the yield of pulses and aftercrop

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The field experiment with one factor in 4 replications was set in 2011-2012 in four region of Poland. In the experiment, the yield of faba bean, pea, lupine and barley or wheat, as forecrops for oilseed rape and triticale were compared. The chemical composition of seeds and yield – seeds and protein were evaluated. After the harvest, oilseed rape and triticale were sown. Average yields of protein (kg · ha^{-1}), depending on the region for narrow lupine was 491-876, pea 637-1293, faba bean var. Granite 980-1293, faba bean var. Olga 1236-1656, yellow lupine 676-744, white lupine 689-1002, and the yield of protein of spring barley 481-545 and spring wheat 746-758. In 2011, the region I, II and IV after the legumes were sown oilseed rape, which not wintered in early 2012 in the region I, in the region II wintering 85%. In the IV region oilseed rape yielded very high from 5.10 t · ha^{-1} after spring wheat to 6.17 t · ha^{-1} after faba bean. In the region III in 2011 were sown winter triticale, which in 2012 wintered in part of 10-15%. The seed yields after yellow lupine, white lupine and barley were similar (5.0 t · ha^{-1}). After pea and narrow lupine seed yields were obtained lower respectively about 7.0 and16%.

Intercropping autumn-sown annual legumes with cereals for forage production

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Intercropping annual legumes such as pea (*Pisum sativum* L.) and vetches (*Vicia* spp.) is one of the most ancient cultivation practice throughout the world, especially in temperate regions of Europe, Asia Minor, Near and Central East and North Africa. This kind of intercropping may serve for both forage and grain production. In Serbia and throughout the Balkan countries, intercropping annual legumes with cereals is used mostly for providing a high quality fodder in feeding ruminants, especially dairy cows. In a small-plot trial, carried out during the trials years of 2009/2010, 2010/2011 and 2011/2012, there were included the intercrops of eight autumn-sown cereals, namely einkorn (*Triticum monococcum* L.), emmer (*Triticum turgidum* L. subsp. *dicoccon* (Schrank) Thell.), spelt (*Triticum aestivum* L. subsp. *spelta* (L.) Thell.), durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.), common wheat (*Triticum aestivum* L. subsp. *aestivum*), barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.) and triticale (×*Triticosecale* spp.), seven spring-sown annual legumes, such as pea, common vetch (*V. sativa* L.), Hungarian vetch (*V. pannonica* Crantz), hairy vetch (*V. villosa* Roth), faba bean (*V.faba* L.), grass pea (*Lathyrus sativus* L.) and white lupin (*Lupinus albus* L.), and the sole crops of each intercrop component. Each intercrop was sown at a rate of 75%n of a legume and 25% of a cereal in comparison to the sowing rates in their sole crops. All intercrops and sole cops were cut in the stages either before spike or raceme appeared in cereals or in full bloom in legumes. Despite the average three-year values of forage dry matter yields that often surpassed 10 t ha^{-1}, barley and hairy vetch had the largest individual contribution in the tested intercrops, being rather aggressive within a stand and competitive for natural resources, such as light, water and nutrients. On the other hand, intercrops such as those of einkorn with Hungarian vetch, emmer and spelt with faba bean or pea, common vetch and grass pea with oat had the highest three-year values of land equivalent ratio (LER), thus proving economically most reliable. Further investigations will be aimed at forage dry matter quality and stress resistance.

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Intercropping spring-sown annual legumes with cereals for forage production

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Growing annual legumes such as pea (*Pisum sativum* L.) and vetches (*Vicia* spp.) in mixtures with cereals is one of the most traditional ways of both forage and grain production in many temperate climates in Europe, Asia Minor and Near and Central East. This practice is considered beneficial kind of intercropping may serve for both forage and grain production. In Serbia and other Balkan countries, intercropping annual legumes with cereals is rather widespread and is extensivelz used for producing quality and protein-rich forage in feeding dairy cows and other ruminants. In a small-plot trial, carried out during the trials years of 2010, 2011 and 2012, there were included the intercrops of three spring-sown cereals, namely common wheat (*Triticum aestivum* L. subsp. *aestivum*), barley (*Hordeum vulgare* L.) and oat (*Avena sativa* L.), six spring-sown annual legumes, such as pea, common vetch (*V. sativa* L.), Narbonne vetch (*V. narbonensis* L.), faba bean (*V.faba* L.), grass pea (*Lathyrus sativus* L.) and white lupin (*Lupinus albus* L.), and the sole crops of each intercrop component. Each intercrop was sown at a rate of 75%n of a legume and 25% of a cereal in comparison to the sowing rates in their sole crops. All intercrops and sole cops were cut in the stages either before spike or raceme appeared in cereals or in full bloom in legumes. Although the average three-year values of total forage dry matter yields in the intercrops were higher than 9 t ha^{-1}, barley and grass pea had the most prominent individual contribution in the tested intercrops and were the aggressive when intercropped and further more competitive for natural resources, such as light, water and nutrients. However, intercrops such as those of pea, common vetch and Narbonne vetch with oat had the highest three-year values of land equivalent ratio (LER), proving its economic reliability. Intercrops of faba bean and white lupin with cereals suffered from a slightly more severe attack by weeds. The goal of further investigations will be assess forage dry matter quality and stress resistance, with emphasis on pests and diseases.

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Intercropping spring-sown annual legumes with brassicas for forage production

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Available references on intercropping brassicas with legumes are rather scarce. However, there are recent reports showing numerous benefits for a brassica component, especially in the form of an easier uptake of less available nutrients because of a positive influence of the legume companion. The goal of this study was to assess the potential of intercropping various spring-sown brassicas with legumes for forage production. A small-plot trial has been carried out in the trial years of 2010, 2011 and 2012 at the Experimental Field of the Institute of Field and Vegetable Crops at Rimski Šančevi in the vicinity of Novi Sad. It included six intercrops of spring-sown brassicas with spring-sown annual legumes. Two brassicas, in this case, rapeseed (*Brassica napus* L. var. *napus*) cv. Jovana and white mustard (*Sinapis alba* L.) cv. NS Gorica played the role of supporting crops for four legumes, namely pea (*Pisum sativum* L.) cv. Jantar, common vetch (*Vicia sativa* L.) cv. Perla, and grass pea (*Lathyrus sativus* L.), acting as supported crops. All six cultivars, developed at the Institute of Field and Vegetable Crops, were also sown as sole crops. The two-year average forage dry matter yield in the sole crop of rapeseed (6.9 t ha^{-1}) was much higher than in the previous trial in the same agroecological conditions, while the two-year average forage dry matter yield in the sole crop of pea was also higher than in the preliminary results, with 8.2 t ha^{-1}. The highest two-year average individual contribution in the total forage dry matter yield among brassicas was in rapeseed (3.9 t ha^{-1}) when intercropped with common vetch, while the highest individual contribution in the total forage dry matter yield among legumes was in grass pea (6.2 t ha^{-1}) when intercropped with white mustard. The two-year average values of LER$_{FDMY}$ ranged between 1.07 in the intercrop of rapeseed with grass pea and 1.25 in the intercrop of whit mustard with pea.

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Some aspects of *amaizing* annual legumes

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Intercropping maize (*Zea mays* L.) with annual legumes is distributed throughout the world, mostly in subtopic and tropic regions of Africa, Asia and South America, including warm season legumes cowpea (*Vigna unguiculata* (L.) Walp.), pigeonpea (*Cajanus cajan* (L.) Huth), common bean (*Phaseulus vulgaris* L.) and soybean (*Glycine max* (L.) Merr.). Numerous studies of maize/legumes intercropping underline its role in soil erosion control, weed and pests management, water use efficiency, light distribution and use, nutrient availability and uptake, and more efficient use of land (LER>1). Exudates from maize stimulate nodulation biomass production of legumes, whereas maize uptake N stimulates nitrogen fixation in conditions of competition for N. In temperate regions, cool season legumes can be intercropped with maize, where different sowing dates and growth dynamic allow different temporal and spatial use of environmental resources. Such intercrops are grown on smaller areas in developing countries, while in China it is practiced on larger scale, especially with pea (*Pisum sativum* L.). In Serbia and other Balkan countries, intercropping maize with annual legumes is present in many regions, especially on small fields and in gardens. It is mostly common bean, faba bean (*Vicia faba* L.) and chickpea (*Cicer arietinum* L.) that are sown between the rows of maize. Local farmers consider this practice beneficial for both components, with maize providing a kind of shelter or support for a legume and a legume filling the space between the maize rows and fighting weeds. Both components are harvested by hand, making the issue of concurring the times of sowing and harvesting irrelevant. Intercropping maize and legumes diversifies agriculture production, increases yield stability and productivity and reduces the risks of pest and disease outbreaks.

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The use of legumes in an aquaponic agricultural system

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Aquaponics is the combination of aquaculture (fish) and hydroponic cultivation of plants without soil. The technology works as a closed loop system that reduces the consumption of fresh water compared to conventional monocultures and uses the nutrients excreted by the fish as fertilizer. Thus all fish feed nutrients can be utilised. Legumes are widely grown for food both as grain (pulses) or fresh as a vegetable. They are an important protein source both directly as food and, to an even higher volume, indirectly as animal feed. In both purposes they yield a high and widely recognised quality. During the winter period 2012-2013, pea plants were grown in an aquaponic based facility examining the cultivar variation in an NFT setting, irrigated with fish water. Nutrient, water and energy balances within the growing period were determined, but also possible reuses of the rockwool substrate after harvesting of the peas. Legumes (mugabean, peas, faba beans and lentils) were grown as microgreens in the used inorganic substrate (grodan) as the second phase of the experiment. Preliminary results at this stage indicate that legumes can successfully grow in an aquaponic facility providing very low use of inorganic inputs, low energy and water use but high prospect on food supply growing in such high-tech systems. This opens the prospect that food production could move from the fields even into the heart of cities.

Nitrous oxide emissions of legume based agricultural systems in Northern and Southern Europe

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The main objective of this paper was to explore the extent to which similar legume based cropping systems could influence N_2O emissions per unit of grain production (emission intensity) in a two-year experimental period (2011 and 2012) within Northern and Southern Europe. Two sites were selected: one in the United Kingdom (Atlantic region) and one in Italy (Mediterranean region). The following leguminous crops were studied: beans, peas, barley and wheat (subsequent crop). Greenhouse gas emissions(N_2O, CO_2 and CH_4) and extractable soil N were measured every 15 days and at key growth stages, from sowing to harvest at both sites. Grain yields were also recorded for the calculation of N_2O intensities. Preliminary results have shown for 2011, in Italy, the legume monocrops had higher intensities when compared with the cereal (barley) monocrop, but in UK, all the legumes had lower intensities than the unfertilised cereal crop. In the second year, the subsequent crop, wheat, significant influenced both the grain yield but also the N_2O intensities. For example, in Italy, the wheat following a previous legume monocrop showed a very high N_2O intensity when compared with an intercrop. The data provided by this study can help to design systems (including low-input and organic systems) that increase their N use efficiency and reduce their environmental impact. Greenhouse gas emission intensities provide a valuable measure of this impact. An improved understanding of different species/varietal combinations and seeding ratios will help to develop management recommendations for optimising the productivity and environmental impacts of legumes in practice.

Effect of organic fertilization treatments on grasspea (*Latyrus sativus* L.)

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A better knowledge of important relationships between soil and crop production is necessary to develop sustainable agricultural systems. It is often stated that only a few staple crops produce the majority of the food supply. This might be correct but the important contribution of many minor species should not be underestimated. Legumes have always been used as a source of nutrient-rich organic matter and nitrogen for crops. The grass pea (*Lathyrus sativus* L.) is a food, feed and fodder crop belonging to the family Leguminosae. This crop has over the past decade received increased interest due to its adaptability to arid conditions and contains high levels of protein, a component that is increasingly becoming hard to acquire in many developing areas. In Italy, after a drastic decline in the last 50 years, there is great potential for an expansion in the utilization of grass pea in the sustainable crop systems of the marginal areas of southern Italy. The diffusion of grass pea cropping is subject to the evaluation of the potential grain yield in the semi arid areas of south Italy. The objectives of this study were to investigate the impact of different organic fertilization treatments on quantity and quality traits of grain and the effects of crop-residue application on yields in a grass pea-durum wheat rotation system. The results show that the grass pea has not been able to take advantage from high fertilizer input. Good yield performance was obtained from cultivar Gecon 15 in the studied crop rotation system.

Field pea (*Pisum sativum* L.) as a companion crop for the establishment of perennial legumes

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Pea is one of the most important crops in the world. Among the many ways of utilization of this crop are that related for forage production. Forage pea may be cultivated alone as a pure crop, or in mixture with other crops, usually cereals. In recent time, several attempts have been made to cultivate forage pea with other legumes, where pea would play the role of cover crop. The results reported here are obtained in a greenhouse experiment aimed to assess the pea as a companion crop for establishment of lucerne (*Medicago sativa* L.), red clover (*Trifolium pratense* L.) and sainfoin (*Onobrychis sativa* L.) as undersown crops. The semi-leafless pea cultivar Jezero with three population densities (30, 60 and 90 plants per m^2) were used in this experiment. Two control treatments also included in the experiment – the perennial legumes grown as a pure stand, and the perennial legumes with oat as a cover crop. The effect of pea on forage yield, chlorophyll content, leaf surface and LER is determined. According to obtained results, the best combination for lucerne is 30 pea plants per m^2, 90 for red clover and 60 pea plants per m^2 for sainfoin.

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Effect of Transplanting Pigeonpea Enhance the Nutrient Uptake through Foliar Nutrition Spray of Water Soluble Fertilizer

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Pigeonpea (*Cajanus cajan*), an important food legume crop in the semi-arid regions of the world and the second most important pulse crop in India. Pigeonpea is a nutritious legume crop that has the ability to fix nitrogen, grow on marginal lands and has multipurpose use for human consumption, animal feed and fuel. India is the largest producer and consumer of the crop. However, there is growing interest to produce the crop by other countries. Field experiment was conducted at Agricultural College and Research Institute, Madurai, Tamilnadu, India during Kharif 2010 and summer 2011 to study the effect of transplanting pigeonpea enhance the nutrient uptake through foliar nutrition spray of water soluble fertilizer. In this study, different age of transplanting seedling combine with foliar nutrition spray get higher yield. Different age of seedling (14 days old seedling raised in pro tray, 21 days old seedling raised in pro tray, 28 days old seedling raised in pro tray) and = Foliar spray of nutrients (DAP, Pulse wonder, polyfed and KNO_3) were plating the seedling at the same time of all age of seedling sprayed as individual and in combination at flowering stages. Planting 21 days old seedling (M_3) during both seasons increased nutrient uptake observed number of flower drops reduced. Foliar application of polyfed (one per cent) and multi K (one per cent) (S_4) foliar spray during both seasons increased growth attributes followed by polyfed foliar spray (one per cent) which was on par with DAP (two per cent) spray treatment.

Impact of synthetic and bio-fertilizers on yield of mungbean (*Vigna radiata* L.)

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Mungbean is the second most important pulse of Pakistan; it fixes atmospheric nitrogen to improve soil fertility as nodules are present in roots of mungbean. It contains essential amino acid such as lysine present in high amount that increases the efficiency of mungbean as compared to other pulses. An experiment to evaluate the impact of nitrogen and phosphorus levels in combination with inoculum sources on yield and quality of Mungbean (*Vigna radiata* L.)was conducted in 2012. Response of mungbean (*Vigna radiata* L.) cultivar NM-2011 with net plot size of 5.0 m × 2.4 m was studied in field condition. Factor A was Nitrogen + Inoculation combinations with three levels [A_1 = N (24 kg ha^{-1}), A_2 = Inoculation and A_3 = N (24 kg ha^{-1}) + Inoculation] and Factor B Phosphorus + Humiphos combinations with 4 levels [B_1 = P (50 kg ha^{-1}), B_2 = Humiphos (24.7 kg ha^{-1}), B_3 = P (50 kg ha^{-1}) + Humiphos (24.7 kg ha^{-1}), B_4 = P (25kg ha^{-1}) + Humiphos (24.7kg ha^{-1})] and treatments were repeated thrice. The yield data was recorded by adopting the standard procedures. Yield components like number of nodules per plant, number of pods per plant, number of seeds per pod, 1000-grain weight (g), grain yield (kg ha^{-1}), harvest index(%) were significantly increased when combination of N (24 kg ha^{-1}) + Inoculation was used and were also significantly increased when combination of P (50 kg ha^{-1}) + Humiphos (24.7 kg ha^{-1}) was used.

The establishment of legume for forage and nitrogen usage for subsequence spring wheat yield formation

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The potential of legume yield influence the complex of factors and their interactions. These are greatly dependent on legume species, successful establishment and management. The experiment was aimed to determine the possibilities of yield formation intensity for forage already in the sowing year by using adequate combinations of legumes and their cover crops and to explore organic N of legumes as a potential N source for subsequently grown spring wheat. Two experiments were carried out on a loamy *Endocalcari-Epihypogleyic Cambisol*. Legume/grass mixtures were sown with and without a cover crop of barley or peas for whole crop and barley for grain. *Trifolium pratense* L. and *Medicago sativa* L. were sown in the mixtures with *Lolium perenne* L., and as sole stand. The yield of swards, its formation rate and crude protein concentration differed due to legume species, different competitive plant in the first year and different climate conditions. In drier years lucerne and ryegrass sward in all cases studied was significantly more productive than the red clover/ryegrass mixtures, and in wetter years significantly more productive were red clover than lucerne swards, except for lucerne mixture that grew without a competitive cover crop. The effect of legume/grass of two years of age on the yield of spring wheat that grew after them and on nitrogen accumulation in grain significantly differed due to the sward composition and practically did not differ due to different swards mixtures and combinations of cover crops.

Intercropping of legumes with *Helianthus* species

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Intercropping, or growing together more species at the same time in the same place is one of the oldest systems of cultivation in agriculture. Today, intercropping is recommended in sustainable agriculture due to various positive effects. The main advantage of legumes in intercropping is the possibility to increase soil fertility and protein levels with companion plants. The most common agricultural species of the *Helianthus* genus are sunflower (*Helianthus annuus* L.) and Jerusalem artichoke (*Helianthus tuberosus* L.). Intercropping of legumes with sunflower has been tested more than with Jerusalem artichoke. Intercropping of Jerusalem artichoke with sweet clover and hairy vetch showed that the level of nitrogen in the soil was not significantly lower than the controls. Intercropped sunflower showed large variations in yield depending on the variety and location. Species like *Vicia villosa* Roth, *Melilotus officinalis* Lam., *Medicago sativa* L. and *Medicago scutellata* (L.) Mill. have been intercropped with sunflower with the same sowing time and at the V4 and V10 sunflower growth stages. Intercropping in later growth stages had no negative impact on the development of sunflower. Intercropping with beans produced no significant effect on yield, while alfalfa, soybean, mung and cluster bean significantly reduced sunflower yield. Increased levels of nitrates in the soil have been recorded when sunflower was intercropped with hairy vetch. Most of the research so far on intercropping of legumes with *Helianthus* species has been done in subtropical area, where the best results were obtained when sunflower was intercropped with bean and hairy vetch.

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Effects of field pea (*Pisum sativum* L.) intercropping with spring cereals on pea aphids (*Acyrthosiphon pisum* Harris, 1776) and their natural enemy occurrences

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Occurrences of pea aphid and its natural enemies (syrphids, mummies caused by entomopathogenic fungi *Beauveria* sp. and by parasitic wasp *Aphidius ervi*) were compared in monocultures and mixtures of field peas and spring cereals in three seasons (2008-12; project NAZV: QH82027). At the beginning of colonization, aphid occurrence was not substantially influenced by intercropping with cereals. However, the numbers of pea aphids located on inflorescences started to decline earlier in mixtures compared with monoculture. More syrphids (eggs + larvae) were found in mixtures than in monoculture, and more syrphid eggs were found in young aphid colonies (10 – 20 individuals) in mixtures. Intercropping did not influence occurrence of fungi mummies (*Beauveria* sp.), but mixtures tended to have more aphid colonies infested by *A. ervi* in 2008, 2009 and 2011.

Eco-friendly early maturing pigeonpea to enhance the livelihoods of recourse-poor farmers of Rajasthan

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Pigeonpea (*Cajanus cajan* (L.) Millsp.) is an important pulse crop in rain-fed agriculture in the semi arid tropics of India. Even though pigeonpea is the second most important pulse crop in Rajasthan, due to the ignorance of farmers about early maturing varieties, the crop is cultivated in very less area as local/desi varieties. In viewpoint the project 'Eco-friendly early maturing pigeonpea to enhance the livelihoods of recourse-poor farmers of Rajasthan' has introduced into the farmer locality of Rajasthan by ICRISAT, in cooperation with SKRAU, Rajasthan to enhance the area production and productivity of pigeonpea. During 2012 cropping season we have covered 212 demonstrations in 212 ha in Jaipur district, in that most of the area (177ha) from Padasoli village. From yield point of view, overall the Jaipur district the highest yield was observed as 1900 kg/ha with an average productivity of 1119 kg/ha. This crop made the farmers of Jaipur district from almost zero return to tremendous returns which they did not expect like before that they got thirteen times more returns than their expenditure. By sure this crop changed the fate of the farmers of Jaipur district.

Competition between spring cereals and faba bean in mixed cropping

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The field experiment was established in Krasne, near Rzeszów (50°03'N, 22°06'E), South-east Poland on good wheat soil complex. The aim of the research was to assess competitive interactions between spring cereals (barley, oat, naked oat, wheat, triticale) and faba bean varieties of varying growth type (undetermined and determined varieties). The reaction of plants cultivated in mixtures was studied using the land equivalent ratio (LER), relative crowding coefficient (RCC), competitive ratio (CR) as well as aggressiveness (A). Faba bean mixtures with spring cereals gave significantly higher yields in comparison with faba bean in pure sowing but did not differ much compared to cereals in pure sowing. The exception was in faba bean mixtures with naked oats, which gave the lowest yields while their yields were close to those obtainable in pure sowings of both components. The highest yields were obtained from mixtures of faba bean with hulled oats, but the grain yield of the leguminous component in the total yield of such mixtures was the lowest. The cultivation of spring cereals and faba bean in mixtures is more effective than in monocultures as indicated by the higher than 1 LER value. The highest value of LER was noted in mixtures of faba bean with triticale, which thus confirms the mutually positive impacts of both species. A higher efficiency of use of environmental resources was demonstrated by cereals. Hulled oats turned out the most dominating in mixtures with both varieties of faba bean. The most favourable conditions for growth and yield for the undetermined growth type variety were in mixtures with triticale and wheat, while for the determined growth type was in mixtures with triticale and barley. The determined growth type variety exhibited greater competitiveness with cereals than the undetermined growth type variety.

A study of soil nitrogen supplies available to winter cereals following pulses compared to winter cereals following oilseed rape

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The value of residual nitrogen through soil nitrogen supply to cereal crops following pulses is currently estimated to be around 30kgN/ha, providing a yield benefit to first wheat. The recommendation for nitrogen fertiliser applications (Defra Fertiliser Manual (RB209) 8th edition) to cereals following pulses is for a reduction of this quantity compared to cereals following cereals, providing a cost benefit to pulse growers as well as the associated environmental benefit. In addition to this pulses do not require nitrogen fertiliser as they are able to fix atmospheric nitrogen. The Processors and Growers Research Organisation in collaboration with Wherry and Sons Ltd. undertook work in 2011 and 2012 to quantify more accurately the nitrogen available to the succeeding crop. The study investigated soil mineral nitrogen to 90 cm depth, and the percentage nitrogen that was measured in the unfertilised crop at harvest (grain and straw) for commercial cereal crops following peas and beans compared to paired crops following oilseed rape. Although the data in 2011 and 2012 were very variable, overall both measures of soil mineral nitrogen in spring and harvested nitrogen indicated that pulses provided more nitrogen to the succeeding cereal crop than oilseed rape. Additional investigations in 2013 and 2014 will clarify whether preliminary conclusions represent typical soil nitrogen supplies and apparent mineralisation following pulse crops.

Session 10

Mechanisms of beneficial legume-microbe interactions

KEYNOTE LECTURE

Mechanisms of beneficial legume-microbe interactions

Daniel Wipf

UMR1347 INRA/Agrosup/Université de Bourgogne Agroécologie, Pôle Interactions Plantes-Microorganismes - ERL 6300 CNRS, Dijon Cedex, France

Understanding mechanisms underlying high nutrients use efficiency and carbon allocation in a context of legumes-microbe interactions at the root level (mycorrhiza and nodules) is critical for sound management of croplands taking care of ecosystem services rendered by beneficial microbes. Indeed, availability, uptake and exchange of nutrients in biotrophic interactions will drive plant growth and modulate biomass allocation, that are central to plant yield, a major outcome, in the context of high biomass production. Transport processes across the polarised membrane interfaces are of major importance in the functioning of the established symbiosis as the relation is based on a 'fair-trade' between microbes and host plant. Uptake and exchanges of nutrient and/or metabolites, at biotrophic interfaces are controlled by membrane transporters and their regulation patterns are essential in determining the outcome of plant microbe interactions and in adapting to changes in soil nutrient quantity and/or quality. In the present talk, the current status regarding transport systems as well as future challenges will be highlighted.

Effect of co-inoculation with *Bradyrhizobium japonicum* and two *Bacillus* strains on soil biogeny parameters and soybean yield

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Plant growth-promoting rhizobacteria (PGPR) enhance plant growth and yield by various mechanisms which involve fixation of atmospheric nitrogen, production of siderophores, solubilization of minerals such as phosphorus, synthesis of phytohormones, etc. The aim of this study was to determine the effect of symbiotic nitrogen fixing bacteria *Bradyrhizobium japonicum,* applied alone or in co-inoculation with two different PGPR strains - *Bacillus subtilis* and *Bacillus megaterium*, on soil biogeny parameters, growth and yield of soybean cultivar Galina. This study was conducted on two-year experiment which was set up in four replications at experimental field of the Institute of Field and Vegetable Crops from Novi Sad. Soil biogeny was determined using the total number of microorganisms, number of azotobacters and dehydrogenase activity. Growth parameters were determined based on the height and weight of the aboveground plant parts and roots, number and mass of nodules, pod number, seed number and seed mass per plant. All treatments showed a positive effect compared to the control in both years of research. Co-inoculation had a greater impact on soil biogeny, growth and yield of soybean plants, while the best effect on nodules and yield parameters was achieved in the variant of single inoculation. On average, a higher percentage of increase was in the total number of microorganisms (5 – 37%) than in azotobacter population size (11 - 14%). The yield increase over the control ranging from 3,2% in the variant with *Bradyrhizobium japonicum*, to 11,7% in the case of co-inoculation with both strains of *Bacillus*.

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Homologs of *Medicago truncatula* symbiotic proteins and the evolution of nitrogen fixing root nodule symbiosis

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Symbiotic association with microbes proved to be a powerful strategy for plants to overcome nutrient limitations of their habitat. An ancient type of coexistence is the arbuscular mycorrhiza (AM) symbiosis, which is present in the majority of land plant families. The root nodule symbiosis (RNS) is more recently appeared in legumes and closely related plants with nitrogen-fixing bacteria. RNS is essential in the natural nitrogen circulation of ecosystems. Crop rotation with nitrogen fixing legumes has been a successful method for centuries, by which crop lands were refreshed for horticultural cultivation, and still, biological nitrogen fixation is cost-efficient and effective solution for the continuously growing nitrogen demands of agricultural cropping all around the globe. Genetic analyses showed that the AM and RNS systems share genes, supporting the idea that already existing elements of the more ancient program were recruited during the evolution of RNS. Besides, homologous counterparts of legume symbiotic genes had been identified in non-legume plants as well. Some are able to accomplish the symbiotic function of their legume homolog, as it was proven by a number of studies. We have searched plant genomes by symbiotic genes to identify the respective homologs, thereby to show to what extent these genes are conserved outside the nodulating clades. We would like to know how the genes indispensable for RNS have specialised for their function during the evolution of plants by studying the structure and regulation of these homologous genes and comparing their protein products.

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Role of the arbuscular mycorrhizal symbiosis on S-uptake and S-starvation resistance in *Medicago truncatula*

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Due to its key role in the biosynthesis of many S-containing compounds, sulphur is a macronutrient essential for plant growth, development, and response to various abiotic and biotic stresses. Sulphate represents a very small portion of soil S pull and it's the only form that plant roots can take up and mobilize through H^+-dependent co-transport processes implying sulphate transporters. Unlike the other organically bound forms of S, sulphate is normally leached from soils due to its solubility in water, thus reducing its availability to plants. Although our knowledge of plant sulphate transporters has been growing significantly in the last decades, little is still known about the effect of the arbuscular mycorrhiza (AM) interaction on S-uptake and S-stress resistance. For this reason our studies focused on the mycorrhizal interaction between the leguminous model plant *Medicago truncatula* and the arbuscular mycorrhizal fungus *Rhizophagus intraradices* (ex *Glomus intraradices*). Carbon, nitrogen and sulphur measurements in different plant tissues and expression analysis of genes encoding putative Medicago sulphate transporters (MtSULTRs) were performed to better understand the beneficial effects of mycorrhizal interaction at different sulphate concentrations. The putative effects of mycorrhizal interaction were also assessed on seed weight and quality through protein content and 1-D gel analyses. Among the 8 putative MtSULTRs in-silico identified; some of them were differentially transcribed in roots and leaves due to sulphate concentration and/or upon mycorrhization, potentially defining a switch between direct (DP) and mycorrhizal (MP) sulphate uptake pathways.

Improving the efficiency of biological nitrogen fixation of chickpea *Cicer arietinum* L.

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Chickpea *Cicer arietinum* L. is characterized by high dietary diversity as a food crop. At the same time it has a high resistance to drought and heat. In the Russian Federation the chickpea is grown on the area over 100 thousand hectares. It can be used as an insurance culture instead of peas in the central regions of Russia with frequent droughts. Introduction of chickpea in the north regions is restrained mainly because of the lack of well-adapted varieties with short growth period and crop management practice. Inoculation with nitrogen fixing bacteria *Mesorhizobium ciceri* (strain 527 ARRIAM) and fungi of arbuscular mycorrhiza *Glomus* was applied in order to increase nitrogen fixation efficiency. Research was carried out in 2010-2012 at the field of the All-Russia Research Institute of Legumes and Groat Crops on the dark-gray forest soils with a humus content of 4,7 - 4,9%. 13 chickpea accessions received from the Vavilov Research Institute were tested in this study. Use of microbiological agents increased plant height by 0,9 – 9,4%. Maximum increase of seed yield per plant of 61,1- 69,4% was observed in variant with dual inoculation with rhizobia and fungi. Maximum amount of nodules formed in case of single rhizobia inoculation and was 79 nodules per plant, maximum nitrogenase activity was recorded at the level of 256 μg N_2/plant/h in variant with dual inoculation. Total seed yield of chickpea in variants with microbe inoculation increased by 0,2 – 0,7 t/ha over control.

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Phenotypic and genetic diversity of rhizobia isolated from nodules of the legume genera ***Hedysarum coronarium*** in North West region of Morocco

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The species *Hedysarum coronarium* L. (tribe Hedysareae, family Leguminosae), known by the Italian name of sulla or Spanish sainfoin, ranges within the Mediterranean basin from northern Africa to southern Spain and in Italy from centrally to southern. In Morocco these phytogenetic resources are currently damaged by severe genetic erosion due to overgrazing, irregular rainfall and reduction of rangeland. Phenotypic and genetic diversity of 60 strains, isolated from root nodules of *Hedysarum coronarium* in North West region of Morocco, were studied using both phenotypic and genetic techniques. The phenotypic tests, (growth rate, tolerance to salinity, pH, temperature, resistance to antibiotics and metals ...), revealed that most of strains are belonging to rhizobia and genetic tests clustered all strains into 26 different profiles. Results of 16S rRNA sequencing revealed that *Hedysarum* rhizobia were phylogenetically related to *Rhizobium sullae* strain IS 123T in 14 groups. In this study, we revealed that a large diversity among the 60 strains.

Antioxidant characteristics of symbiotic association of soybean and different *Bradyrhizobium japonicum* strains

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The ability of legume plants to fix atmospheric nitrogen in symbiotic association with nodule bacteria, have great environmental and agricultural significance. *Bradyrhizobium japonicum* is the most common soybean microsymbiont. In order to overcome the negative effects of drought, more tolerant crops genotypes are created and agricultural practices are improved. Selection of effective strains of rhizobia more tolerant to water deficit is certainly a measure that can increase nitrogen fixation and improve soybean production under drought conditions. This study was focused on the impact of drought on antioxidant characteristics of symbiotic communities of soybean and different *Bradyrhizobium japonicum* strains from the collection of Institute of Field and Vegetable Crops. Drought stress conditions increased the activity of the soluble and ionically cell wall-bound peroxidases in plant roots and nodules. The antioxidant activities in the roots and nodules as well as the content of soluble proteins were significantly increased under conditions of water deficit. Increased protein content in stress conditions can be explained by the synthesis of proteins involved in antioxidant response and accumulation of plant proteins involved in adaptation to drought, as confirmed by a significant correlation between protein content and the activities of peroxidase in roots and nodules. The applied strain significantly affected the activity of soluble and cell wall-bound peroxidase, antioxidant activity and the content of soluble proteins. The applied strain had a greater impact on the studied parameters in plants that were exposed to water deficit compared to plants that were provided optimum water. Significant correlations between antioxidant activity and the activity of soluble and bound peroxidases in nodules and roots indicated a linked antioxidant response of plant and bacteria under stress.

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Grain yield and microbial abundance in the rhizosphere of soybean and bean: conventional and organic system growing

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For higher productivity heavy doses of fertilizers and other agrochemicals are applied. This has led to the emergence of a movement forward specified farming concept based on the traditional farming philosophy known as organic systems. These systems do not use synthetic chemicals and in the long run way be more sustainable than inorganic and conventional farming. The purpose of this study was to investigate microbial abundance in rhizosphere and yield of bean and soybean in conventional and organic system growing. The trial was set up on chernozem soil at the Bački Petrovac experiment field of the Institute of Field and Vegetable Crops. Soil samples of rhizosphere were collected under conventional management and organic management. Samples for microbiological analyses were taken at two dates (1st June and 18th July). Soil microbial abundance was significantly greater in organic farming growing compared with conventional. The total number of microorganisms, number of ammonifiers, azotobacter, actinomycetes and fungi was higher in rhizosphere in organic production of bean than in rhizosphere of bean in conventional production. Similar results were obtained in rhizosphere of soybean in organic production compared to the conventional. Beside the higher of examined groups of microorganisms in rhizosphere of soybean, the higher number of celulolytic actinomycetes was obtained, too. The number of nodules on root of soybean in organic production was three times higher than in conventional. Grain yield was higher in conventional production than in organic, while in organic production greater yield quality, protein and oil content, was obtained.

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Mutually beneficial multi-component plant-microbe systems of legumes and their potential for plant production

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Legumes develop different mutually beneficial symbioses with soil microbes, such as arbuscular mycorrhizal (AM) fungi, nodule bacteria and plant growth promoting bacteria. Symbioses supply the plants with nutrients (predominantly with nitrogen and phosphorus), protect them from pathogens and abiotic stresses and improve soil microbial biodiversity and fertility. The synergistic activity of beneficial soil microbes (BSM) on the plants has great importance for the use of multi-component symbiotic systems in low-input sustainable environmentally-friendly agrotechnologies. However, the complex nature of the AM symbiosis when in a multi-component symbiosis (plant-fungus-bacteria) creates complications for the fungus to produce AM fungal propagules and poses questions (a) about the effectiveness of the fungus per se in interactions with the plants, without associates, and (b) about the necessity of using sterile/axenic conditions for the production of the AM fungi based inoculants because of any mixing and competition by microbes from the inoculants with the local soil microbial consortia. The legume genes controlling interactions with BSM (including genes responsible for effectiveness of such interactions) should be considered as a united genetic system. The plant genome is more stable than that of microbes and therefore crop plants should select beneficial microbes and control the effectiveness of the whole plant-microbe system in the field for the benefit of the crop and therefore of human beings. There is clearly a need to breed legume crops with improved performance under sustainable conditions involving interactions with BSM and optimising the use of agrochemicals.

Application of different *Lactobacillus* strains in production pea seed protein hydrolysates with antioxidative activity

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In recent years antioxidant properties of plant derived protein hydrolysates were established. Antioxidative activity of these hydrolysates largely depends on specificity of the proteinase used for hydrolysis, duration of proteinase treatment, as well as composition and molecular weight of hydrolysis derived peptides. In order to explore potential use of nine proteinase containing *Lactobacillus* strains in production of antioxidative peptides we tested their ability to hydrolyze lyophilized hydrosoluble protein fraction from dehulled and defatted dry pea seeds (*Pisum sativum*), cultivar NS Mraz. We also developed plant protein based liquid medium for cultivation of lactobacilli which was later used as starter for hydrolysis reaction in larger volume. We found that all nine strains could hydrolyze plant hydrosoluble fraction of pea seed proteins but with different efficiency. We also examined the effect of duration of hydrolysis on antioxidative activity in <10kDa fraction of obtained peptide extracts, using most proteolyticaly active strain, human vaginal isolate *Lactobacillus rhamnosus* T10. General conclusion was that antioxidative activity was positively correlated with increase of hydrolysis duration and amount of small peptides, which reached up to 34% of total proteins after 24h of hydrolysis treatment.

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Effect of soybean co-inoculation with *Bradyrhizobium japonicum* and *Azotobacter chroococcum* on yield and nitrogen fixation parameters

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Fixation of atmospheric nitrogen plays a significant role from the point of agricultural production. Therefore, the aim of this research was to determine the effects of soybean inoculation with two different nitrogen-fixing bacteria on yield and nitrogen fixation parameters. A two-year trial was set up on experimental field of Institute of Field and Vegetable Crops from Novi Sad on chernozem soil using a randomized block design with four replicates. The soybean cultivar Galina and three variants of inoculation were tested: 1. *Bradyrhizobium japonicum,* 2. *Bradyrhizobium japonicum* + *Azotobacter chroococcum* + humic acid, and 3. *Bradyrhizobium japonicum* + *Azotobacter chroococcum.* The effect of inoculation on yield, pod number, seed number and seed mass per plant was determined. The effectiveness of nitrogen fixation was determined based on the number and mass of nodules and nitrogen content in aboveground plant parts, roots, nodules and seeds. The average number and mass of nodules, nitrogen content and yield of soybean obtained in response to inoculation were higher compared to the control in both years of research. Inoculation had a positive effect on nodule number (35% increase), nodule mass (40% increase) and nitrogen content (34% increase - roots, 62% - aboveground parts, 27% - nodules, 31% - seeds). Significantly higher yield increase (16%) was registered in the case of co-inoculation with *Azotobacter chroococcum*, while the best effect on nitrogen content, nodules and yield parameters was achieved in the variant with *Bradyrhizobium japonicum.*

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Session 11

Legumes in animal feeds: requirements and impacts

KEYNOTE LECTURE

Forage Legumes and Pulses in Ruminant Diets

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In temperate climates, most forage-based ruminant production systems have members of the Poaceae/Gramineae as the primary feed source. These forages range from highly productive, recently sown monocultures to permanent pastures of diverse botanical composition, and are utilised by grazing, conservation or a combination of both. Forage legumes (Fabaceae/Leguminoseae) occur or are included in some of these graminoid-based swards, and their most compelling benefit is where their presence increases the overall profit of the livestock enterprise. This is most likely to derive from an increase in herbage yield or nutritive value, or a reduction in the cost of providing livestock with feed. The presence of legumes in swards and pastures can also provide environmental benefits, although it may not always be feasible for farmers to obtain an economic reward for this service to society. The objective of this paper is to provide an overview of the impact of forage legumes and pulses in ruminant production systems, and to outline future challenges. These legumes are likely to impact on system parameters such as stocking rate, meat and milk production, meat and milk quality, animal wellbeing and productivity, environmental fluxes and, ultimately, profitability.

Antioxidant potential and total phenolic content of Serbian red clover cultivars

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Red clover (*Trifolium pratense*) is rich in phytoestrogens as well as other phenolic substances which contribute to its antioxidative properties. Still, its antioxidative potential has not been thoroughly investigated. The aim of this study was to determine radical scavenging capacity and total phenolic content of red clover cultivars from Serbia observing different plant parts. Leaves, stems and flowers from four red clover cultivars (Kolubara, Una, Avala, K17) were grounded and extraction with water, 3M HCl and ethanol was performed. Radical-scavenging capacity was measured by reaction with the stable DPPH (2,2-diphenyl-1-picrylhydrazyl) radical and calculation of the amount necessary to decrease initial DPPH concentration by 50% (IC_{50}). Total phenolic content (TPC) was determined according to the Folin-Ciocalteu method and expressed as gallic acid equivalents (GAE). Antioxidant potential and TPC of leaves and flowers were not statistically different, but on average, flower extracts had the highest antioxidant activity (IC_{50} 0.087 mg/ml) and leaf extracts the highest TPC (30.30 mg/g GAE). Sample with the highest antioxidant activity (IC_{50} 0.074 mg/ml) and TPC (32.95 mg/g GAE) was the flower of Kolubara cultivar. This cultivar also had on average the highest TPC (23.06 mg/g GAE) when observing all plant parts, while cultivar Avala had the highest antioxidant potential (IC_{50} 0.176 mg/ml). Leaves and flowers of Serbian red clover cultivars are rich sources of phenolic compounds with antioxidant potential. Stems were poor in phenols as well as in antioxidant activity compared with other plant parts. Cultivar Kolubara had the highest TPC and Avala the highest antioxidant potential.

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Sugar composition of green peas (*Pisum sativum* L.) for human and animal consumption

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The pea (*Pisum sativum* L.) is one of the most important legumes and it is one of the oldest subjects of breeding improvements. Planned breeding dates back to the 18th century when Knight performed the first cross breeding experiments on the pea plant. Since then, through systematic breeding and selection pea species were segregated according to their usage for human or animal consumption. The aim of this paper was to determine if there are differences in structure and content of individual saccharides among the collected samples from pea breeding programs for human and animal consumption. The analysis was conducted on green (immature) seeds of five accessions of both pea types (human and animal consumption) with the HPLC method. No significant differences were determined for α- galactosides (verbascose, stachyose and raffinose) between the tested types. Significant differences in monosaccharaides and disaccharides content in both pea types were determined through variance analysis. Accessions intended for human consumption had double the content of sucrose compared to accessions intended for animal feed with a significance level of $p \leq 0.01$. Types of peas for human consumption had a significantly higher content of glucose than types intended for animals. The content of monosaccharides fructose and galactose varied according to accession in both groups. The overall sugar content in types for human consumption was between 13.42% and 19.28% in absolute dry matter (aDM). The sugar content in pea types for animal consumption was between 7.83% and 9.93% in aDM. The results confirm the assumption that humans have, through continual selection, influenced the segregation of the two pea types according to purpose.

Nutritional value of Polish varieties of narrow-leaved lupine (*Lupinus angustifolius* L.) determined in experiments conducted on broiler chickens

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Seeds of narrow-leaved lupine can provide a good source of protein for broiler chickens (1). The objective of the performed experiments was to recognise the nutritive value of currently cultivated Polish varieties of narrow-leaved lupine and to evaluate possibilities of their application in broiler chicken nutrition. Four cultivars of narrow-leaved lupine (Botuta, Neptun, Sonet, Graf) were analysed. The following parameters were determined in "*in vivo*" experiments (60 Ross 308 male broiler chickens; one bird was treated as one replication): ileal (dry matter, crude protein, amino acids) and total tract (dry matter, crude fat) digestibility of nutrients, nitrogen relation and AMEn. For this purpose the difference method of digestibility determination and ME was used. It can be stated, on the basis of the performed investigations conducted on broiler chickens that the employed narrow-lupine cultivars failed to exert a significant influence on dry matter and crude protein ileal digestibility. The examined cultivars of narrow-leaved lupine were characterised by significantly differing levels of total tract digestibility dry matter - from 50.6% (Graf) to 61% (Neptun) and value of metabolisable energy - from 7.69 MJ/kg (Neptun) to 9.26 (Sonet) in feed as is. Furthermore, significantly different degrees of exogenous amino acid digestibility: lysine - from 79.4% (Graf) to 87% (Boruta), threonine - from 71.9% (Graf) to 78.8% (Boruta). The obtained research results indicate that the examined narrow-leaved lupine cultivars were characterized by high differentials in amino acid digestibility, metabolisable energy, as well as in total tract digestibility of dry matter.

Nutritional value of Polish varieties of yellow lupine (*Lupinus luteus* L.) determined in experiments conducted on broiler chickens

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The goal of the performed experiments was to recognise the nutritive value of currently cultivated Polish varieties of yellow lupine and to evaluate possibilities of their application in broiler chicken nutrition. Four cultivars of yellow lupine (Lord, Parys, Perkoz, Mister) were analysed. The following parameters were determined: ileal (dry matter, amino acids) (1) and total tract (dry matter, crude fat) digestibility of nutrients, nitrogen relation and AMEn. The performed experiments on broiler chickens show an effect of the varieties of yellow lupine cultivars on the significant differentiation of ileal digestibility of crude protein - from 77.5% (Mister) to 84.3% (Parys). The examined yellow lupine cultivars were characterised by differing levels of total tract digestibility: dry matter from 59.8% (Lord) to 64.1% (Parys) and crude fat - from 72.6% (Lord) to 80% (Perkoz) as well as metabolisable energy values - from 8.87 MJ/kg (Lord) to 9.60 MJ/kg (Parys) in feed as is. Moreover, ileal digestibility of amino acids was also determined in the analysed yellow lupine cultivars and it was found to range: threonine from 73.4% (Mister) to 81.1% (Parys). The obtained research results indicate that the examined yellow lupine cultivars were characterized by a high differentials in amino acid and crude protein ileal digestibility, metabolisable energy.

Nutritional value of Polish varieties of pea (*Pisum sativum* L.) determined in experiments conducted on broiler chickens

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The aim of the performed investigations was to expand our knowledge regarding the nutritive value of currently cultivated Polish pea varieties and to assess possibilities of their application in the feeding of broiler chickens. Five pea cultivars (Turnia, Sokolik, Muza, Milwa, Cysterski) were subjected to analyses. The following parameters were determined in experiments (72 Ross 308 male broiler chickens; one bird was treated as one replication): ileal (dry matter, amino acids) and total tract (dry matter, crude protein, crude fat) digestibility of nutrients, nitrogen relation and AME_N. For this purpose the difference method of digestibility determination and ME was used. It was found that the employed pea cultivars did not affect intestinal digestibility of dry matter and crude protein. Crude protein digestibility coefficient ranged from 71.7% (Turnia) to 75.7% (Muza), while that of dry matter – from 57.7% (Sokolik) to 62.3% (Cysterski). The examined pea cultivars were characterised by differing levels of total digestibility: dry matter – from 60.6% (Sokolik, Muza) to 67.1% (Cysterski), crude fat – from 68.0% (Cysterski) to 80.1% (Turnia, Sokolik), as well as the value of metabolisable energy – from 8.78 MJ/kg (Milwa) to 9.48 MJ/kg (Cysterski) in feed as is. Moreover, ileal digestibility of amino acids was also determined in the analysed pea cultivars and it was found to range: lysine from 49.8% (Cysterski) to 60.9% (Sokolik) and threonine from 55.4% (Milwa) to 71.1% (Muza).The obtained results indicate that pea is characterised by a significant variation in the total tract digestibility, amino acid ileal digestibility and metabolisable energy.

Phytase have beneficial effect in lupine diets for broiler chickens

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The aim of the study was to investigate the effect of lupine (yellow) in three different levels (5, 10, 20 %) and phytase addition on broiler chickens performance, Ca and P apparent retention as well as tibia ash. The experiment was conducted with 512 broiler male chickens, divided into eight dietary treatments, 8 replications with 8 birds in each. All of diets were deficient in Ca, available P and lysine and TSAA. Apparent ileal digestibility (AID) and retention (AIR) was determined using TiO_2 as a digestibility marker. Statistical analysis of results was performed using the General Linear Models procedure (GLM) of the SAS®. Use of phytase improved BWG (1747 *vs.* 1872 g), ($p<0.05$) and FCR (1.91 vs. 1.87), ($p<0.05$). There was tendency ($p<0.1$) to decrease BWG and increases FCR ($p<0.05$) after lupine inclusion. Phytase addition improved ($p<0.05$) AIR of P and Ca as well as AID of phytic acid. Tibia ash percentage was significantly ($p<0.05$) higher from birds fed diets supplemented with phytase than for the unsuplemented (45.1 vs. 47.7 %). Calcium retention was not significantly improved with phytase supplementation. Use of phytase improved AME level by over 100 kcal per kg of diet. Present trial is demonstrating that phytase may be used as a method to improve nutritional value of yellow lupine in broiler diets.

Antioxidant characterization of phenolics from *Trifolium scabrum* L. aerial parts

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Phenolic compounds have been recognized for many years as potent antioxidants. Isoflavones are naturally occurring diphenolics found mainly in the legume family. Our preliminary analysis suggest that *Trifolium scabrum* L. (rough clover) can be considered as very good source of these compounds for commercial use. Fourteen isoflavones (aglycones or their glucosylated derivatives) and two flavonol glucosides have been isolated and identified in aerial parts of *Trifolium scabrum* L. Free radical scavenging potential of these substances has been assessed by means of a simple benchtop TLC-DPPH$^\bullet$ bioassay. Standard compounds and the analyzed fraction were applied to aluminium-backed silica gel chromatographic plates. As the analyzed extract contained compounds characterized with a wide range of polarity different chromatographic systems were used to screen the free radical scavenging potential of individual compounds. In this study following mobile phases were used: toluene-ethyl acetate-formic acid (60:30:5, v/v/v) – for the least polar compounds; ethyl acetate-formic acid (95:5, v/v) – for the medium polar constituents and chloroform-methanol-ethyl acetate-water (20:40:22:10, v/v/v/v) for the most polar ones. TLC plates were immersed in freshly prepared 0.2% (w/v) methanolic DPPH$^\bullet$ solution. After removing DPPH$^\bullet$ excess, plates were kept in the dark for 30 min and then scanned by flatbed scanner. A comparison of the results was performed by an image processing program ImageJ. Antiradical activity was shown by following compounds: 3'-hydroxydaidzein-7-*O*-glc, isoquercitrin, calycosin-7-*O*-glc, kaempferol-3-*O*-glc (astragalin), daidzein, genistein and biochanin A.

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Hay quality of some clover (*Trifolium* sp.) species

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Clover (*Trifolium* sp.) plants grown in cool and moist places are available in natural pastures province of Ordu (Black Sea Region, Turkey). In this study, crude protein (CP), ADF, NDF, contents, relative feed value (RFV), Ca:P and K/(Ca+Mg) ratios of *T. hybridum* L., *T. aureum* Poll., *T. arvense* L., *T. meneghinianum* Clem, *T. scabrum* L. and *T. angustifolium L.* species collected from natural range were determined. The values of these plants harvested at flowering, are shown: CP ranged from 17.3 to 21.3%. While ADF varied between 32.5 and 40.2%, NDF ranged from 38.9 to 55.9%. According to RFV of species, hay quality ranged fair to prime (98.2-157.6). Ca:P and K/(Ca+Mg) ratios were calculated as 4.03 to 5.58, 0.80 to 1.52, respectively.

The use of legume crop residues in livestock feeding

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The human population of the world is increasing rapidly in recent. Therefore feeding of world population is becoming big problem in many places. Increasing of human food requirements induce more production. Because of agriculture areas are limited, food output gap try to cover by the obtain more product in unit area. One of the most efficient method in this issue is use of crop residues as livestock feeding. Crop residues are an important renewable resource that can be used in livestock feeding. Crop residue is defined as the non-edible plant parts and they are remain after the grain or marketable portion of the plant is removed. The most common is cereal grain straw from wheat, barley and rye, followed by corn stalks, grain sorghum residue and sugar beet tops. The utilisation of crop residues can be improved in several ways. Generally while cereal residues is grazed in field, legume residues are often collected as hay for sale or stall feeding. In addition, crop residues contain very low crude protein and a large proportion of lignocellulosic cell wall constituents. But the composition of chemical constituent and biological attributes of crop residue vary with different species. For example, legume crop residues have higher concentration of crude protein and digestible energy than cereal residues. So, despite their greater lignification, legume residues are degraded in the rumen at a faster rate than cereal straws, leading to a higher extent of degradation and, consequently, to higher dry matter digestibility. In this review, information about important of legume crop residues was presented.

Effect of soybean variety and processing on growth performance of pigs

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The presence of protease inhibitors prohibits utilization of the raw beans for food and feed. As a result of soybean breeding program at Maize Research Institute Zemun Polje, two cultivars with reduced amount of anti nutritional factors (Kunitz trypsin inhibitor-free cultivars) are developed. Feeding trials were conducted to compare the response of growing and finishing pigs to diets based on different soybean varieties processed in various ways. Feed mixtures were formulated to contain: 20% grits made by extrusion of standard grain quality soybean, 20% grits of extruded grain of Kunitz-free soybean cultivar, 20% row Kunitz-free soybean cultivar. The greatest improvement in growth performance, as indicated by average daily weight gain (DWG) and feed conversion ratio, was noted for the group fed extruded Kunitz-free soybean. Average DWG for this group was by 7.42% and 6.47% higher than for those fed mixture with raw KTI-free and extruded standard cultivar, respectfully. Growth parameters for group of pigs fed raw Kunitz-free cultivar were slightly reduced as compared to groups fed extruded soybean, either KTI free or standard cultivar. Although the nutritional value of raw Kunitz trypsin inhibitor-free soybean varieties is diminished by other heat-labile antinutritional factors present in grain, such varieties might be processed more economically with shorter heating time and lower temperature, or utilized in extensive farming systems.

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Effects of thermal treatments on Maillard reaction products, phenolic compounds and antioxidant capacity of soybean: Evaluation of potential risks and benefits

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Given that soybean must be processed before consumption, the Maillard reaction is the main chemical event occurring in this food ingredient during thermal processing. This study aimed to investigate potential risks and benefits associated with thermal processing of soybean. The browning indicators, furosine (N-ε-fructosyllysine), hydroxymethylfurfural (HMF), acrylamide and color were determined to evaluate heat effects induced during extrusion, infrared and microwave heating of soybean. In addition, the present work aimed to study the changes in the phenolic compounds, as well as in the overall antioxidant properties of different soybean products in relation to heating at 45 to 140°C during the processes. Soybean proteins were highly sensible to Maillard reaction and the Amadori product (furosine) was rapidly formed under slight heating conditions during extrusion and infrared heating. After a short time of extrusion and infrared heating at 140°C and microwave heating at 135°C for 5 min, concentrations of HMF increased to 11.34, 26.21 and 34.97 µg/g, that is approximately 9, 20 and 27 times higher than its initial concentrations, respectively. The present study suggests that the temperature need not be higher than 100°C for acrylamide to form in soybean. In addition, the reduction in moisture during infrared heating of soybean at 140°C to below 4% in the heat-treated soybean flakes was critical for the accelerating acrylamide formation. The results indicate that the complex structure of soya beans provides protection of phenolic compounds from thermal degradation, as well as that the newly-formed Maillard reaction products (MRPs) improved the antioxidant properties of heat treated soybean.

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Stereological analysis of *Medicago truncatula* Gaertn. stem as a pre-breeding technique to improve digestibility

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Structural characteristics of stem, especially the proportion of lignified tissues, have a strong impact on digestibility in many legume species. Anatomical analysis of stem of *M. truncatula* cultivars was performed using stereological method in order to examine genotypic variability in histological parameters that affect digestibility and assess tissue proportions along the stem maturity gradient. Lignification was first recorded in primary xylem walls. Volume densities (*Vv*) of phloem and xylem increased linearly from the top to the bottom of the stems. Lignification of parenchyma pith cells occurred simultaneously with intensive xylem development. On the contrary, the proportions of epidermis, mechanical and parenchyma tissue significantly decreased along the stem. Lignified secondary xylem was the tissue with the highest *Vv* and estimated as the one with the highest potential impact on digestibility. Therefore, the improvement of digestibility could be achieved through the reduction of its proportion. Analyzed parameters showed high among-cultivar variability, which could be useful as a starting point in breeding for improved digestibility. The most favorable ratio of lignified and non-lignified tissues was recorded in Mogul and Jemalong cultivars. Borung and Jemalong were the cultivars with the largest stem cells, which are easier to digest and contain higher amounts of cell solubles. *M. truncatula* cultivars had more favorable percentages of thick-walled cells in stems, compared to *M. sativa*. Stereological method proved to be useful as a pre-breeding technique in the process of the biological evaluation of forage cultivars and an improvement of their digestibility.

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Session 12

Getting the message out: grow, use, feed and eat legumes

KEYNOTE LECTURE

Getting the message out: grow, use, feed and eat legumes

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What is better than a legume? Its biological nitrogen fixation fertilizes the legume crop at low cost and provides high-value protein. It is better at liberating and taking up phosphorus and several mineral nutrients than most other crops, providing both nutrient use efficiency and end-product value. The side-effects of growing a legume crop include a better growing environment for the subsequent crop, improved food and feed security, and improved consumer health. Some things remain to be improved. The indeterminate growth habit of many legumes allows flexible response to the environment but leads to yield instability. Yields lag behind those of small-grain cereals. The amino acid composition of most species is not as good as that of soya bean. Most farmers in Europe are not familiar with growing legumes, and in order to persuade them to try, the message about the benefits to the cropping system, from farm to fork, needs to go not only to farmers and agricultural advisors, but also to policy makers and consumers. Pulse Canada and Pulse Australia do this successfully in their respective countries, and one of the roles of the Legume Society will be to emulate this in Europe. A consortium of some of our members has written a policy document for the European Parliament's Committee on Agriculture and Rural Development on "the environmental role of protein crops in the new common agricultural policy", explaining the farm-to-fork aspects of legume production and its role in agricultural sustainability in a policy context.

Legume proteins and peptides as compounds in nutraceuticals: a structural basis for dietary health effects

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In recent years, legume proteins have been recognized to have a nutritional role far beyond that providing amino acids for growth and mainteinance of body tissues. Not only small peptides, but also partially digested proteins of different size and even intact proteins from several legume seeds -soybean, lupin, lentil, chickpea, pea, common bean- have been found to play important physiological, hormone-like effects. Anticarcinogenic, hypocholesterolemic, glucose-lowering, antioxidant, antimicrobial, immunostimulant activities have been associated to enzymatic inhibitors, lectins, 7S and 11S storage proteins, as well as to peptides deriving from them, notably lunasin and hydrophobic peptides. Chance of modulating these effects through control of protein structure is the challenge of future research. A deep understanding of how structural stability of legume proteins impacts *in vivo* digestibility and release of bioactive sequences, with special concern of structural modifications occurring during technological processing and gastrointestinal digestion is required to improve nutraceutical potential of protein-rich plant foods. Major current knowledge about relationship between structural properties, digestibility and bioactivity of proteins in whole legume seeds will be presented here. Specific secondary structural elements, as recently revealed by Fourier Transform infrared (FTIR) spectroscopic studies, of relevance for protein and peptide bioavailability in processed legumes, will be pointed out and discussed.

Legume Improvement Program at AVRDC –The World Vegetable Center: Impact and future prospects

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Legume breeding atAVRDC– The World Vegetable Center currently focuses on two crops: mungbean (*Vigna radiata* (L.) Wilzcek) and vegetable soybean (*Glycine max* (L.) Merrill). High yielding, short duration mungbean varieties conventionallybred by AVRDC in conjunction with 29 national partners across Asia revolutionized the industry over the last two decades and increased global production by more thana third. To further improve productivity,the genetic base of the crop needs to be broadened by using parents from different backgrounds, and by interspecific hybridizationto tackle problems such as mungbean yellow mosaic disease. New sources of resistance to this disease have been identified in related species such as black gram (*Vigna mungo* (L.) Hepper) and are being introgressed into elite mungbean lines. Improving protein qualitybytransferring high methionine and bruchid resistance traits from black gram is also in progress. Improvement of vegetable soybeanwith higher pod yield, improved seed size and color, higher sugar content andbasmati flavor includes the use of selections from local landraces and the transfer of desirable traits from grain soybean.More than 3000 vegetable soybean breeding lines have been distributed to researchers worldwide, including those that are less sensitive to photoperiod and temperature to extend adaptability to tropical zones.Ongoing efforts to promote vegetable soybean as a nutritious legume in South Asia, Central Asia and Africa are supported by improvedseed supplies and recipes developed toadapt this vegetable to local consumption patterns.

The environmental benefits of incorporating legumes into arable systems

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Cultivated legumes are an important source of protein for humans and livestock. Currently, the EU import approximately 30 million tonnes soya annually, much of which comes from South America. Some of the demand for protein could be supplied from home grown legumes. In addition to meeting the protein demand, home-grown proteins deliver a unique combination of ecosystem services: food, feed, resource conservation and direct mitigation of adverse impacts on the environment. The purpose of this paper was to quantify the impacts of incorporating peas into a typical UK crop rotation to assess the implications on pollutants and pesticide use. The Farmscoper tool has been used to conduct the analysis. The non-legume crop rotation is winter barley, winter oil seed rape, winter wheat, winter wheat. This is compared against a legume rotation where a pea crop is incorporated between the two winter wheat crops. Standard recommendations for fertiliser applications have been assumed. For the winter wheat following the pea crop, the nitrogen fertiliser has been reduced by 30 kg N/ha, thus allowing for a carry-over effect from the nitrogen fixed by the pea. The results indicate that annual average level of ammonia, nitrous oxide and pesticide declines; however, there is an increase in nitrate, phosphorus and sediment. This is likely to be due to the higher nitrogen in the crop residue compared to other crops, and the increased length of time when there was no crop coverage. Nevertheless, these results do not incorporate any effect of legumes on improved soil structure.

Biofortification of pea

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Pulse crop seeds are rich in protein, slowly digestible starch, soluble and insoluble fiber, and are low in fat making them nutritionally outstanding foods. In comparison to cereal grains, pulses are also generally rich in micronutrients such as iron and zinc. Enhancing the concentration of micronutrients through plant breeding is called biofortification. We are exploring biofortification of pea by evaluating natural variation in micronutrient concentration in diverse germplasm, determining genetic control of micronutrient concentration in recombinant inbred lines, and developing germplasm low in phytate, a natural chelator of iron. Research progress will be summarized. Biofortification could further enhance the nutritional value of pulse crops in international food markets.

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www.nsseme.com

What is IFVCNS?

- A public, non-profit, non-educational and research organisation with more a 75-year long tradition
- Rooted in breeding numerous field and vegetable crops such as small grains, maize, sunflower, rapeseed, sugar beet, sorghum, soybean and other grain legumes, lucerne and other forage legumes, sorghum, vegetable and medicine plants
- Also skilled in agronomy, agro-ecology, biotechnology, biodiversity, seed production and organic farming of those field and vegetable crops
- Coordinator and participant of numerous national, bilateral and international research projects in different topics related to agriculture
- Dominant in the agricultural economy of the West Balkans and the South Eastern Europe
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How does IFVCNS contribute to legume research?

- By carrying out a flexible and complex network of diverse field trials
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- By acting as a solid and capable link between basic science and applied research, between the decision-makers and end-users and between the European Research Core countries and the Third Countries
- By developing a research networks within specific crop groups
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Saskatchewan Pulse Growers

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Saskatchewan Pulse Growers (SPG) is a not-for-profit organization representing and supporting more than 18,000 pulse crop growers in Saskatchewan.

Our Mission
Our mission is to provide leadership for a profitable Saskatchewan pulse industry through research, market development and communications. Our vision is to be the world leader in growth, quality, and value in the pulse industry.

Research and Development — Our program provides funding and leadership for ongoing research including: disease management, genetic improvement and quality assessment, agronomy and weed control, processing and utilization of pulse crops.

Variety Commercialization — SPG supports the development of new pulse varieties through the University of Saskatchewan's Crop Development Centre's pulse breeding program. Our Variety Release Program offers breeder seed without royalties to Select-status seed growers in Saskatchewan and Alberta, while specialty varieties are tendered to seed companies who bid on the opportunity to grow, disseminate, and commercialize seeds that are in a unique or niche market class.

Agromarket

www.agromarket.rs

Agromarket company is a private company founded in 1991 with its headquaters in Kragujevac, Serbia.

It is a leading company in sales and distribution of raw materials for agricultural production. It produces, imports, and distributes a range of agricultural and garden items meeting the high quality requirements of its customers. Besides the representation of leading manufacturers brands, Agromarket has developed its own brands **Villager** and **AGM**.

Agromarket is a leading supplier of pesticides on the Serbian market with a share of over 25%. It supplies the products of numerous prestigious companies such as DuPont, Syngenta, BASF, Bayer CropScience and Dow AgroScience.

The company's long-term goal is the development in the agrarian sector of Serbia and actively participation in raising the competitiveness of Serbian farmers. Its ultimate goal is to attain the position of one of the three most successful companies in the agricultural sector in the Western Balkans with a leading role in pesticide distribution and to achieve the high position of Villlager brand on the market of garden goods.

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In the rich world of global agriculture, diverse legumes can play key roles to develop environment-friendly production, supplying humans and animals with the products of high nutritional value.

The Legume Society was initiated in 2011 with two primary missions. One of them was to treasure the rich legume research tradition of the European Association for Grain Legume Research (AEP), with emphasis on carrying out its the triennial legume-devoted conferences. Another one is to fulfill a long-term strategy of linking together the research on all legumes worldwide, from grain and forage legumes pharmaceutical and ornamental ones and from the Old World to the Americas.

We do anticipate that the First Legume Society Conference will be a unique and genuine contribution to our common goals: to promote the legume research and all its benefits into all spheres of the society, linking science with stakeholders and decision-makers, and to demonstrate how an efficient, useful and firm network of the legume researchers of the world is possible and sustainable.

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