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ORAL SESSIONS

Challenges and opportunities for innovative research on legume nutrition and stress adaptation: an ecophysiologist's and phenotyping point of view

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Agroecology needs to reconcile agronomy and ecology, preserving and valorizing plant and microbe biodiversity. Legumes have merits for agroecology considering their ecological services. They constitute a protein source and their production relocation give merits for feed and food. However, they are still under represented due to both biotic and abiotic constraints. Research need to increase their profitability through higher and more stable yield and protein content, and new uses in a fluctuating environment [2].

Mechanisms which control nutrient use efficiency have to be highlighted i) considering nutrient acquisition, storage, remobilization [3] ii) under various conditions of water deficit or depressed nutrient availability, and iii) assessing plant and soil microbe interactions [4] which have important effects on the growth, nutrition and health of plants as well as on biogeochemical cycling.

This rely on using multidisciplinary approaches and different legume species, depending on the characteristics to be improved and available genetic and genomic resources. Some phenotyping platform under controlled conditions can modulate both plant genetics and microbiome. Phenotyping tools and methods involving also some "omic's" [5] can so generate large data sets concerning plant and possibly microbial traits. These are completed by environmental characterization which is part of phenotyping and need also both accurate tools and methods [6].

These data in turn feed models [7] which are crucial for a better understanding of plant physiological functions under various environmental conditions. This is essential to identify plants that are better adapted or resilient to stress and to characterize (beneficial) plant and microbiome interactions.

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Golden age of genebanks: using genetic diversity in the genomic era

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Keywords: genetic diversity, germplasm, genotyping

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Ensuring global food security now and for the future is one of the greatest challenges of our time. Food sovereignty and security, livelihoods, landscapes and environmental integrity are underpinned by agricultural biodiversity and its component genetic resources for food and agriculture. It is widely recognized that the genetic diversity of cultivated plants has narrowed as a result of domestication and connected bottlenecks. To avoid a permanent loss of diversity, conservation of plant genetic resources in form of *ex situ* collections were initiated by the Bureau of Applied Botany in Russia (1894) and United States Departmen of Agriculture (1862) and proliferated during extensive collecting missions of N. I.Vavilov in 1920-40 or F. Meyer (1905-44). These resulted in accummulation of over 7,400,000 accessions of most cultivated plant species in global network of germplasm collections today. World-wide, there are 1,750 genebanks and 130 of them preserve more than 10,000 accessions. CGIAR centers [1] collectively held around 800,000 seed accesions, 55,000 vegetatively propagated or living plants and 20,000 *in vitro* or cryopreserved accessions of 30 crop species and their wild relatives. After the cereals, legumes are the most abundant (155,000 acc.). Globally there are around 1 milion of legume accessions including forages [2]. In addition to genebanks, there are 2,500 botanical gardens which are important in the preservation and study of a broader spectrum of species, yet still relevant to agriculture. As security backup, the Global Seed Vault was established in Svalbard with a current stock of nearly 1,000,000 seed accesions of 6,000 species.

To efficiently catalogue such vast diversity, access to the information is criticaly needed. Several systems were established, the most universaly used being Germplasm Resource Information Network (GRIN)-Global. The Genesys online portal provides a single entry point to data on plant genetic resources maintained around the world. From 2017, Genesys brings together data from the CGIAR, European network (Eurisco), USDA and other genebanks into more than 3.6 million accession records from 434 crop collections.

The International Treaty on Plant Genetic Resources in Food and Agriculture [3], to which 144 countries are now party, has established a global Multilateral System to provide farmers, plant breeders and scientists with access to plant genetic materials and to ensure that recipients share benefits they derive from the use of these genetic materials, although there are controversial view on its effectivity and perhaps even preventing further plant exploration.

With some exceptions, the majority of germplasm maintained accessions are representatives of cultivated crops, of either registered varieties, breeding material or historical landraces. Although we know that Crop Wild Relatives (CWR) are a rich source of novel genetic diversity for crop breeding, their representation in collections and use is still largely poor.

So we have large amount of genetic diversity stored but not so much has been used so far. Despite we know that germplasm provides the resources for breeding to mitigate biotic and abiotic stresses, including climate change, breeders have been reluctant to use especially more primitive material such as landraces and CWRs. Knowledge derived from plant models as well as advancements in genotyping offers rapid selection of desired allelic combinations.

Traditionally, germplasm classifications were made by morphological descriptors, together with known pedigree and passport data. Owing to the progress in molecular methods, in past two decades genetic structures of major germplasm collections have been investigated. The historical phenotypic data [4] might now be efficiently exploited in combination with up-to-date genotyping using toolkits such as association mapping, as a strategy to gain insight on genes and genomic regions underlying desired traits.

So how can the available genomic technologies help us to better assess and access the genetic variation? We have reference genomes for most of the crop species. There are efforts to re-sequence also respective CWRs, since it has been shown that plant reference genomes might lack a number of agronomically important genes, restricting the gene space available for analysis. It became clear that for efficient trait discovery we need pan-genomes, e.g. resequecing and collating hundreds, perhaps thousands of genomes [5]. These results have shown that wild relatives indeed represent a significant source of new genes and alleles that were lost during domestication. Similarly important are advances in geoinformation technologies, to describe the environments from which genetic resources were originally collected and to which they might be adapted. These of course push further the issue of databases and data access. Results from genomics and agronomic research must be connected to the communities that are creating new cultivars of crops and germplasm diversity held in germplasm is instrumental.

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Development of genomic resources for narrow-leafed lupin

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Keywords: *Lupinus angustifolius,* grain legume, reference genome

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Narrow-leafed lupin (NLL; *Lupinus angustifolius* L.) is the main grain legume grown in Australia and forms an important part of sustainable farming systems, reducing the need for nitrogenous fertilizer, providing valuable disease breaks and boosting cereal yields. Over the last years we have developed a number of genetic and genomic resources for NLL. These include a draft genome sequence [1], development of various transcriptome libraries [2, 3] and a dense reference genetic map [1, 2]. The use of these resources has led to the identification of candidate genes for key domestication traits. In addition, candidates genes involved in alkaloid biosynthesis and regulation have been identified [4]. A new four-year project has recently started to expand these resources, including the generation of a pan-genome and a TILLING resource for the species. These resources will significantly improve and accelerate NLL breeding programmes and an overview of the developed resources to date will be presented.

Acknowledgments: This research is a Grains Research and Development Cooperation (GRDC) investment (9176622).

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ORAL SESSION

1.3

The origin and distribution of faba bean (*Vicia faba*) – new insight from the past

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Faba bean is a staple in the diets of many societies from North Africa to China and India. Despite its agronomic and economic importance, very little can be done to improve the crop sensitiveness to pests, diseases and environmental stress. All the subspecies are domesticated and useful genetic traits, often preserved in the wild type, cannot be selected to increase the crop resistance.

This work aims to identify the original distribution of the wild progenitor of faba bean and the routes used by prehistoric farmers to spread the domesticated types across the Mediterranean. The study is based on the survey of the findings of faba bean in archaeological sites, where the seeds can be preserved for thousands of years.

The data collected show that the wild progenitor grew on Mount Carmel (Israel) about 14,000 years ago and that the earliest domesticated types were cultivated in Lower Galilee (Israel) around 10,200 years ago. From this point onward, remains of faba bean started appearing in the Middle East, then in the Mediterranean, and later, in central Europe, following the dispersal routes of the Neolithic farmers.

Walking on the wild side: widening the genetic & adaptive diversity of domestic chickpea

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Keywords: wild & domestic Cicer, collection, adaptive diversity

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The narrow base of chickpea is a constraint to further improvement, reflecting the crop's unique evlutionary history [1]. The annual wild relatives have long been recognized as an excellent source of biotic and abiotic stress resistance [2], but until recently were themselves constrained by extremely limited collection. This bottleneck has been resolved by comprehensive surveying/collection of annual wild *Cicer* populations across altitude, temperature and rainfall gradients throughout SE Anatolia (Fig. 1). The world collection has been increased by > 1200 single plant accessions from 84 locations covering the Anatolian distribution range: *C. reticulatum* (n=589, 42 sites), *C. echinospermum* (n=282, 17 sites), *C. pinnatifidum* (n=252, 25 sites), *C. bijugum* (n=85, 6 sites).

These accessions are the basis of an international effort to phenotype adpative traits and introgress these into domesticated chickpea. Traits of interest include plant responses to water & VPD deficits (measureing both water use and productivity), tolerance to climatic extremes (cold, heat, chilling), low pH, regulation of flowering through photoperiod, ambient & vernalizing temperature, and resistances to ascochyta blight, phytophthora, and a range of nematode species. In each case, the screening of wild material is returning an impressive array of diversity much wider than found in domesticated chickpea, offering real opportunities for crop improvement.

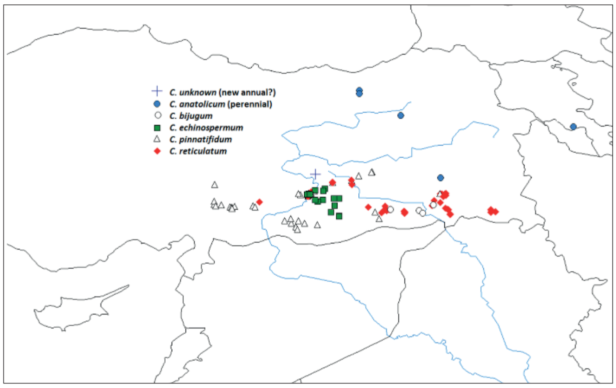

Figure 1. The annual wild Cicer collections from 2013-16 cover the SE Anatolian species range, sambling a broad array of habitats and underlining their discrete distributions

Acknowledgments: This work was supported by GRDC CSP00185: Collection, phenotyping and exploitation of wild Cicer genetic resources for chickpea improvement.

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The strange case of beans: *Phaseolus* domestication and convergent evolution

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Keywords: *Phaseolus*, convergent evolution, shattering

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Phaseolus can be considered as a unique model for the study of crop evolution, and in particular, for an understanding of the convergent phenotypic evolution that occurred under domestication.

The almost unique situation that characterizes the *Phaseolus* genus is that five of its ~70 species have been domesticated (i.e., *Phaseolus vulgaris*, *P. coccineus*, *P. dumosus*, *P. acutifolius*, and *P. lunatus*), and in addition, for *P. vulgaris* and *P. lunatus*, the wild forms are distributed in both Mesoamerica and South America, where at least two independent and isolated episodes of domestication occurred [1-7]. Thus, at least seven independent domestication events occurred, which provides the possibility to unravel the genetic basis of the domestication process not only among species of the same genus, but also between gene pools within the same species. Along with this, other interesting features makes *Phaseolus* crops very useful in the study of evolution, including:

(i) their recent divergence, and the high level of collinearity and synteny among their genomes;
(ii) their different breeding systems and life history traits, from annual and autogamous, to perennial and allogamous; and
(iii) their adaptation to different environments, not only in their centers of origin, but also out of the Americas, following their introduction and wide spread through different countries.

In particular for *P. vulgaris* this resulted in the breaking of the spatial isolation of the Mesoamerican and Andean gene pools, which allowed spontaneous hybridization, thus increasing of the possibility of novel genotypes and phenotypes.

Moreover, a key aspect of domestication is the convergent phenotypic evolution that is associated with the adaptation to a novel agro-ecosystem, and to human needs. The loss of seed shattering is a key trait in crop domestication, particularly for grain crops such as legumes. For wild plants, seed shattering is a crucial mechanism to achieve greater fitness, although in the agricultural context, this mechanism reduces harvesting efficiency, especially under dry conditions. Loss of seed shattering was acquired independently in different crop species by 'convergent phenotypic evolution', leading to similar low dehiscent and indehiscent phenotypes and to similar functional changes.

Figure 1. Wild (left) and domesticated (right) seeds and pods of common bean

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Development of orphan legumes for sub-Saharan Africa

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Keywords: underutilised, food security

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The Genetic Resources Centre (GRC) of the International Institute of Tropical Agriculture (IITA) has important collections of African underutilised legumes particularly Bambara groundnut (*Vigna subterranea*) and African yam bean (*Sphenostylis stenocarpa*) . We are establishing a platform for breeding of these crops by carrying out diversity analysis and phenotyping of key traits including yield, yield stability, nitrogen fixation, drought tolerance, nutritional profile and cooking time. Both of these crops have the potential to play a greater role in the smallholder agriculture of West Africa particularly given the likely impacts of climate change.

Bambara groundnut has centres of diversity in Nigeria and Cameroon and is grown in these countries as well as in the Sahelian region of West and Central Africa. It has significant potential in these areas as a drought and heat tolerant legume. Work in GRC is building a platform for the development of this crop. It is carrying out diversity analysis of accessions conserved in GRC using DArTseq. At the same time phenotypic evaluation of these crops is focused on addressing the key constraints to greater use. This includes analysis of yield, yield stability, drought tolerance, cooking time and nutritional quality including protein content Significant variation between accession s was seen for all these traits. At the same time, we have focused on developing a robust methods for crossing this species, which is very difficult. To date seed numbers from crosses remain low but they are increasing and we intend to undertake QTL mapping studies to complement GWAS for key traits. Research also focuses on nitrogen fixation-quantity fixed, characterisation of nodulating Rhizobia.

African yam bean is widely grown in sub-Saharan Africa. It has both edible seeds and tubers. Again, the potential of this crop in the development of resilient farming systems supplying a high-quality diet has not been developed. The GRC collection has been characterised and evaluated for key traits: yield, nitrogen fixation, nutritional quality of tuber and seed drought tolerance. Significant variation has been observed between accessions for key traits including yield, nitrogen fixation, drought tolerance and seed and tuber composition. DArTseq has been used to analyse the genetic diversity between accessions.

As described above the basis for pre breeding and breeding in these two crops have been made. However, much more is needed to develop their full potential. This requires awareness raising, innovation and capacity development across the value chain including processing. IITA has been involved ia number of discussion with partners, particularly, within Nigeria on these subjects and was instrumental in the creation of the Society of Underutilised Legumes bringing in many stakeholders across the academic, private and farming sectors in Nigeria.

These two crops are likely to be come of increasing importance with the expected and ongoing impacts of climate change in the Savannah and Sahelian regions of West Africa. Enhancing food security in these areas requires the development of resilience systems that can deliver nutritional value as well as calorific content. As legumes they will also of course have an important role in Integrated Soil Fertility Management.

Soybean breeding for early maturity groups

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Soybean (*Glycine max* (L.) Merr.) is used to a large extent as high-protein feed ingredient in livestock production. The increasing demand in Germany and Europe for GMO-free soybean products against the backdrop of the increasing cultivation of genetically modified seeds requires the expansion of national breeding activities and the development of domestic soybean varieties. Securing the domestic protein supply, in particular in the organic production of livestock and poultry feed, presupposes the availability of climatic adapted, high and constant yielding varieties with high protein content.

Due to the climatic conditions in (Southern) Germany, relevant breeding material has to be developed in the early to very early maturity gene pool of maturity groups MG00 to MG0000. For our activities at LfL, around 300 unselected and less characterized soybean lines from gene banks of the United States (GRIN-USDA), Canada (GRIN-CA) and Germany (IPK-GB) as well as approved elite varieties from France, Canada, Austria and Switzerland was used as prebreeding material. So far, this material has been repeatedly evaluated in field for agronomic traits and for their performance in Southern Germany. In parallel, the genetic diversity of these soybean lines and varieties was analyzed genome wide by using high-throughput genotyping (Soy6kSNPChip) and examined in selected candidate genes for breeding-relevant traits.

A set of 80 varieties and 214 gene bank accessions was genotyped with the Illumina Soy6kSNPChip containing 5,403 SNPs that are evenly distributed across the 20 chromosomes of the soybean genome. More than 1.4 Mio data points were generated and used to determine the genome-wide genetic diversity. A cluster analysis based on 4,869 SNPs with minor allele frequence (MAF) >5% and missing values <5% revealed a classification mainly according to origin separating Japanese, Chinese and European accessions. The current elite varieties cluster in a distinct branch and thus are genetically very close in comparison to the studied gene bank material. Our findings are in agreement with diversity studies of Hahn and Würschum [1] and Bandillo and coworkers [2]. No clustering according to maturity groups is observed.

Missing or low sensitivity to photoperiod is necessary for short-day crops, such as soybean, to adapt to high latitudes. Photoperiod insensitivity in soybean involves three important genes: *E1*, *E3* and *E4* [3]. We were assessing the genotypes of these maturity genes in our gene bank material to investigate the molecular diversity of these genes. Several different genotypes/alleles of the analysed genes *E1* (*E1*, *e1-as*, *e1-fs*, *e1-nl*), *E3* (*E3*, *e3-fs*) and *E4* (*E4*, *e4-SORE-1*) were found in the above mentioned soybean material. Beside the dominante alleles also the non-functional alleles as described in the literature were present. Especially for the candidate gene *E1* the non-functional allele *e1-as* is associated with early flowering [3]. Hence first molecular markers are successfully established and identified very early flowering and ripening accessions from Canada, Japan and China. Now the results have to be validated for further different environments.

Our results show that the current elite varieties are genetically very close in comparison to the studied gene bank material. In a next step, the narrow genetic base of the elite material will be extended by crossing selected high-protein and high yielding elite varieties with genetically divergent, early flowering and timely ripening as well as uniform maturation gene bank accessions. With our investigations we will lay the foundation for a powerful soybean variety development by optimizing the breeding and selection methodology, using efficient molecular marker approaches for breeders, farmers and consumers.

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Translational genomics for accelerating genetic gains in legumes

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Legume crops such as chickpea, pigeonpea and groundnut contribute to livelihood as well as human nutrition and are mostly grown in semi-arid regions in many Asian and African countries. Exposure of these crops to different biotic/abiotic stresses in marginal environments results in low crop productivity. Although several varieties have been developed through conventional breeding, the rate of genetic gains has been quite low in legume improvement programs in developing countries. Recent advances in sequencing, automation/robotics and computational biology have started an era of –omics sciences in legumes. A number of –omics approaches have been deployed to understand the genome architecture, genome diversity and complexity of trait. Modern trait mapping approaches have been used to map a number of agronomic traits. Molecular markers and genes identified through various approaches have been used to enhance precision and efficiency of breeding programs. A number of legume lines with improved traits related to production constraints as well nutrition developed through molecular breeding are in advanced stage of field trials in India and Ethiopia. In summary, our efforts highlight the role of translational genomics for developing climate resilient and nutritious varieties. In my opinion, accelerated and coordinated efforts in the area of translational genomics as well as fast-track release policy of molecular breeding products in combination of better agronomy will be helping to realize faster genetic gains in farmers fields.

Long non-coding RNA landscape of soybean and identification of lncRNAs potentially associated with agronomic traits

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Keywords: soybean, long non-coding RNA, three

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Recently, it has been revealed that eukaryotic genomes, including plant genomes, encode a multitude of non-coding RNAs. One class of ncRNAs are long noncoding RNAs (lncRNAs), which are defined as transcripts > 200 bp in length with no discernible protein-coding potential [1]. Multiple lines of evidence point to lncRNAs as important regulators, controlling the expression of protein-coding genes. lncRNAs are found both in the nucleus and cytoplasm, which suggests a diversity of modes of action, including chromatin modification acting as decoys preventing access of regulatory proteins, including splicing machinery, and microRNAs to their true RNA and DNA targets and acting as scaffolds for assembly of larger protein-RNA complexes.

Soybean is an important legume crop providing over 50 percent of oilseed production globally and can fix atmospheric nitrogen. Soybean plant exhibits many vegetative and floral development complexities. However, the lncRNA repertoire and the functional significance of lncRNAs in soybean are still mostly unknown. In this study we report genome-wide discovery, characterization, and functional annotation of lincRNAs in the soybean genome. Genome-wide lincRNA identification was performed using a combination of *de novo* and reference-guided assembly approaches generating a most comprehensive lincRNA database. Comparative analysis between soybean lincRNAs and other legume species was performed to identify lincRNAs, which could play universal roles in all legumes and the lincRNAs, which are soybean-specific [2].

We used over one billion RNA-seq read pairs from 37 samples representing nine tissues to discover 6,018 lincRNA loci. Our results showed that the lincRNAs had lower expression levels but show highly tissue specific expression patterns. Few of the loci were observed to be conserved in two other legume species chickpea (*Cicer arietinum*) and Medicago (*Medicago truncatula*), but almost 200 homeologous lincRNAs in the soybean genome were detected. Protein-coding gene-lincRNA co-expression analysis suggested the involvement of lincRNAs in signal transduction, developmental, and stress response. Positional analysis of lincRNA loci pointed in transcriptional regulation.

Our analysis also revealed that several lincRNAs potentially associated with agronomic traits. Six of the lincRNAs overlapped trait-associated SNPs, and the remainder were found in close proximity. The SNPs proximal to candidate lincRNA loci were related to traits such as number of days to flowering, number of days from flowering to maturity, and number of seeds per pod. In conclusion, the soybean genome encodes several thousand of lincRNAs, and several lincRNAs may be associated with agronomic traits. Further comprehensive investigations on their function and regulation, including identification of interacting partners and regulators of the lincRNAs, will reveal their mechanism of action in controlling plant development [3], [4].

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Lupin evolutionary stories through chromosome and transcriptome analyses

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Keywords: chromosome rearrangments, evolution, phylogeny

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Lupins (*Lupinus* genus, Genisteae tribe) belong to the important grain legumes along with soybean (*Glycine*), common bean (*Phaseolus*), and peanut (*Arachis*). *Lupinus* diverged from other legumes approximately 60 million years ago. They consist of approximately 270 species, divided into two groups known as Old World Lupins (OWL) and New World lupins (NWL). OWL include agronomically important crops, such as *L. angustifolius* (narrow-leafed lupin), *L. albus* (white lupin) and *L. luteus* (yellow lupin) and wild species as *L. micranthus*, *L. pilosus*, *L. digitatus*, *L. palaestinus*, *L. atlanticus*, *L. cosentiniiL. princei* [1]. Interestingly, OWL are geographically and phenotypically diverse, mainly represented in Mediterranean region and North Africa that highlights their adaptation to very different environments. In contrast to the NWL, OWL show a higher variability not only in chromosome number, but also in the basic chromosome number and genome size [2].

Here we report a first global view of the chromosome structure variation in entire OWL group, using available genetic resources for *L. angustifolius* [3, 4]. For this purpose, we established the complete genome-based cytogenetic map of *L. angustifolius* through assignment of its chromosomes and whole genome assembly. Comparative BAC-FISH mapping revealed puzzling chromosome evolution and highlighted the complex patterns of rearrangements leading to chromosome number reduction in lupins.

However, to better understand the mechanism that shaped lupin genome evolution we developed the transcriptome database for all OWL and also selected Genistae species. To get insight into lupin relationships we have studied homeologous (polyploidy-derived) genes using transcriptome sequencing. The transcriptomes were assembled into species-specific sets of genes and then compared based on average orthogroup count for each species. The well-resolved phylogenetic tree illustrated complexity of the structure and function of lupin genomes. Moreover, high level of similarities between lupins illustrate that diploidization process(es) could play pivotal role in shaping current wild and crop lupin genomes.

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Genetic analysis and gene identification of seed coat color integrating GWAS and mapping population in adzuki bean

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Keywords: adzuki bean, GWAS, seed coat color genes

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Adzuki bean is one of important legume crops. Seed coat color is an important domestication trait and morphological marker in genetics and offspring evaluation of breeding in adzuki bean. Seed coat color is closely relate with flavonoid and anthocyanin metabolism pathway. Flavonoid biosynthesis result in difference of seed coat color. The flavonoids have oxidation resistance, medicine and nutrition value. Seed coat color of adzuki bean influence on the paste quality for cake, commodity and nutrition quality. The abundant flavonoids mainly anthocyanin and *vigna c*yanidins make adzuki bean show diversity of seed coat color [1]. The almost all of wild adzuki bean has black mottle on grey seed coat. The vast majority of cultivated adzuki bean is red seed color, very few cultivars with black mottle on grey or red seed coat, and some cultigen with seed coat colors of beige, light brown, brown, ivory, greenish yellow, green, black, and golden. Seed coat pigmentation play a role in stress tolerance and resistance to disease.

We constructed thirteen mapping population of seed coat colors in adzuki bean. Genetic analysis predict the number of loci controlling seed coat color by phenotype analysis and chi-square test on F_1, F_2 individual plants, and F_3 families between seven seed coat color and red seed coat color. Ivory color is recessive to red color controlling by a single R locus. Black, black mottle, light brown, golden, brown are all dominant to red color. Red with black mottle, light brown, golden, brown color phenotypes were dominant to red and controlled by a single locus. Grey with black mottle was dominant to red and controlled by two loci. Black was dominant to red and controlled by two loci. Black loci showed dominant epistasis to another one. The genetic model of these seed coat colors was predicted.

We resequenced 331 accessions of wild adzuki bean (*V. angularis var. nipponensis*) and cultivated adzuki bean (*Vigna angularis*) including the accessions of eleven seed coat colors at average 8.42× depth, and identified 8,259,201 SNPs. A *VaBm* gene of black mottle on Seed coat color was associated on chromosome 8 using GWAS (Figure 1), and then was verified in the $F_{2:3}$ mapping population with this intragenic SNP markers. *VaBm* is uncharacterized, and has the conserved domain of *B-Bolivia* and *b1* alleles in maize. *B-Bolivia* confer aleurone-specific pigmentation, and *b1* encodes a transcription factor that regulates anthocyanin pigment expression [2]. Red seed coat color (*SDC2*) and black seed coat color (*SDC1*) were associated on chromosome 1 and chromosome 3, respectively (Figure 1), which are located on the same region of *SDC2* and *SCD1* mapping [3]. GWAS of purple mottle flower, purple mottle calyx, and purple mottle receptacle all associated to the same loci of *VaBm* on chromosome 8.

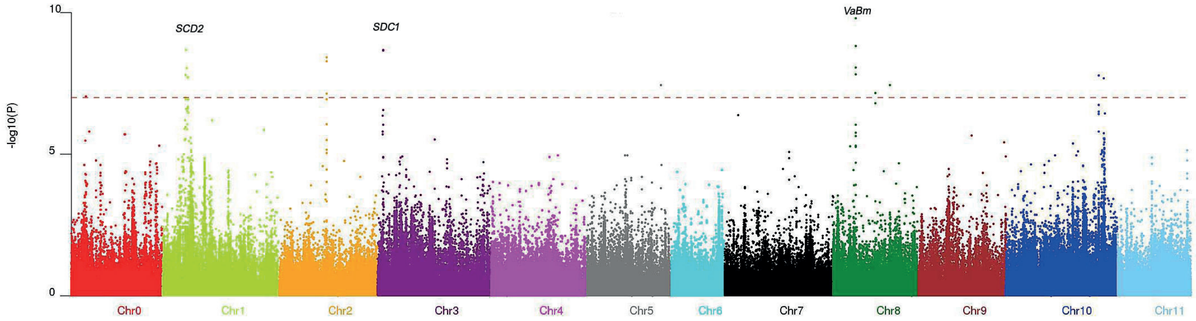

Figure 1. GWAS of seed coat color

Genetic transformation and functional verification of *VaBm* are being done. Genetic analysis of seed coat and gene identification in adzuki bean are very useful for further insight into the trait domestication, gene evolution, and genetically dissecting regulation mechanism. This research will supply the theoretical basis for improvement of functional nutrition quality in adzuki bean.

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Using genomic resources in grasspea (*Lathyrus sativus*) and pea (*Pisum sativum*) for comparative trait discovery

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The world population is expected to reach about 9.8 billion people by 2050, with Africa doubling its population in the next thirty years [1]. The impending climate change scenarios put further pressure on the sustainability of agriculture at the global level to feed the increased population. Plant sources, especially legumes, will have to play an important role in providing protein nutritional security. In order to achieve this, there is a critical need to develop legume varieties that are resilient to abiotic and biotic stresses.

Grasspea (*Lathyrus sativus*) is a hardy legume grown by resource-constrained farmers under adverse conditions for its tolerance to many abiotic stresses such as drought and soil salinity [2]. However, under these conditions its yield is low. It is also a relatively neglected crop [3]. The closely related pea (*Pisum sativum*) is a more widely grown cool season legume with high yielding potential. It is however susceptible to many abiotic and biotic stresses which preclude its wider adoption by farmers in developing countries. We have developed genomic resources, including whole genome and transcriptome sequences, for grasspea and pea. Analysis of the 8.1 Gbp genome of a European variety of grasspea, alongside the transcriptomes from three varieties of grasspea and pea genome and transcriptome sequences, is supporting trait discovery and genetic studies. The comparison of the two related genomes is facilitating the identification and characterisation of genes involved in resilience to abiotic and biotic stresses in both crops.

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Seed development in *Phaseolus vulgaris* L.: post-transcriptional regulation mediated by miRNAs

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Keywords: seed development, *Phaseolus vulgaris*, miRNA

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Phaseolus vulgaris, the common bean, seeds are rich in protein, carbohydrates, fibers, vitamins and minerals, being crucial for food security and nutrition [1].

Seed development is one of the most relevant developmental processes in plants. Our team has described the molecular mechanisms acting during seed development in the common bean [2], [3]. Despite these advances, it remains to be understood the regulatory mechanisms that underly the control of gene expression seen during seed development. Among those, post-transcriptional regulation of gene expression mediated by microRNAs (miRNAs) has been implicated in seed development. The knowledge of miRNAs that act during seed development is still limited, namely in the grain legume *P. vulgaris*.

In order to identify and understand how the accumulation of miRNAs may regulate gene expression, the dynamics of miRNAs expression (small RNA-Seq) were analyzed in 4 times points during seed development. The points represent different developmental phases from late embryogenesis to seed desiccation. Additionally, target prediction analysis and degradome sequencing were performed.

Expression of 72 previously described miRNAs as well as 39 new candidates was found during seed development. Target prediction, degradome and molecular interaction network analysis revealed several metabolisms under the putative regulation by miRNAs. Most of target genes belong to the MapMan functional categories of 'RNA', 'Stress' and 'Development'.

Genes encoding the NUCLEAR FACTOR Y (NF-Y) transcription factor subunits were predicted as targets of the MIR169 family members. One of those, the *NUCLEAR FACTOR Y, SUBUNIT A3* (*NF-YA3*) was previously shown to be required for embryo development in *Arabidopsis* [4] NF-YB and NF-YC. In *Arabidopsis thaliana*, NF-Y subunits are known to play roles in many processes, such as gametogenesis, embryogenesis, seed development, drought resistance, ABA signaling, flowering time, primary root elongation, Endoplasmic Reticulum (ER). Our results show higher expression of *NF-YA3* at late embryogenesis, where the abundance of the MIR169 members is lower.

The *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* (*SPL2* and *SPL12*) genes were predicted as targets of MIR156 family members. The network miR156/SPL has been shown to affect developmental phase transitions in plants [5] SQUAMOSA PROMOTER BINDING PROTEIN (SBP). The *SPL2* expression was found lowest at seed desiccation, in agreement with the highest accumulation of miR156 members.

In another case, the *STRESS INDUCED PROTEIN* (*EM1*) was found to be targeted by the candidate PC-3P-1761_3790 in our degradome analysis. The *EM1* expression was found highest at seed desiccation, where the lowest accumulation of the candidate miRNA was observed. The EM1 is a LATE EMBRYOGENESIS ABUNDANT protein which may have a role in seed desiccation [6] recalcitrant seeds are unable to survive drying. These desiccation-sensitive seeds constitute an interesting model for comparative analysis with phylogenetically close species that are desiccation tolerant. Considering the importance of LEA (late embryogenesis abundant).

Furthermore, qPCR expression profiles of selected miRNAs and target genes were performed to validate the sRNA-Seq analysis and target prediction. Our results present possible points of post-transcriptional regulation of gene expression by providing an overview of miRNAs and respective targets acting during seed development in *Phaseolus vulgaris*.

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Developing molecular markers for herbicide tolerance traits for the Australian pulse industry

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Keywords: herbicide tolerance, pulse, marker-assisted selection

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Pulse crops have become a significant component of Australian broadacre farming systems, with rotational benefits of nitrogen fixation, diversification and disease management. Weed control is a major constraint to pulse production in Australia due to a lack of in-crop herbicide options for broadleaf weed management, and this remains one of the main limitations to their adoption [1]. To address this, we have employed mutagenesis techniques to produce large M2 populations (~0.2 – 8 million seeds) of chickpea, lentil, faba bean and field pea as a source of novel genetic diversity. Bulk destructive field screening of these populations has identified several mutagenised lines with tolerances to various herbicides including AHAS-inhibitors, Photosystem II inhibitors and synthetic auxins.

To date, we have identified diagnostic genetic markers for target site tolerance to AHAS inhibitors and a PSII-inhibitor, as well as QTL and candidate genes for non-target-site tolerance to PSII-inhibitors. Through implementation of markers and high-throughput phenotyping methods from this project, an imidazolinone-tolerant faba bean variety, 'PBA Bendoc', became commercially available in spring 2018, only seven years after the identification of a single plant selection following mutagenesis.

Using mutagenesis techniques, we have developed over 250 mutagenised lines with putative synthetic auxin tolerance, with some lines able to tolerate up to 4x the maximum recommended herbicide rate for weed control in cereals. Linkage mapping and candidate gene sequencing in these novel pulse germplasm is expected to give a valuable insight into the genetic mechanisms of synthetic auxin herbicide tolerance - a trait with significant agronomic potential that is still poorly understood in any crop or weed species in the world [2].

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The pea genome and beyond

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Keywords: *Pisum*, genomics, breeding

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The recently generated pea genome sequence is a significant step for the pea research community towards unravelling functional diversity and establishing genome-enabled breeding. Re-sequencing data reveal the considerable diversity present in the *Pisum* genus. High-throughput genotyping is now available to explore large collections using the exome capture technology in genome-wide association studies (GWAS) or tackle map-based QTL cloning. Furthermore, genomic selection strategies have been developed in order to tackle complex traits such as yield regularity and improve selection efficiency. We will present snapshots of these results and discuss potential transfer of knowledge from pea to related crops.

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Using molecular marker diversity to infer morphophysiological and adaptive diversity of germplasm accessions: an alfalfa case study

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Keywords: landraces, *Medicago sativa*, molecular characterization

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Germplasm collections of major crops may include thousands of accessions whose morphophysiological and agronomic evaluation is prevented by high costs. Molecular marker-based characterization, which is getting increasingly affordable, may be used to infer morphophysiological and/or adaptive diversity, to discard sets of accessions expected to be agronomically similar from future evaluations or from core collections. Genotyping may exploit SSR markers, or SNP markers issued by arrays or more recent techniques, such as genotyping-by-sequencing (GBS). Genotyping DNA of large numbers of bulked plants instead of several single plants per accession was proposed for outbred species, such as alfalfa (*Medicago sativa* L. subsp. *sativa*) [1], or for heterogeneous populations of inbred species [2].

This study aimed to compare 4 genotyping strategies, as represented by SSR or GBS-generated SNP markers assessed on sets of single plants or bulked plants, for ability of their genetic diversity information to predict the morphophysiological and adaptive diversity of a set of alfalfa landraces from Northern Italy (Figure 1).

For each accession, DNA samples undergoing SSR marker and GBS analyses derived from (i) 5 single plants and (ii) 3 bulks of 100 independent plants (acting as replicates). Genotyping of bulked samples, described elsewhere [3], was based on 41 SSR markers showing polymorphism out of 65 derived from EST sequences or genomic DNA libraries, and 1274 polymorphic SNP markers showing reading depth per accession > 50 and no missing data. Genotyping of single plants relied on a subset of 34 polymorphic SSR markers, and 237 polymorphic SNP markers with no missing data. The morphophysiological evaluation was based on 11 traits described in [4]. Adaptation patterns were assessed for 10 accessions according to dry matter yield over 3 years across 4 contrasting environments, which displayed remarkable genotype × environment (GE) interaction [5]. Accession diversity was expressed by the dissimilarity matrix outputed by Pattern analysis of environment-standardized yield data for the adaptive responses [6], and the Euclidean distance for marker-based or morphophysiological diversity. We also considered the Euclidean distance for extent of drought stress at collecting sites (as the difference between potential ETP and available water in summer) because of its impact on GE interactions [5], and the geographical distance between collecting sites. The correlation across diversity measures was assessed by Mantel's test.

An AMOVA of single-plant marker data highlighted the small size of the between-population variance, whose size was 7.5% based on SSR and less than 1% based on SNP markers relative to the within-population variance.

High correlation between diversity measures outputed by the 4 genotyping strategies was found only for SSR- and SNP-based measures obtained from DNA of bulked plants ($r = 0.67$, $P < 0.01$). No marker-based diversity measure was correlated with morphophysiological diversity. Only the genetic diversity based on SNP markers from bulked samples tended towards correlation with adaptive diversity ($r = 0.34$, $P < 0.10$), whereas both SNP-based and SSR-based diversity measured on bulked samples were related to diversity for drought stress at ($r \geq 0.43$, $P < 0.05$) and proximity of ($r \geq 0.30$, $P < 0.10$) collecting sites. Adaptive and morphophysiological diversity exhibited modest association ($r = 0.29$, $P < 0.10$). In conclusion, no marker-based diversity could reflect properly the diversity layers of practical interest to breeders. However, the assessment involving wider sampling of accession DNA (via bulked sampling) and genomic data (via GBS-generated SNP markers) provided information fairly related to adaptive diversity.

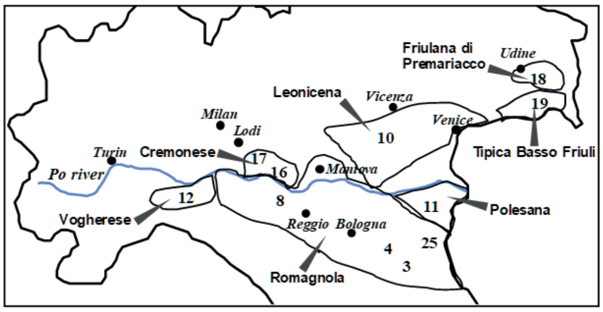

Figure 1. Collection site of 11 alfalfa landraces and production area of 7 historical commercial ecotypes from Northern Italy

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Application of genomic selection in Australian Pulse Breeding Programs

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Keywords: genomic selection, lentil, field pea

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Genomic selection charactertises the genetic diversity within a breeding pool and combines this with phenotypic data to allow the prediction of phenotypic performance of individual lines. This has the potential to significantly increase efficiencies within a breeding program and is exemplified through implications on the breeder's equation which states:

$$\text{The response to selection} = \sigma_g \times i \times r / L$$

Where
σ_g = genetic variation,
i = selection intensity,
r = selection accuracy
L = generation length.

Genomic selection can 1) provide more detailed knowledge of genetic variation to improve design of hybridization and increase genetic diversity, 2) increase selection intensity, particularly in early generations where optimal haploid values [1] can be determined on a large number of progeny seed such that only a few of the very best perfoming lines are advanced, 3) improve selection accuracy through the use of multiple phenotyping trials to increase prediction equation accuracies and 4) dramatically decrease generation length down to as little as 1-2 years as the phenotypic performance of F_2 progeny can be predicted from their genetic composition.

As a first step in evaluating genomic selection strategies, we used c. 2,000 and 1,500 advanced breeding lines from the Australian lentil and field pea breeding programs, respectively. A genotyping-by-sequencing approach was used to genotype the breeding material and over 150,000 SNPs were identified from both lentil and field pea. The lines were evaluated in a range of environments from 2010-2017 for lentil and from 2013-2017 for field pea, for economically important traits including grain yield, grain weight, disease resistances and abiotic stress tolerances. The ability to genomically predict the observed phenotypic performance was explored by forward prediction and applying a range of genomic selection models as well as incorporating GxE components. Moderate to high prediction accuracies have been attained for traits such as yield, grain weight, boron and salt tolerance as well as botrytis grey mould resistance in lentils (0.35-0.70).

Hybridisation strategies designed to increase genetic gain and reduce the overall generation interval are well developed. We are currently advancing F_4 seed from the first round of 4-way crosses and plan yield trials in 2020 as a proof of concept. We also have F_2 seed from our second round of intercrossing since the program began in 2016, highlighting the reduced generation length.

Field peas have shown much higher levels of GxE in regard to yield than was observed in lentils. We have therefore focused on more heritable traits such as ascochyta blight and downy mildew resistance, boron and salt tolerance, where prediction accuracies ranged from 0.23-0.52. Significant progress has also been made in defining economic values for these traits and data will be presented that allows the development of selection indicies in both crop that support genomic based htybridisation and selection programs for multiple traits simultaneously.

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Individual-based modelling as a tool to identify combinations of traits promoting overyielding in grass-legume mixtures

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Keywords: Individual-based model; grass-legumes mixtures; overyielding; trait divergence; complementarity; competition

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The role of many plant traits involved in carbon (C) and nitrogen (N) economy on species balance and total aboveground biomass production of grass-legume mixtures is still to be determined. A modelling approach using the Virtual Grassland model [1, 2] was developed to explore key trait combinations favourable to overyielding and mixture persistence.

The model was previously calibrated for alfalfa-based mixtures [3]. Taking this calibration as a departure point, a first step consisted in running a sensitivity analysis. This allowed us to identify the model parameter (i.e. plant traits) most sensible to inter-specific competition among 30 candidate traits. A second step then consisted in testing the impact of sensible parameter combinations (selected to account for distinct physiological functions: light acquisition, mineral N acquisition, kinetics of growth and resource use efficiency) on virtual mixture performance. Each trait combination was assessed in a virtual experiment based on the de Wit's replacement design (Fig. 1A) that allowed us to assess density-dependent overyielding (Fig. 1B). Three pedo-climatic conditions, differing by their N fertility level (0N, 120N, 300N), were analysed.

Based on our simulations (Fig. 2), maximal overyielding was achieved in cases where trait values were divergent for N acquisition (i.e. allowed complementarity in the use of different N pools) but convergent for light interception (i.e. limiting the asymmetric competition for light). The best combination of traits was not the same in all the pedo-climatic conditions tested and depended on the level of mineral N available into the soil. Random trait combinations could frequently lead to sub-optimal yields (i.e. less than a neutral situation of competition, Fig. 1) and even to under-yielding situations (i.e. less than the average of monocultures). Such situations were frequent when legumes displayed late growth kinetics.

Overall, our study demonstrates how heuristic modelling can help to identify the most favorable combination of traits in a given pedoclimate.

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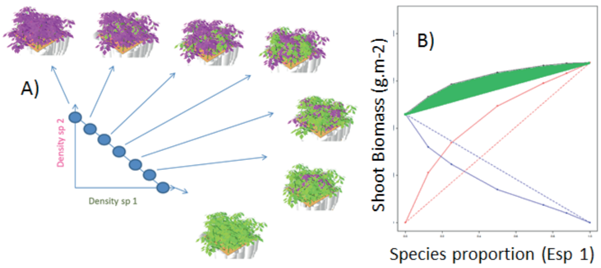

Figure 1. Virtual experiment design (A) and overyielding estimate (B, green area) used to asses a binary mixture – The example is for a neutral situation of competition where species differ by a single trait affecting N fixation

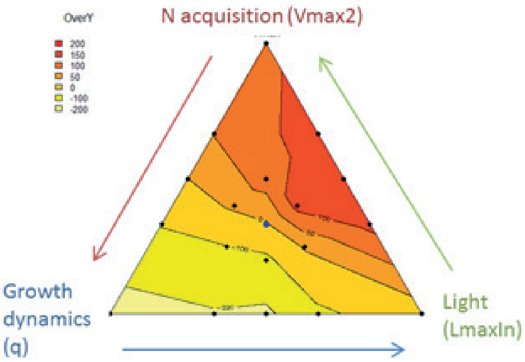

Figure 2. Example of overyielding map for the 120N fetility level – The central blue point represents a neutral situation of completion; each corner indicates the maximal divergence for a single trait value in favor of grasses

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A rapid gene-introgression platform to transfer *Cicer* wild alleles to cultivars (or a geneticist's paradise for legume evolution and adaptation studies)

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In legumes, the generation of rapid homozygousity through double haploid techniques have proven difficult to achieve [1]. Until recently, incorporating genes conferring valuable traits remained a slow and time-consuming process in the major crop legume species. Our group developed accelerated single seed descent (aSSD) platforms for chickpea, lentil, pea, lupin [2,3] and faba bean. The platform is now well-integrated into the Australian national pulse breeding programs handling over 40,000 individuals over three years. We cycle 5-7 generations per year from segregating breeding populations with gene fixation times equivalent to doubled haploidy. We apply phenotyping for herbicide, biotic or abiotic stress to individuals germinated from immature seed maintaining turnover times [4] and use genetic markers to customise the materials delivered to breeders.

In practical terms, when dealing with introgression of wild alleles into cultivars, often a major obstacle is interspecific hybridization barriers. In chickpea (*Cicer arietinum*, Ca), an additional impediment (and study opportunity) is the difference in life cycles between wild types and cultivars both in length and seasonal adaptation. The wild species in the chickpea's primary gene pool, *C. reticulatum* (Cr) and *C. echinospermum* (Ce), are long season plants with autumnal germination, spring flowering and summer maturation cycle while the cultivar is a short season plant, traditionally sown in the spring and maturing in summer [5].

Here we report on the adaptation of the aSSD platform to rapidly introgress wild *Cicer* alleles into cultivars and on derived findings on cytogenetics, phenology and evolution.

The rapid gene introgression protocol (RGI) for *Cicer* is based on the acceleration and compression of time to flowering and podding using long day length, tailored LED light spectra and temperature regimes, and the truncation of seed fill. The generation turnover is of 4-6 generations per year for segregating hybrid populations including short and long season types [6]. The protocol was validated using the large collection of wild accessions (described in [7]) and elite chickpea cultivars adapted to different Australian cropping areas. Through the modification of our aSSD platform we effectively synchronized time to flower and improved flower quality to efficiently achieve high proportions of true hybrids, accelerated homozygousity in parental lines, and developed F2-F7 populations derived from crosses between *C. reticulatum*, *C. echinospermum* and *C. arietinum*. The populations are available through the Australian Grains Genebank.

Beyond accelerated gene introgression and breeding outputs, there are substantial advantages in tailoring the platform for genetic studies varying in complexity, breadth and depth. For example, (i) accessibility to numerous F1 per Ca/Cr x Ce combinations facilitates the evaluation of chromosome pairing and identification of loci transferrable to subsequent generations and of effective value to the breeding programs; (ii) through the synchronisation of the plants' physiological state [8,9], the platform also facilitates the screening of phenology-dependent traits such as disease resistance and response to chilling at reproductive stage; and (iii) through cloning of F1 plants to produce large seed numbers and miniaturization of F2 individuals, we generate large numbers of recombinant families which enables us to identify the underlying genetic factors affecting adaptation of wild and cultivated *Cicer*.

Acknowledgments: This work was supported by the Australian Grains Research and Development Corporation (GRDC projects UWA00175 and CUR00024).

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Genes underlying early flowering trait in white lupin (*Lupinus albus* L.) and yellow lupin (*Lupinus luteus* L.)

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Early flowering is a crucial agronomic trait enabling successful cultivation of seed crops in temperate climate. In facultative long day plants like lupins, it is primarily conferred by lowering or elimination of vernalization requirements. Deciphering of genetic basis of flowering time control in particular crop has high potential to accelerate breeding process and development of new cultivars.

Lupins are legume plants rich in seed protein and appreciated in crop rotation due to improvement of soil structure and fertility. There are three main Old World lupin crops, i.e. narrow-leafed (*Lupinus angustifolius* L.), white (*Lupinus albus* L.) and yellow (*Lupinus luteus* L.) lupin.

In *L. angustifolius*, early flowering is conferred by dominant genes named *Julius* (in Europe) and *Ku* (in Australia). Recent studies revealed that just a single gene from *FLOWERING LOCUS T* (*FT*) subclade, named *LanFTc1*, underlies locus *Ku*/*Julius* [1][2].

Genes conferring this trait in *L. albus* and *L. luteus* remain unknown, thus we make efforts to elucidate the genetic basis of flowering time control in these species.

We performed genomic and genetic analysis of white lupin germplasm differing by time to flowering, including mapping population derived from Kiev Mutant (early flowering) and P27174 (late flowering). Thirty six flowering induction pathway genes were subjected to molecular marker development and linkage mapping. Markers representing 24 homologs were localized in 17 linkage groups. Four quantitative trait loci (QTLs) of flowering time were localized in three linkage groups. Three QTLs absolutely co-localized with *GIGANTEA*, *FLOWERING LOCUS T* and *SEPALLATA 3* gene-based markers whereas one neighbored the *FRIGIDA 3* marker by 3.6 cM. Analysis of the narrow-leafed lupin genome scaffolds, syntenic to the white lupin linkage group regions carrying QTLs of early flowering, evidenced to that these homologs are the best candidate genes for early flowering in *L. albus* [3].

Similar approach is being exploited to identify genes underlying early flowering in *L. luteus*. 21 flowering induction pathway genes have been chosen for molecular marker development. Two independent recombinant inbred line populations are being used for linkage mapping: Australian population derived from a cross P28213 x Wodjil and Polish population descending from Parys x PRH444/14.

Initial results obtained from this approach, supported by differential gene expression profiling using RNA-seq (60 unique RNA isolates, NovaSeq6000 method - paired ends, read length 100bp, 30M reads) indicated that early flowering in these accessions of *L. luteus* is conferred by several genes including one gene from the vernalization pathway and two genes from the autonomous pathway.

Elucidation of genetic control of flowering time in all three Old World lupin crop species: *L. angustifolius*, *L. albus* and *L. luteus* will enable improvement of future breeding programmes by implementation of marker-assisted selection targeting particular alleles. It will also provide valuable comparative data for studies on evolution of flowering induction pathway genes in legumes.

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Grain legume plant breeding in the EU

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The European Commission have published in November 2018 a new European protein plan that need to be implemented through National plan in preparation by the Member states, especially in France, Germany and Poland. The grain legumes are one of the targeted plants that the Commission wants to promote and support. In the meantime, we are witnessing the development of various niche or local value chains versus commodity (from feed to food). Moreover, several projects have been recently funded under the H2020 programme to look after a more diversified cropping systems, in which legumes will play a major role. Therefore, plant breeding remains the key factor to provide suitable varieties well adapted to the various pedoclimatic conditions with appropriate quality to meet the needs of these value chains.

There are 23 public research institute involved in grain legume breeding and 46 private plant breeders in the EU, with a total of 137 breeding programmes distributed among several grain legume species.

Grain legumes	**Field pea**	**Fababean**	**Soybean**
public breeding programme	11	9	11
"small" private breeding programme	22	13	11
"major" private breeding programme	7	3	4
TOTAL	**40**	**25**	**26**
Grain legumes	**Lupine**	**lentil**	**chickpea**
public breeding programme	11	2	4
"small" private breeding programme	6	2	2
"major" private breeding programme	1	0	0
TOTAL	**18**	**4**	**6**

Figure 1. Inventory of plant breeding programmes of grain legumes in the EU

The breeding programmes are located all over the EU, generally closed to the areas of production:

– Field pea and fababean in the Northern part of the EU, both in spring and winter types.

– Soybean in the Danube region with Italy and France, with several new programmes started in the North

– lupine mainly concentrated in Germany and Poland

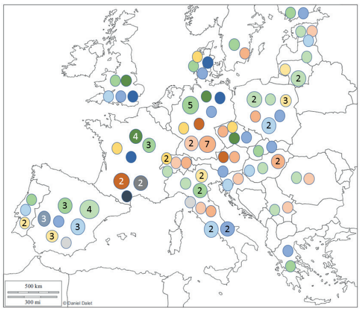
– Lentils and chick pea in the Southern part of Europe

A proposed typology of plant breeding is as follows, composed in three groups:

1-The public plant breeding programmes, mostly located in the South and East of the EU, with four main missions: registration of varieties in the absence of private breeders, proof of concept in genetic innovation (methodology, tools, new ideotypes), collaboration with private breeders (mainly pre-breeding) and finally the management of genetic resources.

2-The « small » breeding programmes that generally respond to local needs and for whom the registration of new varieties is quite slow. They are "astonishment" numerous in pea, fababean and soya, and they are precious relays to penetrate the different European territories.

3-The « major » plant breeding programmes with a targeted EU market and who rely on a significant return on investment . They often collaborate with the public research. It can be seen that there is no large breeding programme of lentils and chickpeas, and only one in lupine, and not so many in pea, fababean and soya.



Breeding programme	Public	Private « small »	Private « major »
FIELD PEA	●	●	●
FABABEAN	●	●	●
SOYBEAN	●	●	●
LUPINE	●	●	●
LENTILS	●	●	
CHICKPEA	●	●	

This first preliminary inventory requires a deeper analyses and surveys, but it hallowed the identification of several networks that strengthen the durability of the grain legume breeding in the EU. That is illustrated with the two following examples:

In Germany, a soya breeding program is set up at the state institute (Bayerische Landesanstalt für Landwirtschaft-LFL) in collaboration with private Bavarian breeding companies. Together, methods for the selection of breeding strains regarding early maturity, grain yield, protein yield and cold tolerance are developed, as well as a basis for a marker assisted selection. LFL produces the developed breeding material which is introduced into the breeding programs of the private partners and is to some extend prepared for variety approval.

In France, the GSP (Groupement des Sélectionneurs de Protéagineux), gathering 6 private breeding companies, collaborates with INRA for more than 30 years in pre-breeding in pea on particular traits difficult to handle alone. The long story of research efforts on *Aphanomyces euteiches*, started in 1995, is now reaching successful results with the first pea tolerant varieties at the stage of the registration process in France. Such kind of collaboration could be set up in lentil and chickpea, to accompany breeding programmes targeted to suitable European regions.

Thes examples demonstrate how it can be efficient to develop collaboration between public and private research.

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Diversification of feed protein sources for the food security – facts and myths

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Keywords: animal feeding, legume seeds, protein security

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Plant protein is a strategic stock in meat production. Its significance drastically increased as a result of the BSE disease and prohibition for using meat flour in animal feeding. Today the main source is a post-extraction soy meal. Its global production increased from 88 mln t in 1995 to 179 mln t in 2013 with about 60 mld € value. Main exporters of the soya meal (77%) are Argentina, Brazil, USA and the main importer of the global soya seed production is China (over 62%). An increased world population as well as meat consumption *per capita* resulted in the increased soya meal demand. In some countries with alarge population meat consumption *per capita* in 2000-2013 increased: for pork +40-100% and for poultry +40-180%. For meat producers the above-mentioned facts could be a sufficient reason to look for alternative sources of feed protein. Therefore, useful sources particularly in a moderate climate countries can and should be the rape meal and grain legume seeds.

Grain legume advantages are well known – high protein content in seeds, N-fixation (no N-fertilizing – savings and environment protection), an advantageous influence on soil fertility and yield of successive crops (legumes should be grown every 4-5 years on a given field = 20-25% in a crop rotation). Field experiments in Poland show that profit from grain legumes growing can be equal to cereals.

Thanks to a decreased content of anti-nutritional compounds legume seeds can be directly used in animals feeding (tripsine inhibitors must be removed from soya seeds) and results in pigs and poultry feeding are comparable or even better.

In countries with a large animal production for maintaining the so called protein safety the ±50% of domestic protein should be used. Decreased soya meal import by 1 mln t per yer gives above 300 mln € of savings. In the meantime in many countries a share of own protein sources is about 25% but grain legumes in crop rotation 2-3%. Which scientific disciplines can help to solve above paradoxes – genetics/breeding, growing and animal feeding technologies or economy/business?

Paradox and puzzle: legume-based agri-food and -feed systems in Europe

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Paradox: properly managed legume-supported agri-food and feed-systems are sustainable, and in Europe such systems are heavily legume-reliant. However, these legumes utilised are not home-grown but are imported and mainly as soybean for use as animal feed. Such high import dependence means that while the multiple 'societal benefits' of legumes are acknowledged and include: the provision of nutritious feed and food, natural nitrogen cycling, improved soil qualities, lowered greenhouse gas (GHG) emissions, plus, restoration and conservation of biodiversity and food culture; these benefits are forfeited as the legumes are not home-grown - and are used only at very low levels for direct human consumption.

Puzzle: coupled with this is the issue of potential feed-insecurity which stems from this high protein import dependency, as well as intensive farming which is geared mainly towards the use of synthetic nitrogen fertiliser dependant crops. This predominantly produces additional feed for farmed animals and drives a puzzle that must be solved. This is: *how do we supply food and feed in the face of growing global demand whilst also reducing high environmental and societal costs?* These costs include eutrophication, contributions to GHG emissions, biodiversity loss and the disease implications for humans, and farmed animals, of unsustainable diets. It is also noteworthy, given the current estimates of decarbonisation rates within the energy and transport sectors, that within the next 5 years agriculture will be the largest contributor to global GHG emissions. Yet, there appears to be no joined-up policies to tackle this 'perfect storm' of challenges, which are as local as they are global. Thus, legumes remain marginalised and fail to be recognised as essential components of sustainable agri-food systems.

The development of effective policies and strategies to help realise legume-supported agri-food systems is therefore critical. Historically, many consumers have found themselves passive recipients at the end of the supply chain. However, there is now growing social pressure encouraging consumers to engage in more sustainable consumption. In response, increasing numbers of consumers are questioning the nature of the food they consume, and the values associated with the food chain actors. Their concerns relate to nutritional value, food safety, authenticity and provenance, use of agrochemicals and antibiotics, and their collective socioecological impacts. Consequently, many consumers are now rationalising their meat and dairy consumption, and also aim to optimise the types of carbohydrate they eat to help offset obesity and diabetes epidemics. Yet, while this scenario is helping increase the consumption of legumes as food, there is no guarantee that these legumes are produced locally, ethically or in the most environmentally sustainable manner possible.

The EU-funded TRUE project was therefore established (www.true-project.eu) to identify and enable transition paths to help realise more-sustainable legume-supported agri-food systems in Europe. The TRUE consortium comprises 24 partners which span a balance of academics and non-academics from 10 different EU countries and Kenya. TRUE embodies the transdisciplinary approach, since transition from the current agri-food system paradigm demands that various 'lock-ins' are clearly identified and addressed in a holistic fashion.

Here we go beyond identifying some of the possible transition paths and explore mechanisms by which the transitions may be implemented using pilot studies and Work Package activities. We describe the resources and findings generated by the project to date and highlight a diversity of innovations. These range from breeding and precision agriculture, to outputs from the network of European Legume Innovation Network (ELIN) workshops which have been established.

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Strategies for production of organic winter peas in Germany and Austria

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In the beginning of the 2000s, winter peas were introduced into organic agriculture in Germany and Austria as a completety new crop. Meanwhile, winter peas are an important grain legume on many farms especially on poorer soils and under dry weather conditions. Introduction of winter peas helped to increase the amount of organic pea production.

There are two types of winter peas available in Germany and Austria: The first types are short straw varieties from France with relatively low winter hardiness, low weed suppression, white flower and large seed size. The second types are long straw forage type varieties with mostly good winter hardiness, good weed suppression, white or coloured flowers and often small seed size. This type is called Austrian field pea in North America. Conventional farmers prefer to grow French types for seed production, while organic farmers prefer long straw types. Long straw types are always mixed with a cereal partner to reduce lodging. Partners are triticale, rye, wheat or barley. Sowing density depends on the region and location. An average quantity is 150 seeds of cereals with 40 seeds of peas. At dry locations the amount of cereals is reduced and the amount of peas is increased. In wet regions or on soils with good quality, the amount of peas is reduced. Some organic farmers use the mixture with peas and cereals for silage.

Trials in Lower Saxony in 2013 and 2014 examined different varieties, seed densities and sowing dates [1]. Mixtures were sown between the middle of September and the end of October. In all cases damage during winter was lower with the later sowing dates. The early sowing dates enhanced biomass production, but the longer plants had a lower straw stability. The later sowing dates resulted in shorter plant height but higher grain yields of peas. In 2013, sowing between the end of September and the middle of October resulted in the highest yields, in 2014, sowing between the middle and end of October performed best. The seed density was between 50 and 70 seeds per m² for the peas, for triticale between 100 and 130 seed per m² on the sandy and dry soils. Highest yields of the peas could be reached with 60 seeds of peas and 100 seeds of triticale. For the earlier seed dates, triticale seeds had to be enhanced to 130 seeds for a better stability.

In 2017 and 2018, an exact variety trial was conducted in Ramsthal within the German knowledge transfer network for cultivation and utilisation of field peas and field beans (DemoNet ErBo). French varieties were compared to long straw varieties with white and coloured flower and with different cereal partners. Long straw varieties were sown with 40 seeds per m² and 150 seeds of triticale. Short straw varieties were sown with 80 seeds and 80 seeds of triticale like it is usually done for spring peas sown with cereals in mixed stands. In winter 2016/2017, the lowest temperature was minus 13°C but with a lttle snow cover. All varieties survived. The higher yield potential for French varieties was observed both in yield of the peas and total yield of the mixture. In winter 2017/2018, the lowest temperature was minus 17°C but without snow cover. French varieties died off completely and most long straw varieties with white flowers were reduced in number of plants after winter. Myster, Dexter and Flokon are French varieties. E.F.B. 33 is an old long straw variety with coloured flower, Pandora is a new long straw variety with white flower.

Table 1. Yield of peas and total yield of mixtures of selected varieties.

Variety E.F.B.33 = rel 100	Pea yield per plot (15m²) in kg	Relative to all varieties of peas (%)	Total mixture yield per plot (15m²) in kg	Relative to all varieties of total mixture (%)
2017				
Myster	4,05	166	5,15	141
Dexter	3,61	148	5,55	152
E.F.B. 33	2,44	100	3,64	100
Pandora	2,34	96	4,42	121
2018				
Dexter	0	0	6,38	81
Flokon	0	0	6,56	83
E.F.B. 33	4,18	100	7,87	100
Pandora	1,37	33	7,50	95

Grain yield of peas was highest with rye as partner in the mixture in 2017, but in 2018 it was best with triticale. For total yield of the mixture, results were heterogenous. More trials have to be conducted, especially as there is a high variation in results on different locations.

Acknowledgments: The project is supported by funds of the German Federal Ministry of Food and Agriculture (BMEL/BLE) under the Federal Protein Crop Strategy.

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Legume cover crops for soil conservation

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Keywords: legumes, cover crops, soil conservation

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One of the main challenges in agricultural practice in Pannonian plain of the Vojvodina Province is to maintain soil fertility within exciting crop rotation. Intensive agriculture in this region have caused significant decline in soil productivity. Thus, further production requires being more sustainable oriented. The content of soil organic matter directly influences the soil moisture retention which is important issue in the semiarid regions with increased precipitation variability.

There are several management practices that can be used to maintain or to increase soil fertility, such as reduced tillage, cover crops, and use of organic fertilizers. Growing winter cover crops seems to be the one of the most promising practices in the conditions of Vojvodina Province. Legume cover crops and mixtures of legumes with cereals are usually grown because they provide biologically fixed nitrogen (N) into the cropping system and improve soil organic matter [1].

The aim of the research was to analyze impact of winter legume cover crops on the soil water balance and water availability for the main (subsequent) crop.

The field experiment was conducted at the experimental station Rimski Šančevi in the Vojvodina Province, Serbia (45°19´ N, 19°50´ E, 80 m a.s.l.), between 2011 and 2013. The climate of the area is moderate continental with mean annual temperature of 11.4 °C and annual precipitation of 578 mm (long-term data, 1971-2015). The years 2011 and 2012 were considered as extremely dry, while the year 2013 was with moderately increased to normal values of moisture.

The experiment was conducted as a randomized block design with three replications and included winter cover crops used as green manure - common vetch sole crop (*Vicia sativa* L.), triticale sole crop (x *Triticosecale* Wittm. ex A. Camus) and their mixture, two N rates (120 kg N ha^{-1} and 160 kg N ha^{-1}) and the control (unfertilized and without cover crop). Cover crops were planted at the beginning of October of 2011 and 2012 and ploughed-in in May 2012 and 2013. Mineral N fertilizers were applied together with ploughing-in. After the cover crops were ploughed-in, silage corn was sown.

The soil samples were taken in four soil layers (0-30, 30-60, 60-90, and 90-120 cm): after the winter period (March), after cover crops ploughing-in (end of May), and after harvest of silage corn (September/October). The soil water balance was analyzed for the period before silage corn (March-May) and during the growing season of silage corn (June-September/October) using the formula by Krstić et al. [2].

The soil water balance was higher at plots with cover crops primarily because of a plant water uptake and of evapotranspiration, while fallow treatments (N and control) had water losses only by evaporation and leaching. However, more water use/losses were noticed in 2013 with favorable weather conditions. In both years silage corn on fallow treatments have a higher soil water balance, but values in extremely dry 2012 indicate lower productivity of silage corn plants. In the year with adequate precipitation distribution, like in 2013, cover crops had positive impact on soil water content and yield improvement of the cash crop.

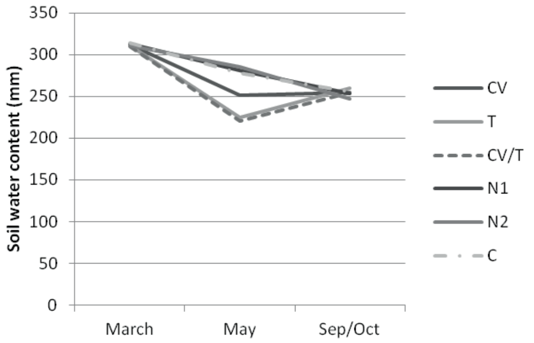

Figure 1. The effect of cover crops and nitrogen rate on soil water content in the soil profile from 0 to 120 cm at the locality of Novi Sad-Rimski Šančevi, CV – common vetch, T – triticale, CV/T – mixture of CV and T, N_1 – 120 kg ha^{-1}, N_2 – 160 kg ha^{-1}, C - control

Acknowledgments: This work was supported by the Provincial Secretariat for Science and Technological Development of the Vojvodina Province, project number 114-451-2180/2016.

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Improved grain yield of chickpea (*Cicer arietinum* L.) inoculated with arbuscular mycorrhizal fungi and plant growth promoting bacteria in a field trial under different water regimes

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Chickpea (*Cicer arietinum* L.) is widely cultivated and one of the most important grain legumes. It is a low-cost source of vegetable protein for human consumption. Once chickpea is mostly grown in rainfed environments, water deficit is a major constraint to plant growth and development [1].

To enhance productivity, irrigation is required for crop growth and development. Nevertheless, due to the global water scarcity it is important to reduce the use of water resources for agricultural purposes.

Plant growth promoting bacteria (PGPB) and arbuscular mycorrhizal fungi (AMF) have been employed as a biotechnology tool for ameliorating the impacts of drought on plants.

The aim of this study was to assess the effects of single and dual inoculation with PGPB and an AMF inoculant on the biomass, grain yield and protein content of chickpea in diverse water regimes.

After a screening in the greenhouse for selection of the best PGPB isolates, a field trial was conducted at Vila Real (41°28´N; 7°73'; 430 m) with the chickpea line CHK 3357. Three treatments were imposed: T1 = Control (non-inoculated), T2 = PGPB mix (*Mesorhizobium ciceri, Burkholderia* sp. and *Pseudomonas* sp.) inoculated through seed coating, and T3 = PGPB mix (*Mesorhizobium ciceri, Burkholderia* sp. and *Pseudomonas* sp.) inoculated through seed coating and a commercial AMF conventionally inoculated in the soil. The irrigation regimes applied during the crop cycle were: 1) 100% of plant water requirements (WR), 2) 50% of WR, 3) 25% of WR, 4) irrigation only during flowering (DI) and 5) rainfed (R). Each plot area was 2.4 m^2 and the seed rate was 12.5 m^2. The trial was set up in a randomized complete block design with three replicates. After harvest, number of seeds per plant, weight of seeds per plant, 100 seed weight, number of pods per plant, pods weight per plant, total plant weight, harvest index, grain yield and crude protein content were determined.

This study revealed an increase ($p<0.001$) of the total crude protein content in single and dual inoculation when compared to the non-inoculated controls, regardless the irrigation regime.

The results showed an increase in the number of seeds per plant, weight of seeds per plant and grain yield for plants inoculated with PGPB mix and PGPB mix+AMF, being the differences statistically significant ($p<0.05$) when compared to the non inoculated plants.

The plants inoculated with PGPB+AMF with suplementary irrigation during flowering (DI) presented the highest values for grain yield, number of seeds per plant, weight of seeds per plant, weight of pods per plant and total plant weight. The non-inoculated control under rainfed conditions showed the lowest values for the same parameters.

In conclusion we can say that single and dual inoculation improved grain yield and enhance grain quality, regardless the irrigation regime.

In drought scenarios, these beneficial microorganisms have the potential to tackle problems arrising from water scarcity and can be used as biotechnology tools to benefit sustainable agricultural production of chickpea.

Acknowledgments: This work was supported by portuguese national funds through Programa Operacional Competitividade e Internacionalização (POCI), Project 3599 – Promover a Produção Científica e Desenvolvimento Tecnológico e a Constituição de Redes Temáticas (3599-PPCDT) and Fundo Europeu de Desenvolvimento Regional (FEDER) under Project POCI-01-0145-FEDER-016801 and by FCT under Project PTDC/AGR-TEC/1140/2014". This work is supported by National Funds by FCT – Portuguese Foundation for Science and Technology, under the project UID/AGR/04033/2019. The first author also acknowledges the financial support provided by the FCT-Portuguese Foundation for Science and Technology PD/BD/128271/2017, under the Doctoral Programme "Agricultural Production Chains – from fork to farm" (PD/00122/2012).

Benefits of legumes/non-legumes intercropping: improved symbiotic N_2 fixation, nutrient use efficiency and soil fertility

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Keywords: chickpea, faba bean, intercropping, soybean, maize, symbiotic N_2 fixation

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Intercropping, especialy legumes/non-legumes intercropping, has been considered as one of sustainable agriculture practices. What we concern is the contribution of legumes to agroecosystems functioning, such as productivity, symbiotic N_2 fixation, efficient P utilization, and soil fertility in a long-term scale. Based on a long-term intercropping field experiment established in 2009 in Wuwei city, Northwest part of China, we analysed the collected data from 2015 to 2017, to address these aspects. The field experiment was a split-plot design, where main plot is P application rates (0, 40 and 80 kg ha^{-1}) and the split-plot was cropping systems (faba bean/maize, soybean/maize, chickpea/maize and rapeseed/maize intercropping, and faba bean, chickpea, soybean, oilseed rape and maize monocropping), with three replicates. There were 81 plots totally. Grain yield, aboveground biomass, nutrient uptake, biological N fixation, and soil physical and chemical properties were measured.

Grain yields of intercropped maize was significantly enhanced by intercropping with all three leguems, compared to monocropped maize. At the same time, the grain yields of intercropped legumes did not significantly differ from corresponding monocropped leugmes, with exception for soybean, whose grain yields were decreased by intercropping.

Intercropping significantly incerased P acquisition of two crops, compared with the weighted means of corresponding monocropped species. As a consequence, intercropping enhanced P fertilizer recovery by about 5 - 10 percentage points for faba bean/maize, chickpea/maize, soybean/maize, and rapeseed/maize intercropping. The grain yield, aboveground biomass, and nutrient uptake increased with increasing P application rates.

Intercropping increased significantly percentage N derived from air (%Ndfa) and amount of N derived from air (Ndfa) of legumes, compared with monocropping legumes (Fig. 1). The N surplus of soils under leguems/maize intercropping was significantly greater than monocropping.

Intercropping significantly reduced soil saturation infiltration rate and had no significant effect on soil bulk density. Under the intercropping condition, the content of soil available P and soil exchangeable K was reduced due to the increase of crop yield, compared with monocropping. Phosphorus fertilization had no significant effect on soil bulk density. Increasing P application significantly increased soil Olsen P, decreased soil available K, and had a significant effect on soil saturated infiltration. There was a positive correlation between soil P balance and Olsen P as well as between soil K balance and available K concentration in soils.

In summary, intercropping can significantly increase grain yield, aboveground biomass, and above-ground N, P, K nutrient acquisition, compared to monocropping. Intercropping significantly increased the biological N fixation of legumes and maintained the soil N balance. In addition, intercropping can better maintain the balance of soil P and improve the recovery of phosphate fertilizer. However, intercropping will accelerate the loss of soil K if there is sufficient K fertilizer application. Reasonable intercropping has positive effects on soil N and P balance and physio-chemical properties of soils. Thus, intercropping is one of the important practices for sustainable development of agriculture.

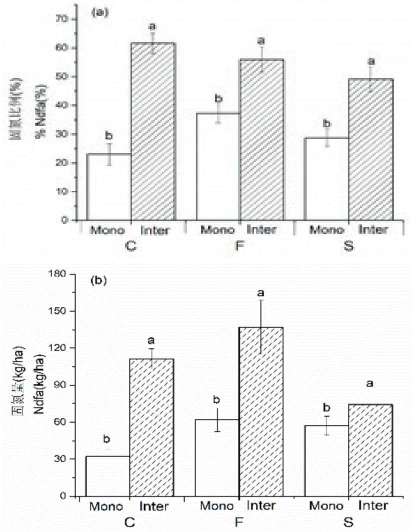

Figure 1. Percentage of N derived from air (%Ndfa) (a) and amount of N derived from air (Ndfa (b) of chickpea (C), faba bean (F), and soybean (S) under intercropping and monocropping across three years (2015-2017)

Acknowledgments: This work was supported by NSFC.

Intercropping of grain legumes with spring oat (*Avena sativa* L.) increases weed control and total yields in organic farming

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Keywords: intercropping, yield, weed suppression

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One of the challenges in the system of organic farming is the weed management. A possibility to reduce the density of weeds is the intercropping system that means the combination of at least two crops on the same field [1]. Intercropping reduces resources like nutrients and light for weeds and increases total yield per area and maintains yield stability [2,3]. In organic farming legumes play an important role in crop rotation due to their ability of nitrogen fixation and as protein rich feedstuff [4].

The objective of the 2-year field experiment focused on the differences in weed suppression and total yield of four grain legumes in sole and intercropping cultivation.

The field trial was conducted in a randomized block design with four repetitions at Trenthorst, located in northern Germany (sandy loam, 8.7°C, 740 mm), in the years 2015 - 2016. Two varieties of the grain legumes faba bean (*cv.* Fuego and Divine), field pea (*cv.* Muza and Lisetta), blue lupin (*cv.* Boruta and Boregine) and spring vetch (*cv.* Slovena and Ina) were tested sole cropped (SC) and intercropped (IC) with oat (cropping system). The faba bean and the blue lupin were intercropped in a legume:cereal seed ratio of 100:25 related to the sole cropped seeding rate. The seed ratio of the field pea and the spring vetch were intercropped in a seeding ratio of 50:50 related to the sole cropped seeding rate and due to the lack of lodging resistance of these both crops. The grain legumes and the spring oat were sown mid to end of April and harvested mid August til end of September depending on the maturity of the crops. Only the sole cropped faba bean, pea and blue lupin were harrowed if necessary. The data of weed biomass were measured by a manual harvest of one square meter in each plot at crop maturity. After harvest total yield and yield parts were recorded. The data set was evaluated using the statistic software SAS 9.4 and PROC GLM.

The ANOVA illustrated for the weed biomass under pea and spring vetch an interaction of year x cropping system. For both species, only in 2016 the weed biomass was lower in IC than in SC (Fig. 1). The choice of pea variety showed an additional effect: the normal-leafed *cv.* Lisetta with long stems had less weed biomass compared to the *cv.* Muza with shorter stems. A year x variety and cropping system x variety effect were given for vetch. In both years *cv.* Ina showed more weed biomass than *cv.* Slovena, but this effect was only significant in 2015. Faba bean and blue lupin showed significantly higher weed biomasses in SC compared to IC in both years (Fig. 1). Blue lupin showed an additional effect of cultivar due to the type of variety. The branched blue lupin (*cv.* Boregine) had a better weed suppression than the terminated cultivar (*cv.* Boruta). In addition to the cropping system, the type of variety is also an important factor for weed suppression.

The total yield was mainly influenced by interactions of year x cropping system, year x crops and year x cultivars. Among the crops faba bean showed the significant highest total yield in both years (2.52 t ha^{-1}, 5.04 t ha^{-1} respectively), but in both cropping systems total and part-ial yield of faba bean was higher in 2016 than in 2015. Total yield of all tested grain legumes was higher in the IC than in SC. Although spring vetch was sown in a low-er seed density, the partial yield of intercropped spring vetch (1.11 t ha^{-1}) was similar to the SC variant (1.22 t ha^{-1}).

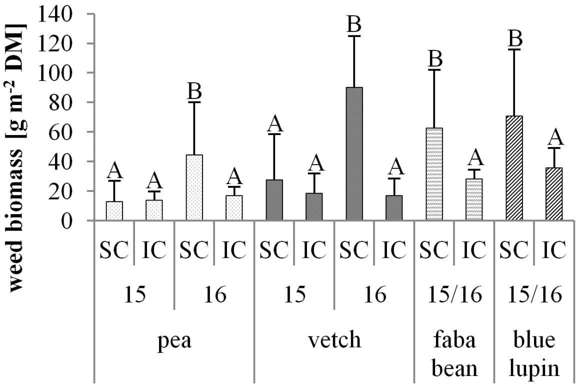

Figure 1. Weed biomass at harvest time [g m^{-2} DM] in sole cropped (SC) and intercropped (IC) grain legumes with oat in the years 2015 and 2016 (15, 16). Significant differences and interactions between sole cropped grain legumes and the respective intercropped x year are highlighted with capital letters for each grain legume ($P<0.05$)

The results demonstrate in the most cases a better weed suppression in intercropping systems, but type of variety plays an important factor, too. The diversity of the interactions points out the complexity of intercropping systems. Nevertheless, intercropping is an alternative way for reducing weed density while maintaining high total yields.

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Legumes in nutrition: understanding and applying scientific data today

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Keywords: bioactive molecules, food, in vitro assays

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During the last decades, a huge amount of data has been made available to the scientific community and to the society about the effects of legume consumption on human and livestock nutrition and health. Attention was especially placed on grain legume as such or on single isolated components. As a consequence, legumes have been seen under a new light in Western countries. This positive perception also bathes legume byproducts.

It is now accepted that several molecules from legumes that have been described as toxic or unhealthy components may have positive functions if considered from a wider point of view. The role of some of these molecules have been reconsidered and their biological activities exploited in pharmacological, medical, cosmetic and food applications. Because of the vastity of the seed components and the many facets of the topic, two very different approaches are feasible to produce scientific results about the biological activities and the effects of legume-derived molecules.

The first one is to put forth the most prominent results about each legume class of molecules, namely proteins, carbohydrates and non-nutrient compounds. The second one is to analyze separately every disease against whom legume seed components have been proved to be effective. Both have pros and cons.

The topic is hot and thus this area of study is rapidly expanding and new knowledge has been accumulating from day to day. The search for novel activities is pursued along with the elucidation of the underlaying molecular mechanisms of the observed effects, including intestinal absorption and bioavailability. One reason for this aliveness is the burst of consumers' interests toward nutritional/dietary issues and the increased industrial expectations that ensue. Another reason is the growing availability of analytical in vivo/in vitro methodologies, which allow more accurate analyses even on minor food components or when the sample is very small. Searching for a direct cause/effect relationships between food components and bioactivities has been providing a deeper understanding of the effects themselves and of the related mechanisms of action. The role of synergic effects of several legume seed components is now emerging.

Chemical, nutritional and functional characterization of Apulian black chickpeas

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Keywords: *Apulian black* chickpea, chemical characterization, chickpea flour, functional properties

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Black chickpeas historically cultivated in Apulia (South of Italy) were shown to display peculiar phenotypic and genetic features compared to the *desi* and *kabuli* chickpeas[1]. There are no scientific reports on the chemical and nutritional composition and the flour functional characteristics of *Apulian black* chickpeas. This ultimately limits their full exploitation by the food processing industry.

The aim of this work was to assess the chemical, nutritional and fuctional characteristics of the Apulian black chickpea in comparison with *desi* and *kabuli* chickpeas.

A wide germplasm collection of 57 different chickpea accessions (17 desi, 19 kabuli and 21 Apulian) from the ex situ repositories of the United States Department of Agriculture (USDA), the Department of Plant, Soil and Food Science of the University of Bari, Italy (DiSSPA), and the Institute of Biosciences and Bioresources of the Italian National Research Council (CNR-IBBR) was examined. Proximate composition, fatty acids profile, bioactive compounds and antioxidant activity were assessed together with fuctional properties of the flours.

As reported in Figures 1 and 2, the majority of the *Apulian black* chickpea accessions were clearly separated from both *desi* and *kabuli* chickpeas, showing peculiar chemical composition and functional properties of the flours. *Apulian black* chickpeas were characterized by higher content of dietary fiber, PUFA, bioactive compounds, and antioxidant activity than *kabuli* type, comparable to those of *desi* type; on the other hand, they were characterized by lower content of proteins and ashes than *desi* chickpeas. Regarding the functional properties of the flours, the pigmented accessions were characterized by high values of the Water Absorbtion Index, which makes them suitable for mixing with cereal flours to produce cereal-pulses foods, such as vegetable/vegan burgers or bread and other bakery products, maintaining a soft texture of the end-product. Low values of Oil Absorbtion Capacity were determined in *Apulian black* chickpeas, thus making them less suitable for food applications where oil incorporation is required, such as meat extenders.

Our findings highlight the nutritional and technological potential of local landraces of *Apulian black* chickpeas, which are particularly suitable for human nutrition and for the preparation of legume-based foods.

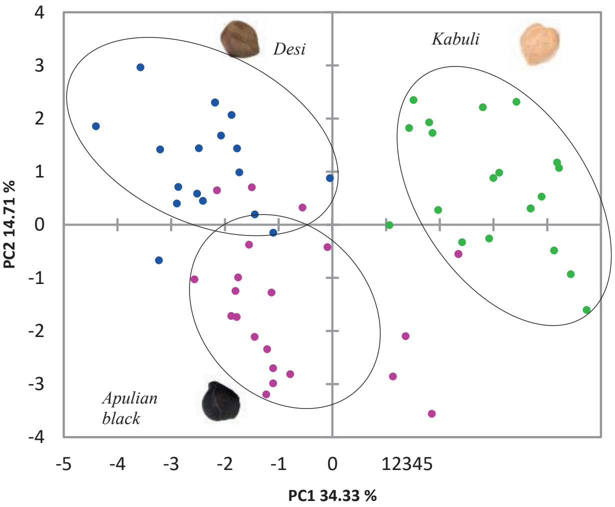

Figure 1. Loading plot of the PCA analisys of 57 chickpeas accessions considering the chemical, nutritional, and functional data

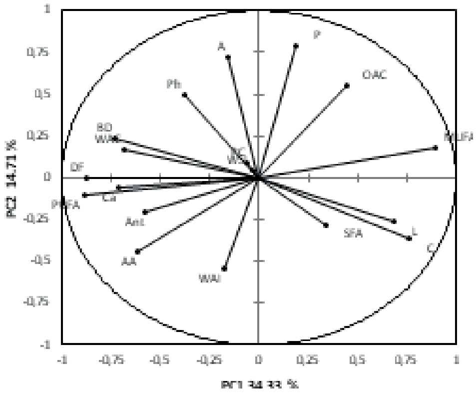

Figure 2. Score plot of the PCA analisys of 57 chickpeas accessions.

A=Ash; P= Protein, L=Lipid; DF=Dietary fiber; C=Carbohydrates; PC= Phenolic compounds; Ca=Carotenoids; Ant= Anthocyanins; AA=Antioxidant activity; Ph=phytate; SFA= Saturated fatty acids, MUFA=Monounsaturated fatty acids; PUFA=Polyunsaturated fatty acids; BD=Bulk density; WAI= Water Absorbtion Index; WAC= Water Absorbtion Capacity; OAC= Oil Absorbtion Capacity

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Pea biofortification update

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Keywords: pea, *Pisum sativum* L., biofortification, phytate, carotenoids

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Field pea seeds, like those of other pulse crops, are rich in protein and slowly digestible carbohydrates. To further enhance their nutritional value, research is in progress to support breeding for increased concentration and bioavailability of key micronutrients. In previous research, we identified low phytate pea lines and demonstrated that these lines deliver up to 2-fold greater bioavailable iron to human cells in an *in vitro* assay. Carotenoids may also enhance iron bioavailability. Current research aims to evaluate the effect of low phytate/high carotenoid pea diets on iron bioavailability in chickens. This will be followed by a study with female athletes, who often have low iron levels which affect health and exercise performance. From a breeding point of view, advanced pea lines have been developed carrying the low phytate trait combined with good agronomic performance. Using genomic approaches we have identified SNP markers in pea associated with seed concentration of iron, zinc, selenium, and folates and these will be utilized for marker-assisted selection for improved nutritional value.

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New varieties of *Lupinus angustifolius* as a source of interesting nutrients for human

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Keywords: *Lupinus angustifolius,* nutritional value, bioactive compounds

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Currently three lupine species of are used in food technology, mainly due to their excellent functional properties. However, in Poland lupine is still an ignored component of food, and although products with the lupine addition are produced in Europe and present in Polish grocery stores, some consumers are unaware of the lupine nutritional properties.

Therefore, as part of the SEGENMAS Project, nutritive value of new Polish varieties of *Lupinus angustifolius* were studied. The seeds were delivered from two cultivation places and two harvesting years. They were compared in terms of the content of: protein, amino acids, trypsin inhibitors, carotenoids, tocopherols, oligosaccharides, fatty acids as well as in terms of immunoreactivity and digestibility.

The protein content in the studied seeds was 28%-41%. Regardless of the place of harvest, the seeds from 2015 were characterized by a higher protein content compared to those from 2016, which must be explained by the weather conditions.

The qualitative and quantitative analysis of the amino acid composition of seeds of cultivars lines was carried out. The obtained results showed that the tested lupine seeds are particularly rich in leucine, threonine and lysine. A significant effect of precipitation sum on the amino acid composition of seeds was observed. Importantly, in the seeds of some of the varieties studied, not sulfur amino acids (Zeus, Salsa and Graf) but valine was a limiting amino acid. The quality of the lupine proteins were controlled also in the digestibility *in vitro* test. The average digestibility determined was 45.8%, and it was decreased compare to some literature data because of whole seeds (with hull) used in the digestion experiments; but still it was significantly decreased in the second year of harvesting.

The percentage of fat in lupine seeds was in the range of 3.37% - 6.65%. It was found that oleic and linoleic acids dominated in the lipid composition. The seeds contained significant from nutritional point of view acids form Ω-3-family, but also the presence of saturated acids (palmitic and stearic) was determined.

Four oligosaccharides were found in the seeds: sucrose, raffinose, verbascose and stachyose (which was the dominating one). As usually, the place of cultivation significantly influenced the content of those compounds.

The total content of all phenolic compounds was 96.50-300.73 mg/100 g d.m., significance of cultivation place and year of harvesting was noted. From the phenolic acids gallic acid content was the highest, while protocatechic, p-hydroxybenzoic, caffeic acid, p-coumaric acid were present in trial amounts. However, compare to those acids, larger amount of specific flavonoids were detected: apigenin 6,8-di-C-β-glucopyranoside, 7-O-β-apiofuranoside-6, apigenin 8-di-C-β-glucopyranoside, as well as four not identified.

It was found that the narrow-leafed lupine seeds contain four carotenoids: polytaxin, lutein, zeaxanthin and β-carotene. γ- and α-Tocopherol were detected in the seeds, while no β- and trace amounts of δ-tocopherol were noted.

Immunoreactivity of the varieties resulted mainly from the cross-reactivity of sera obtained from individulas suffering from soy allergy with the *Lup-an 2* fraction, although they did not react with the allergic fraction *Lup an 1*.

In conclusion, the significant impact of both genotype and phenotype on the analyzed features of lupine seeds was noted. Therefore, in spite of the fact that the tested seeds showed favorable content of pro-healthy constituents, it is not possible to indicate a variety with outstanding beneficial properties.

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Faba beans (*Vicia faba* L.) and field peas (*Pisum sativum* L.) as feedstuffs for broiler chicken: precaecal protein and amino acid digestibility

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Keywords: amino acids, grain legumes, poultry

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Especially in areas with suboptimal conditions for the cultivation of soybeans, homegrown grain legumes, like faba beans and field peas, are very important protein feedstuffs for monogastric animals, like swine and poultry, in organic farming. Homegrown grain legumes contribute to the circular ecology of organic farms and add to the supply of essential amino acids to the diet.

However, their value is not only determined by the crude protein content and amino acid profile but also by the bioavailability of crude protein and amino acids. Since only precaecally absorbed amino acids are potentially available in the metabolism, the precaecal digestibility of the crude protein and the amino acids can give a hint on their bioavailability.

The aim of the study was to determine the precaecal digestibility of each a tannin-free frequently used faba bean (*Vicia faba* L.) and field pea (*Pisum sativum* L.) variety in young broiler chicken.

The precaecal crude protein and amino acid digestibility of the single feedstuffs was determined in 21-day old broiler chickens using a linear regression approach [1].

Crude nutrient analyses were conducted using VDLUFA methods (crude protein with N*6.25, [2]), amino acid contents were analysed with HPLC modified Cohen and Michaud [3], and TiO_2 contents were determined photometrically [4].

Diets containing three different levels (30, 50, and 70%) of the test feedstuff in exchange for maize starch (40, 20, 0%) and a defined amount of the marker TiO_2 were fed to birds for one week until they reached 21 days of age. Each diet was fed to six groups of 18 broiler chicken. The test feedstuff was the sole source of additional crude protein and amino acids. The birds were killed by asphyxiation with CO_2 and the digesta was gently flushed from the terminal two thirds of the ileal section between Meckel's diverticulum and 2 cm prior to the ileo-colonic junction with distilled water. The intake of the crude protein and amino acids was related to the precaecally digested amount of the crude protein and amino acids by linear regression analysis. The slope of the regression is used as predictor for the precaecal digestibility coefficient.

Bird performance was comparable to previous studies and high inclusion rates of faba beans and fiel peas did not lead to feed refusal or health problems. The relation between ingested and precaecally digested amounts of crude protein and all amino acids was linear. Figure 1 shows exemplary regression lines for lysine.

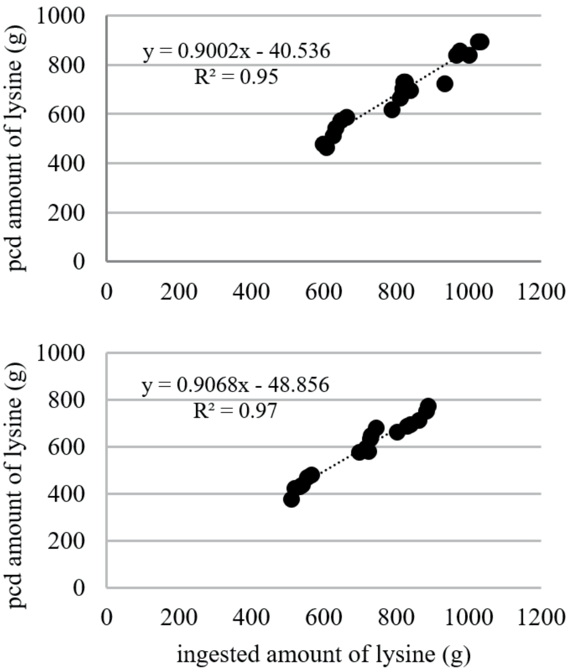

Figure 1. Linear regression of lysine ingestion and precaecally digested (pcd) lysine in faba beans (above) and field peas (below) in 21 day old broiler chickens

The standardized precaecal digestibility of the amino acids was comparable or higher compared to literature results. Crude protein digestibility reached 84% and 81% for the faba beans and the field peas, respectively.

The digestibility of the crude protein and amino acids of organic field peas and faba beans is good. Thus, organic field peas and faba beans are suitable feedstuffs for young broiler chickens.

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Health benefits and potential uses of narrow-leafed lupin (*Lupinus angustifolius*) seed beta-conglutin proteins in functional food

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Keywords: inflammatory-related diseases, insulin resistance, molecular nutraceutics, sweet lupin, type 2 diabetes, vicilin

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Lupins are an important PULSE in many parts of the world, with a wide range of agricultural benefits, i.e. seed β-conglutin protiens have anti-fungal activity [1]. Current interest for lupin seeds is growing motivated by the increasing knowledge of the health benefits (nutraceutical properties) that lupins provide [2], as promising innovative food ingredients and as an important source of proteins for humans.

Furthermore, *Lupinus angustifolius* (narrow-leafed lupin, NLL) seeds are a valuable source of proteins for human consumption with nutritional and nutraceutical properties, particularly those from the vicilin or β-conglutin family, which is the most abundant proteins in NLL seeds [3].

In our studies, we have assessed at the molecular level the capacity of different β-conglutin isoforms (b1 to β4, and β6) to improve the pro-inflammatory state that cause the development and maintenance of type 2 diabetes (T2DM) using *in vitro* and *ex vivo* approaches.

In addition, conglutins b1, b3 and β6 are newly discovered bioactive molecules with antioxidant and anti-inflammatory activities [2, 4], due to their capacity to induce the transcriptional decrease of iNOS expression and the reduction of nitric oxide production; as well as the decrease in the production of pro-inflammatory cytokines (TNF-α, INF-γ, IL-1β, IL-2, IL-6, IL-8, and IL-12); the inhibition of chemotaxis and cell adhesion capacity [4].

Interestingly, our studies also demonstrated that the conglutin isoforms β1, β3 and β6 are candidates to help fight T2DM through their modulatory activity (at the transcriptional and translational levels) of the main players (IRS-1, Akt) and downstream targets (Fig. 1) of the insulin molecular signaling pathway [5]. These properties could be attributed to the ability of these isoforms to interact with insulin [5] and their distinctive structural characteristics [6] from other legumes.

In this regard, insulin resistance is the main causative problem leading to the development of the long-term T2DM complications, and legume-based nutraceutical therapy could enhance the effectiveness of dietary management of T2DM. In this regard, we have unvealed the molecular basis by which NLL conglutins b1, b3 and β6 are capable of reversing insulin resistance in pancreatic cells [7].

These proteins increase insulin sensitivity through different pleiotropic effects at the molecular level such as: 1) increasing glucose uptake; 2) increasing expression of key genes in the insulin signaling pathway; 3) activation of the IRS-1/PI-3-kinase pathway through phosphorylation of its activators (IRS-1) and downstream mediators (Caveolin, CBL). This in turn promotes vesicular transport to the plasma membrane of glucose transporters; 4) decrease in cellular oxidative stress; 5) as well as promoting metabolic homeostasis and cell signaling [7].

Therefore, β-conglutin proteins are a promising source of innovative ingredients for functional food with possible uses for the prevention and treatment of diseases such as T2DM and other inflammatory-related diseases.

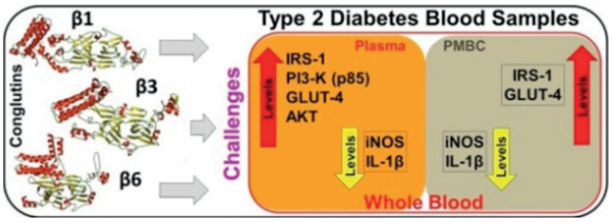

Figure 1. NLL β-conglutin proteins improve inflammatory and T2DM states. Blood samples from type 2 diabetes diagnosed subjects were challenged with b1, b3, and β6 conglutins resulting in a transcriptional up-regulation of insulin signaling pathway modulatory genes, and a decrease of oxidative estress and pro-inflammatory cytokines genes expression.

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A scenario for more legumes and less meat in the Swedish diet: possibilities and challenges related to increased domestic grain legume cultivation

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Keywords: environmental impact, human nutrition, food system

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Grain legumes are climate-smart and health-promoting sources of protein, and their cultivation provides valuable inputs of biologically fixed nitrogen as well as positive break-crop effects in cereal-dominated cropping systems. Despite their benefits, grain legumes are minor crops in European agriculture, representing less than 2% of arable land. In Sweden, grain legumes represent only 1% of the protein intake in human diets, compared to the global average of 5%. This situation is contrasted by the high meat consumption in Western diets (Sweden included), which has negative consequences on several environmental impact parameters as well as on human health.

In a recent study [1], we investigated a scenario in which Swedish average meat consumption would be reduced by 50% and replaced by grain legumes grown in Sweden; common beans, faba beans, lentils and peas. The scenario implies changes both in consumption and production in Sweden, along with a complete reduction in meat imports. Our analyses show that the average daily intake of energy, fat, protein, total iron, vitamin B12 and zinc would be within the Nordic Nutrition Recommendations also after replacing 50% of the meat consumption with legumes. Moreover, the dietary change would lead to a considerable increase in the intakes of dietary fiber and folate, which are currently too low according to the nutrition recommendations.

Regarding changes in production, the area required for growing the grain legumes to replace half of the current meat consumption corresponds to 1% of Swedish arable land. Adding to the current Swedish grain legume cultivation, which is mainly uesed for animal feed, the total area of grain legume cultivation in the scenario would be nearly 86 000 ha (Figure 1), which corresponds to 3.2% of the total Swedish arable land. At the same time, the reduction in domestic chicken and pork production in the scenario would lead to less land required for cereals and oilseeds used as feed for these animals, and this reduction surpasses the increased land requirement for grain legumes. In addition to the small domestic land use change, land used in other countries for producing animal feed would be reduced by more than 20%, which shows that the simulated change in the Swedish diet has a large land saving potential. The analyses also showed a strong mitigation of food-related greenhouse gas emissions by the dietary change, corresponding to a 20% reduction in climate impact. Since the pre-crop effect of grain legumes allows for reduced nitrogen fertilizer applications to the following crop in the rotation, 3.5% of the currently used nitrogen fertilizers in Sweden would no longer be needed.

Our evaluation of the meat reduction scenario shows clear environmental benefits while maintaining or improving human nutrition. Nevertheless, implementing the scenario involves important challenges, notably related to consumer habits and farmers' possibilities to cultivate more beans, lentils and peas. Obstacles that need to be overcome include access to legume varieties that suit the Swedish climate, investments in harvest machinery and post-harvest processing that meet food industries expectations, as well as successful value addition of Swedish grain legumes compared to imported equivalents.

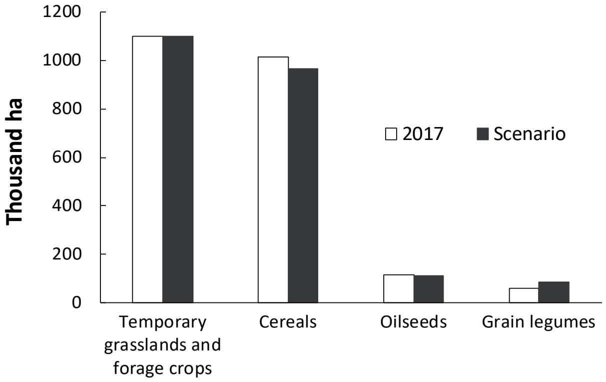

Figure 1. Land use for major crop categories in Swedish agriculture, in 2017 and according to the scenario. In addition to these categories, Swedish agricultural land comprised around 450 000 ha semi-natural grasslands (mainly pastures), 160 000 ha fallow, 30 000 ha sugar beets, 25 000 ha potatoes and 55 000 ha other crops (*e.g.* vegetables and energy crops) in 2017 [2]

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Improving nutritional and health-associated traits in *Pisum sativum* (pea)

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Keywords: mutants, pea, seed protein

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Seed quality traits in pea have been studied historically at many levels, where their impact on improving the nutrition of farm animals was often a primary driver for research. More recently the adoption of pea and other pulse crops for producing widely consumed human foods, as a source of valuable nutrition for specialist diets, and as high-value ingredients for sports nutrition, has resulted in a demand for increased diversity among the profile of constituents that are deposited during seed development and stored within mature seeds.

High-throughput screening of natural germplasm resources and mutagenized populations of pea has identified null variants for several seed proteins, including pea albumin 2, lectin, trypsin-chymotrypsin inhibitors and five classes of gene encoding vicilin proteins. A set of mutations derived by fast-neutron mutagenesis include large genomic deletions, of which at least two affect additional unrelated genes [1]. Combinations of mutations are providing novel germplasm with major changes in protein composition, in turn impacting on protein digestibility and amino acid composition and with implications for nutritional and functional properties.

New prospects for improving the nutritional profile of seed carbohydrates for food use are presented [2]. Recent discoveries from human intervention trials have demonstrated the impact of changes in carbohydrate digestibility and pea seed structure on blood glucose levels. Advances in technologies for characterizing starch [3] and cellular structure are facilitating the screening of pea germplasm and the identification of novel variants.

The acceleration of breeding programmes using novel germplasm is now facilitated by advances in 'speed breeding' [4], enabling rapid generation times which, when coupled with advances in selection, should reduce dramatically the cost and timescale for the development of new varieties.

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The anti-proliferative properties of TI1, a major Bowman-Birk isoinhibitor from pea (*Pisum sativum* L.) seeds, on HT29 colon cancer cells are associated with both trypsin- and chymotrypsin-like protease inhibition

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Keywords: Bowman-Birk inhibitors, pea, anti-proliferative properties

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Bowman-Birk inhibitors (BBI) from legumes, such as soybean, pea, lentil and chickpea, are resistant to both acidic conditions and the action of digestive proteases; their ability to inhibit serine proteases is not significantly affected by the metabolic/enzymatic activity of intestinal microbiota [1]. *In vitro* and *in vivo* studies have demonstrated that BBI might exert a protective and/or suppressive effect against inflammatory and carcinogenic processes within the gastrointestinal tract *via* protease inhibition [2,3]. In this work, TI1, a major pea isoinhibitor with the ability to inhibit trypsin and chymotrypsin, and novel engineered BBI mutants (inactive or those having only anti-trypsin or anti-chymotrypsin activity) were expressed as recombinant proteins in the yeast *Pichia pastoris*. Only the double-headed chymotrypsin inhibitor was in a monomeric state while the other rBBI variants were dimers. Using confocal microscopy, the internalization of fluorophore rBBI in HT29 colon cancer cells was observed. Active rBBIs affected negatively the proliferation of HT29 cells in a dose-dependent manner; despite being internalized, the inactive mutant did not affect HT29 cell growth. The anti-proliferative properties of the novel engineered mutants revealed that both trypsin- and chymotrypsin-like proteases involved in early stages of colorectal carcinogenesis should be considered as potential therapeutic targets.

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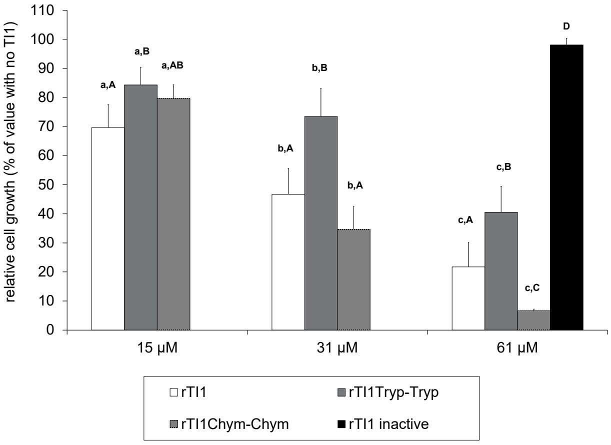

Figure. Effect of TI1 and their derivative mutants, rTI1$_{Tryp-Tryp}$ and rTI1$_{Chym-Chym}$, on the *in vitro* growth of HT29 human colorectal adenocarcinoma cells. Growth media were supplemented with protein in the concentration range 0–61 mM and cells harvested after a period of 96 h. Values are means, with standard deviations represented by vertical bars of at least three independent experiments, each having four technical replicates. ^{a,b,c}Mean values with different letters were significantly different at several treatments; ^{A,B,C,D}mean values with different letters were significantly different between recombinants proteins inside the same treatment. (P < 0·05; Bonferroni's test)

Characterization of the biosynthesis of saponins during seed development in peas (*Pisum sativum*)

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Keywords: protein, off-flavour, saponin

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The use of pulses as ingredients for the production of food products rich in plant proteins is increasing. However, protein fractions prepared from pea or other pulses contain significant amounts of saponins, glycosylated triterpenes which can impart a bitter taste to the final food product. Bitter flavours are currently either removed by energy-requiring physico-chemical treatments or masked by additives. We are in the process of identifying and characterizing the genes involved in saponin biosynthesis during pea seed development, with the objective of identifying mutants in which seed saponins no longer accumulate. To do this we have applied a saponin extraction protocol to follow the biosynthesis of these compounds during the development of pea seeds, and have identified mutants in a Pea TILLING population (1) that correspond to key steps in the saponin biosynthetic pathway. A mutation in a previously identified β-Amyrin Synthase gene (2), which is highly expressed in maturing pea seeds, reduced mature seed saponin content by 97% (3).

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Expression patterns of key hormones related to pea (*Pisum sativum* L.) embryo physiological maturity shift in response to accelerated growth conditions

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Keywords: embryo physiological maturity, hormone regulation, precocious seed germination

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The role of hormones in regulating seed development is poorly described in legumes when compared to Arabidopsis [1]. Recent advances in LED technology have enabled the development of protocols for rapid generation turnover of temperate legumes under conditions optimized for day-length, temperature and light spectra. These conditions act to compress time to flowering and seed development across diverse genotypes, but the effect on the hormone profile of developing embryos remains unknown [2, 3]. To address this gap, we measured hormonal profiles (ABA, GA_{20}, GA_1, IAA and 4Cl-IAA) of developing seeds at 10-22 DAP (days after pollination) from phenologically diverse pea genotypes grown under phytotron-based conditions optimized for rapid plant development and compared this to the profiles of equivalent samples from glasshouse conditions. Our results confirm exposure to conditions optimized for faster development alters the seed hormone content by shifting the timing of expression of key hormones known to regulate seed maturity by up to 4-8 days, resulting in the reduction of time to embryo physiological maturity. In the phytotron environment, we observed a reduction in auxins concentrations and an increase in ABA levels from seeds 12 DAP, indicative of the end of embryo morphogenesis and initiation of seed desiccation. ABA levels were up to three-fold higher at 18 DAP in this environment compared to glasshouse-derived seeds. GA_{20} was detected in both environments with concentrations up to 20-fold higher in seeds from the glasshouse. GA_1 was only detected in seeds produced in the glasshouse. To elucidate the biological relevance of these hormonal fluctuations to the attainment of physiological maturity, we precociously germinated developing seeds (12-22 DAP) *in vitro* with and without the use of plant growth regulators. The extent of sensitivity of developing seeds to exogenous ABA was strongly genotype-dependent. Concentrations between 5-10µM inhibited germination of physiologically mature embryos (18 DAP). Premature germination of seeds 12 DAP was enhanced 2.5-3 fold with the addition of 125µM GA_3 to the medium. The results presented herein provide further insights on the fundamental physiological mechanisms underlying seed development and precocious germination in legumes and information relevant to the expanding application of growing conditions optimized for accelerated breeding.

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Interactions between lupin seed proteins and native flavonoids

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Keywords: lupin seed, proteins-phenolic compounds interactions, flavonoids

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Lupin seeds contain a number of valuable nutrients. The main distinguishing feature of all lupin seeds species is the high protein content. Lupin seeds, similarly to other legumes, are also a rich source of phenolic compounds. Recent research indicates numerous evidence of flavonoids and proteins interactions, which might lead to the formation of complexes between them. Interactions between phenolic compounds and proteins are mainly considered in two aspects. Firstly, these interactions have an influence on enzymatic activity and physicochemical properties such as solubility or thermal stability of proteins. However, the most important fact resulting from protein-phenolic interactions seems to be the reduction of the nutritional value of proteins. Secondly, the formation of protein complexes can significantly reduce the potential health-promoting properties of phenolic compounds by masking their antioxidant properties.

Considering the about mentioned facts, proteins-polyphenols complexes can affect the digestibility and bioavailability, and thus the functional and nutritional properties of products derived from lupin seeds can change. Therefore, our research group has undertaken a number of research tasks aimed to characterize interactions between the main fractions of lupin seeds proteins and native flavonoids [1-3]. The results indicated that lupin seed proteins bind native phenolic compounds present in the seeds [1]. Simultaneously, digestion of lupin seed proteins by various proteolytic enzymes causes a release of these polyphenols (Fig. 1) [1, 3]. The most abundant compounds released from the digested proteins belong to flavonoids. These flavonoids represent also the major phenolic compounds present in seeds of different lupin species. Another important finding was that the unique flavonoid binding phenomenon was attributed to γ-conglutin. Future analyses indicated that γ-conglutin can form a static complex with vitexin (flavonoid standard) with a stoichiometric ratio of 1:1 at pH 7.5 [2].

We assume that the studies of lupin seed proteins-phenolic compounds interactions provide a better understanding of factors affecting the nutritional value of lupin seeds which are necessary to optimize the use of this plant for human nutrition.

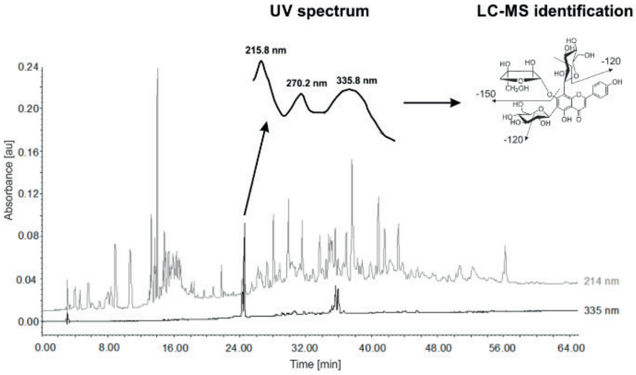

Figure 1. An example of RP-HPLC chromatogram of digested lupin seed proteins by proteolytic enzymes with detection at two wavelengths (214 nm, characteristic for peptide bond; 335 nm, characteristic for flavonoids) (Czubinski *et al.*, 2012). Characteristic UV spectrum of peak eluting about 24 min indicates presence of phenolic compounds. LC-MS identification confirmed presence of flavonoids glycosides

Acknowledgments: This study was financially supported by the National Science Centre, Poland (Project No. 2015/19/D/NZ9/00065).

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Alkaloid biosynthesis in lupins

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Keywords: lupin, alkaloids, biosynthesis

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Lupin seeds accumulate lysine-derived quinolizidine alkaloids (QAs) [1]. Due to health constraints, arising from their toxic effect on humans and animals, QA content in seeds is limiting the use of lupins as food and feed. Therefore low QA content is considered to be one of the pivotal seed quality traits in lupins.

The QA biosynthesis in lupins is still obscure compared with other plants that produce alkaloids, and the information on genes and enzymes involved in the pathway is still limited. The aim of this study was to increase our understanding on the biosynthesis and accumulation of alkaloids in the three lupin crops: narrow leafed lupin (NLL, *Lupinus angustifolius* L.), yellow lupin (*Lupinus luteus*) and white lupin (*Lupinus albus*) through RNA-seq-based comparative transcriptome analysis of genotypes with contrasting seed alkaloid content.

Recently, in NLL we have described APETALA2/ethylene response transcription factor, RAP2-7, which is likely a master gene regulating the accumulation of alkaloids in this species [2]. RAP2-7 was found to co-segregate with a major locus that confers reduced QA content in seeds, *iucundus*, and was located within a region of major QTLs that affect alkaloid composition. Identification of a gene underlying the trait of interest is a critical step affecting marker assisted selection (MAS) robustness [3]. The analysis of RAP2-7 genomic sequence of bitter and sweet NLL accessions confirmed that SNP polymorphism in the fourth exon was credibly associated with alkaloid content in NLL seeds, what enabled us to develop a new molecular marker which can be used in MAS, as a powerful tool in selecting low-alkaloid plants.

Preliminary results of transcriptome-derived investigation of QA biosynthesis in white and yellow lupin suggest that the mechanism of QA biosynthesis in these two species differs when compared to NLL. The finding of our study provide significant progress in the elucidation of QA biosynthesis in lupins.

Acknowledgments: This work was supported by the Polish Ministry of Agriculture and Rural Development within the Multiannual Program (RM-111-222-15).

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The influence of soya bean ingredients in dairy cattle fodder on milk immunoreactivity

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Keywords: soya bean, milk, allergy

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Cow's milk allergy (CMA) affects 2 to 3% of infants [Rona et al. 2007]. Currently, there are over 50 fractions of protein involved in the allergic reaction to milk [Restani et al.1999]. CMA patients demonstrate allergic reaction to soybeans (cross-reactivity of casein and glycine S5 A5-B3), which is a phenomenon that is yet to be invesigated [Natale et al. 2004]. The composition of cow's milk is influenced by many factors: genetics, age, breed of cattle, lactation period, health and diet [Tyasiet al. 2015]. Milk proteins are created in 90% from free amino acids and in 10% from short peptides entering the bloodstream from food. The phenomenon of the child's digestive system being penetrated by peptides from food via female milk, has already been recognized. However, we do not have literature data on how increasing the supply of feed that is the source of allergenic proteins influences changes in milk allergenicity.

The aim of the research was to analyze potential differences in the immunoreactivity of milk of animals fed with feed containing soybeans and their effect on the protein profile, peptide profile and amino acid composition.

The material tested were six milk samples from local farms which had used the declared diet: the sample 3 with the addition of soybean feed, 2 without the addition of soy and organic goat milk. The research methods used were: SDS-PAGE electrophoresis (1-120 kDa), UPLC chromatography, immunodetection-based Slot-blot using 10 sera of patients diagnosed with allergy to soy or milk (*ex vivo* method). Additionally, *in vitro* studies used 3 commercially purchased antibodies fractions of vegetable and animal origin.

Studies have shown that the use of feed containing legumes - soy, does not cause significant changes in the protein content but in its amino acid composition. Higher content of exogenous amino acids was identified in milk samples where soy was added to the feed.

The profile of the peptide (1-120 kDa) of milk from the cows fed with the feed containing soya protein fraction is further observed with a mass of approx. 18 kDa, which is 5-15% of total peptides. The results obtained with the Slot-blot method indicate differences in the immunocompatibility potential of milk samples from local breeders, depending on the addition of soy to the feed. The peptide fragment detected by antibodies contained in sera or commercially purchased antibodies is not a fragment of major milk proteins: casein and lactoglobulin.

The analysis of samples of milk using the Slot-blot method suggests the presence of peptides in the obtained extracts, which may come from incomplete soy digestion in the digestive tract of the tested animals or the synthesis of new peptides due to the altered amino acid supply.

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ORAL SESSION

7.1

The infectosome: a novel protein complex required for polar growth of rhizobial infection threads

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During root-nodule symbiosis in legumes, intracellular accommodation of rhizobia within host tissues is a prerequisite for nitrogen fixation. For many plant hosts, the colonization process initiates in the root hair by the formation of a transcellular infection thread. In *Medicago truncatula*, both Vapyrin (VPY) and a putative E3 ligase LUMPY INFECTIONS (LIN) are required for infection thread development but their roles at the molecular/cellular levels are obscure. Here we show that LIN and its close homolog LIN-LIKE (LINL) interact with VPY and VPY-LIKE (VPYL) in a subcellular complex, termed the “rhizobial infectosome”, that localizes to puncta both at the tip of the growing infection thread and at the nuclear periphery in rhizobia-inoculated cells and that the accumulation of VPY in puncta is positively regulated by LIN. We also show that VPY interacts in the infectosome with an exocyst subunit, EXO70H4, usually present in nuclear and cytoplasmic compartments. Genetic analysis shows that defective rhizobial infection observed in *exo70h4* mutants is similar to that seen in *vpy* and *lin*. Together, our results indicate that the infectosome is a specialized form of the exocyst machinery required for polar growth of infection threads.

Unravelling cluster root development in white lupin

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Keywords: lupin, cluster root, phosphate

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Plants show a strong level of developmental plasticity that is controlled by a complex combination of perception, integration and response. Root systems are a fantastic tool to study this plasticity since the number and position of lateral roots is deeply altered by the environment. In order to understand the fundamental mechanisms governing lateral root development and its control by the environment,our research is focused at understanding the formation of cluster roots in white lupin. These roots are specific lateral roots that are dedicated towards efficient phosphate acquisition and are produced as a response to its deficiency. From a developmental point of view, they consist in the induction of numerous rootlet primordia that will emerge to produce a “bottlebrush”-like structure. We believe that studying these extraordinary structures will help us understand plant organ formation as a response to their environment. Beyond its fantastic root development, white lupin is a model of interest because of its high protein seed content. In order to understand how cluster roots are formed, we described their development by classical histology approaches [1]. We generated and screened an EMS mutagenized population. This allowed us to identify constitutive cluster root (ccr) mutants and we are now trying to find the genes responsible for their phenotype. We sequenced white lupin genome and generated a high-quality assembly at the chromosome level (2n=50). The genome size is 450Mb and the N50 of the final assembly is 17Mb. Also, we have performed detailed time course transcriptomic analysis by RNAseq that we are using for establishing Gene Regulatory Networks in order to find important candidates regulating cluster root formation and validate their role during this developmental process.

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Local and systemic effect of cytokinins on soybean nodulation and regulation of their isopentenyl transferase (*IPT*) biosynthesis genes following rhizobia inoculation

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Keywords: cytokinins, nodulation, symbioses

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Cytokinins are important regulators of cell division and differentiation. A role in nodulation establishment has been demonstrated for this group of phytohormones to induce cortical cell divisions resulting in nodule primordia formation [1]. It was also suggested that LjIPT3, a member of the *IPT* gene family involved in the rate-limiting step of cytokinin biosynthesis, could be involved as the shoot-derived inhibitor signal acting downstream of the leucine-rich receptor-like kinase in the autoregulation of nodulation (AON) pathway [2].

The application of exogenous cytokinins to soybean plants through petiole and root drenching assays demonstrated an inhibitory effect of high concentrations on legume nodule formation while lower concentrations were found to promote nodulation [3].

The complete *IPT* gene family consisting of 17 members was identified in soybean including the orthologous duplicates of LjIPT3, called GmIPT5 and GmIPT6. Gene expression analysis using RT-qPCR analysis revealed *GmIPT5* was upregulated in the shoot following rhizobia inoculation of both wild-type plants and AON-defective mutants (*nts382*). This indicates GmIPT5 is likely not involved in AON, but instead may be involved in upregulating cytokinin biosynthesis to stimulate shoot growth in preparation of an influx of nitrogen from the nodules [4].

In addition, *GmIPT4* expression was upregulated in the root following rhizobia inoculation. This is consistent with its orthologue, *LjIPT2*, being required for an initial cytokinin burst in nodule establishment [5]. Interestingly, its duplicates, *GmIPT3/15*, are induced by nitrate indicating neofunctionalisation has occurred.

Findings from the bioinformatic and functional studies, and the role of cytokinin in nodule development, will be discussed.

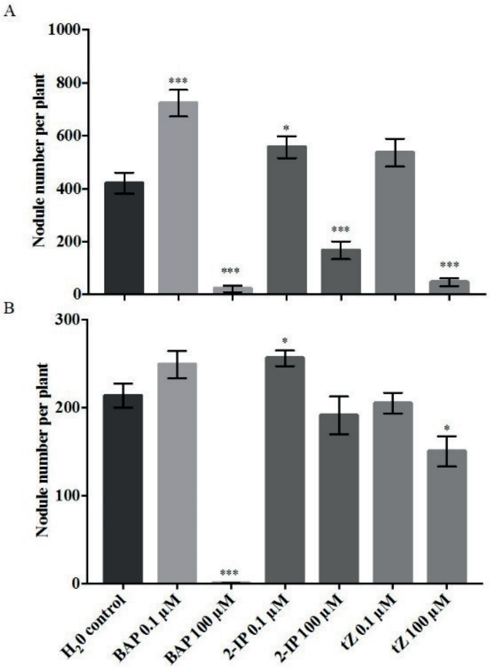

Figure 1. Effect of cytokinin on nodule number

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Characterization of the full-size ABCG transporter from *Medicago truncatula* that influences strigolactones secretion and arbuscular mycorrhiza formation

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Keywords: strigolactones, ABC transporters, arbuscular mycorrhiza

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Strigolactones (SLs) are plant-derived signaling molecules that stimulate spore germination and hyphal branching of arbuscular mycorrhizal fungi (AMF) in the rhizosphere [1]. Up-to-date, our knowledge about the molecular mechanism of SLs transport is restricted to the *Solanaceae* family and it has been shown that excretion of SLs occurs through the hypodermal passage cells (HPCs). Since, the *Fabaceae* family, exhibit a marked difference in root anatomical organization compared to other angiosperm plants, we were interested to figure out if lack of exodermis may influence SLs exudation.

Analyses of transcriptomic and phylogenetic data allowed us to identify ABC proteins in the model legume plant *Medicago truncatula* that potentially participate in the translocation of SLs. We have shown that one of them MtABCG59 exhibits root specific expression and is up-regulated by phosphate starvation and synthetic SL (rac-GR24). Spatial expression pattern analyses with GUS and GFP reporter genes revealed that the promoter of MtABCG59 is active in all over cortical cells and root tip. Heterologous expression in *Arabidopsis thaliana* leaf mesophyll protoplasts showed that the corresponding protein is localized in the plasma membrane. Loss-of-function mutation in *MtABCG59* negatively influences interaction with the AM fungus but does not affect arbuscule structure, suggesting that MtABCG59 is required for initiation of AM. Finally, root exudate from *mtpdr23* stimulates, to a smaller extent than WT, SL-inducible germination of *Phelipanche ramosa* seeds. Interestingly, the effect observed concerned only seeds being not directly in touch with the roots. The latter suggest that SLs can passively diffuse on the root surface, since in Medicago do not encounter the exodermis, being an apoplastic barrier located beneath the epidermis.

In the present work we provide evidence that SLs active export in *Medicago* mediated by MtABCG59 is required to achieve high SLs concentration around the root and thus assuring the full extent of AMF colonization.

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MicroRNAs expression profiles during *Medicago truncatula* somatic embryogenesis: embryogenic vs. non-embryogenic lines

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Keywords: *Medicago truncatula,* miRNAs, somatic embryos

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Medicago truncatula emerged as the model legume due to its short life cycle and autogamous behaviour, its ability to establish symbiosis [1], and relatively small sequenced genome. Moreover, *M. trucatula* is amenable to *in vitro* culture and genetic transformation; however plant regeneration in highly genotype dependent.

Plant Cell Biotechnology laboratory (PCB) of the ITQB-NOVA has a long experience with *M. truncatula*. During the last two decades this laboratory developed several resources for this model plant including an efficient protocol for somatic embryogenesis and genetic transformation [2], [3]. These tools have been used to study the physiological and molecular responses to drought stress in this model plant [4], [5], including the role of microRNAs (miRNAs) in response to water deficit stress [6], [7].

In addition to the importance of miRNAs in various abiotic stress responses they are also important in complex plant development processes, including plant somatic embryogenesis (SE). MiRNAs, which are small, 19–24 nt non-coding RNA regulatory molecules, act by binding to specific target mRNA transcripts promoting their cleavage or inhibiting their translation. MiRNAs play crucial roles in plant SE since they modulate gene expression to enable pattern formation during plant embryogenesis [8].

In PCB lab a highly embryogenic line of *M. truncatula* cv Jemalong (M9-10a) was derived from a non-embryogenic line (M9), as a result of somaclonal variation. These two lines are ideal tools for studying embryogenesis as they share an extremely similar genetic background, differing essentially at the level of SE ability [9]. Using these lines we profiled the expression of four conserved miRNAs (miR156a, miR166a, miR398a and miR408) during 21 days of SE induction. We studied 8 time points corresponding to samples in three different phases identified during the 21d SE induction in M9-10a genotype (early induction, expression and development phase, according to [9]). Real-time quantitative reverse transcription PCR (qPCR) was the methodology used for quantifying miRNA due to its specificity and reproducibility. Our results show significantly differences in miRNAs expression profiles between M9/M9-10a lines over time. While some miRNAs seem to be important for the early induction others are more expressed during the stage of development of the first pro-embryogenic masses.

Targets for those miRNAs have been *in silico* predicted using the psRNATarget tool (https://plantgrn.noble.org/psRNATarget/analysis) and we are currently quantifying their relative expression to corroborate miRNAs profile data.

The results obtained in this work improved our level of understanding on the complicated process of SE, and showed the importance of miRNA regulation thought the comparison of *M. truncatula* lines with different embryogenic competence.

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Characterization of elite groundnut lines for fresh seed dormancy and storage quality

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Keywords: *Groundnut*, *storability*, seed longevity

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In-situ sprouting, because of lack of fresh seed dormancy is very common in groundnut and has been known to cause high pod loss [1] in some genotypes. It predisposes seed to aflatoxin contamination. Being an oil crop, groundnut seed easily loses viability in storage especially under conditions of high temperatures and relative humidity. Meanwhile, a lot of inroads have been made in developing and advancing promising groundnut lines with important attributes such as higher yields, earliness, tolerance to drought and aflatoxin among others. The objective of the present study was to select groundnut breeding lines with appropriate seed dormancy and superior seed storage quality attributes.

A total of 22 groundnut genotypes, representing the best performing genotypes in various breeding pipelines and eight commercial varieties were evaluated under field and laboratory conditions for fresh seed dormancy and storability. A field experiment was set up in Nyankpala to screen the 30 genotypes for in-situ sprouting, during the main cropping season of 2017. The groundnut plants were sampled for analysing for fresh seed dormancy, 10 days after maturity. Good intact pods were selected and dried to a moisture content of 8% and stored.

The lines tested showed a wide variation in in-situ sprouting (Figure 1) ranging between 0% to 25%. The pods of Kpanieli and ICGV-91317 recorded up to 25% sprouting 10 days after maturity while Nkatiesari recorded 0% sprouting.

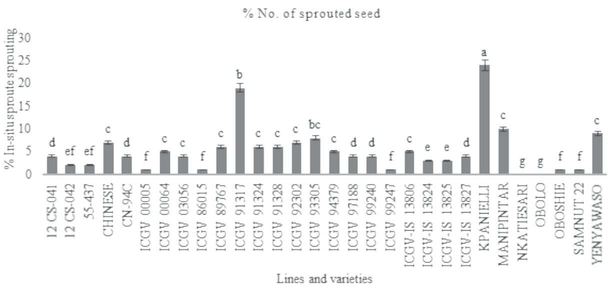

Figure 1: % sprouted seed of groundnut genotypes samples a week after maturity date. Figures with same letters are similar at p ≤ 5 %

Laboratory analysis of six-month-old seed of the test lines and varieties showed variations in germinability and health. The genotypes recorded maximum number of seeds germinated on the 5th day after sowing, and no new seedlings emerged after the 7th day. Germination percentage ranged from a minimum of 20.00% for ICGV-99247 to a maximum of 88.33 % for ICGV-91328. Seed health test conducted on seeds of the groundnut genotypes evaluated revealed the presence of *Aspergillus niger* and *Aspergillus flavus*. The percentage of seed infected with *A. niger* was higher than *A. flavus*. Between 2 to 25% of seed per genotype was infected by *A. niger* while the infection rate was 0 to 27% for *A. flavus.* The groundnut variety, Chinese recorded the least infection for both *Aspergillus* spp. whereas the line ICGV-91328 recorded the least infection rate for *A. flavus*. Among the elite lines, ICGV-91328 also performed well with respect to the other parameters measured.

A wide variability exists among the genotypes tested for dormancy and storability. Delay in harvesting of some of the genotypes could cause pod loss up to 25% because of in-situ sprouting or low fresh seed dormancy. Closer attention needs to be given at maturity and during post-harvest handling to minimize seed quality deterioration. The line ICGV-91328 was found to be superior compared to the elite lines and most of the varieties with respect to the parameters measured. Closer attention needs to be given at maturity and during post-harvest handling to minimize deterioration.

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Genomic and molecular analysis of soybean clock genes shows a novel role in male reproductive development and seed set

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Keywords: *Glycine max,* legume, male reproduction soybean, flowering, pollen

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The world's population is expected to be more than 9 billion by 2050. Feeding this massive population requires a doubling of food production from the same amount of arable land. Further, climate change threatens our food security since plant reproductive development like flowering and grain production are sensitive to the changes in the external environment. Plants being sessile have to integrate exogenous signals with endogenous rhythms to ensure the best time for progression to the reproductive stage to maximise reproductive success [1]. The circadian clock system is known for its role in synchronising environmental signals with endogenous rhythms to ensure proper timing of flowering and seed set. Our knowledge of molecular components of the circadian clock system controlling flowering of food legume crops is lacking [2].

Soybean is a major oilseed legume crop used for human and animal feed, and its genome palaeopolyploid has been duplicated several times. Moreover, legume crops such as soybean have unique vegetative and floral complexities. Expanding the cultivation areas of crops such as soybean requires optimization of environmental and development responses suitable for changing climate regimes.

The internal circadian clock has emerged as a key regulator of different biological functions. Studies have shown that the circadian clock enhances the adaptation, fitness, and survival of plants. However, it is not known whether clock genes regulate plant development directly. The evening complex, a tripartite protein complex of ELF4-ELF3-LUX, is an integral component of a plant circadian clock for maintaining circadian rhythms. *LUX ARRHYTHMO* (*LUX*) has been shown to play a key role in circadian rhythms and flowering. However, it is not clear how the evening complex connects with other pathways to regulate plant development and physiology. Our study on soybean *LUX* [3] revealing the novel function of a clock gene in controlling fertility and seed yield by affecting stamen development and function will be presented.

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Battle of the stresses: seeking more pods and seeds

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Keywords: stress, ovule, pod

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Abiotic stress, most frequently adverse water, temperature and salinity, reduces yield in crops. Vegetative and reproductive growth is compromised even when genotypes possess traits to limit stress effects on metabolism, physiology, and morphology. Responses are best understood by manipulating plants exposed to one stress at a time, but multiple stresses can occur concurrently. Pulses crops may behave similarly or differently to soybean and Arabidopsis. Here we illustrate strategies used by lentil and pea in yield formation, and explain how these crops respond to water deficit and heat stress.

1: A short lifecycle versus prolonged flowering duration. Determinate cultivars are effective in drought when their lifecycles are shorter and they escape the worse of the stress. In Canada new lentil cultivars in the early 2000s were ultra-short season, but flowering duration is longer now. Long reproductive phases aid crop yield recovery from short spells of stress. Flowering racemes were tagged in early or late flowering in field studies. Lentil set more yield early, and later produced nodes had diminished flower numbers, meaning that a lot of late produced nodes are required to boost yield.

2: Flowers and pod setting. Lentil and pea have far less flowers per raceme than soybean and faba. Are pod-setting patterns similar among crops? Lentil cultivars have up to 3 flowers per node. In early flowering, lentil nodes had more flowers (2.6) with 80% of flowers being set to pods. The 80% pod set was consistent across nodes, but later nodes started with 2.1 flowers resulting in less pods. Soybean and faba have more flowers per node and low pod setting. In lentil, late nodes produced the largest pods when water was plentiful, nodes started off with less flowers; pod growth rate was greater because leaf size was likely the same. Water deficit and heat reduced pod setting (60%) and shortened pod filling times, with similar sized or smaller pods. The boost in pod size did not contribute to yield as much as pod number.

3: Gametophyte susceptibility – pollen versus ovule. Gametophytes are pollen grains (male) and the embryo sac (female) of an ovule. Pollen is highly susceptible to drought and heat stress, where stress slows and starves pollen grain development in the anther tapetum, resulting in small, low viability grains. Pollen may be more susceptible to heat than the embryo sac but little is published on the embryo sac because manipulating it is technically difficult. At similar high temperature and growth conditions, pea pollen was more susceptible to stress. Heat reduced pollen viability in the field between 28 to 32°C, especially with several days of heat and if water deficit occurred concurrently. In growth chambers, 35°C for several days produced a similar reduction in pollen viability (<50%), and ovules were more robust (<10% reduction). Callose damage was evident on ovules 4 days after open flower, ovules failed to expand, and the embryo failed to proceed to the globular stage.

4: Ovule position in pods. Over many genotypes in our pea mapping panel, pods contained less seed in stress, an effect linked to ovule hierarchy within pods. (Most yield reduction is caused by lack of flower buds and aborting flowers in stress). Pods lost seeds by ovule position, with more seed loss at stylar and basal ends. Medial positions were likely to have seeds. Basal loss was due to failed fertilization and stylar loss was from maternal assimilate failure. Cultivars with more and small-sized seeds per pod, particularly if normal leafed, had less seed failure. Tiny residual ovules that failed to develop were either non-viable prior to pollination or unfertilized.

5: Heat, water deficit and both. Pea is sensitive to both heat and water deficit stress. From canopy studies, ample water supply ameliorates 2°C of heat stress. Exposure to both heat and drought compounds stress and produces more devastating effects than either stress as a single stress. We also see differences in embryo sac development when we compare heat (growth chamber) and heat plus drought (field) on pea ovaries and ovules. Embryo sacs swell and are damaged in the field (heat and water deficit), but are likely to stop developing and remain smaller in the growth chamber (heat) when water is sufficient. Maternal assimilate supply appears to be part of the problem in field stressed samples, with a swollen funiculus, changed embryo sac size and volume.

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Genotypic variation in lentil (*Lens culinaris* Medikus) for transpiration response to vapor pressure deficit

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Keywords: lentil, transpiration, VPD

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Plants are exposed to several stressful conditions, and drought is one of the most common stresses that limit crop production especially in the dry and semi-arid regions. Under the atmospheric high vapor pressure deficit (VPD) conditions, limited transpiration by reducing stomata conductance is considered the key water saving trait that enhance tolerance to water deficit conditions by crops [1,2].

The present study was carried out to compare transpiration rate in 20 selected lentil (*Lens culinaris* Medikus) genotypes by whole plant measurement under controlled environments during a high VPD period.

The results showed significant variation among lentil genotypes for transpiration rate in reponse to VPD with eleven genotypes exhibiting a breakpoint at about 3.3 kPa. Two genotypes (ILL7835 and ILL7833) registered the lowest breakpoint at 2.8 kPa. These two genotypes with low breakpoint show the prospect of breeding lentil varieties high yield and water use efficiency. The figure 1 shows the transpiration rate of ILL7835 response to high vapor pressure deficit.

The remaining nine genotypes had a linear response to VPD and showed continued increase in transpiration rate as VPD increased. These genotypes are being genotyped to identify the QTLs associated with transpiration rate in lentils.

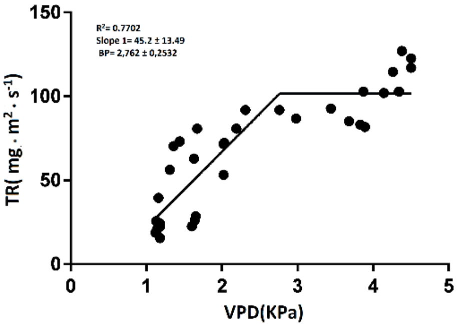

Figure 1. Segmented regression of transpiration rate (TR in $mg.m^2.s^{-1}$) response to vapor pressure deficit (VPD in KPa) for the genotype ILL7835

Acknowledgments: This work was undertaken as part of, and funded by the CGIAR Research Program on Grain Legumes and Dryland Cereals (GLDC).

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How does pea (*Pisum sativum*) recover from water deficit?

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Pea (*Pisum sativum*), like other legumes, has the unique ability to fix atmospheric dinitrogen (N_2) via symbiosis with soil bacteria known as rhizobia in root nodules. This particular feature makes the pea crop an essential component of sustainable cropping systems because of the reduction of nitrogen fertilizers it affords. However symbiotic nitrogen fixation (SNF) is very susceptible to abiotic stresses and particularly to water deficit, which is becoming an increasingly common threat in the current context of climate change.

Water deficit impacts negatively SNF (Prudent et al., 2016), affecting both nodule number and growth (i.e. structural components of SNF) and their N-fixing efficiency (i.e. fonctional component of SNF). Such negative effects can even remain when optimal water conditions are restored.

Although the ability of a plant to recover after a water deficit period may determine both its survival and its yield at harvest, the mechanisms occurring during the rewatering phase in terms of nitrogen nutrition and growth recovery remain poorly addressed.

The aim of this study was to evaluate the capacity of pea genotypes to recover after a water deficit and assess which processes, whether structural or functional, underlie this recovery. Two pea genotypes, Kayanne and Puget, were selected based on their contrasted nodulated root system architecture and their ability to recover after a water deficit period. Plants were cultivated in 4PMI[2] (Dijon – France) and subjected to a two-week water deficit period during the vegetative stage, followed by an optimal rewatering until physiological maturity. During the water deficit period and the two first weeks of re-watering, a sampling time-series was performed. Physiological mechanisms potentially involved in plant stress tolerance and in its recovery were analysed by using a structure-function based ecophysiological framework. This analysis was complemented by a study of the carbon and nitrogen fluxes within the soil-plant-atmosphere continuum.

Both genotypes responded similarly during the water deficit period. Plant growth was negatively affected, and among plant compartments, nodule growth was particularly impacted resulting in a decrease of the nodule biomass over nodulated root biomass ratio and leading to a reduction of plant nitrogen nutrition. Conversely the two pea genotypes displayed contrasted recovery strategies during the re-watering period. Kayanne increased carbon allocation to the nodule compartment allowing a restoration of the nodule biomass over nodulated root biomass ratio. Puget also favored nodules for carbon allocation but at the expense of the roots leading to an overcompensation of the nodule biomass over nodulated root biomass ratio compared to the control. Differences in the kinetics of nitrogen acquisition recovery were also observed, with Kayanne initiating a nitrogen uptake recovery earlier than Puget during rewatering. Interestingly, Puget's yield but not Kayanne's was significantly reduced in response to water deficit suggesting that Kayanne's strategy during rewatering after a water deficit could be more efficient.

Perspectives of this work are to use transcriptomics and metabolomics to identify key genes and metabolic pathways underlying these contrasted recovery strategies. This study will generate key knowledges allowing us to contribute to designing pea ideotypes which are better adapted to fluctuating water constraints.

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Progress in improving black gram (*Vigna mungo* (L.) Hepper) against biotic stresses

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Keywords: black gram, disease, pest

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Black gram (*Vigna mungo* (L.) Hepper), also known as Black Matpe or Urdbean is grown over 3 million ha in South and Southeast Asia. It has a protein content of about 24% and is also a source for minerals like iron, calcium and potassium. Dishes made from black gram include *dal*, *vada* and it is one of the major ingredients in preparation of *idli* and *dosa* (which involves fermentation of the batter made from grains). In addition, it is grown for sprouts, as livestock feed and as a green manure crop. Major black gram producing countries include India, Myanmar, Bangladesh and Pakistan. Black gram easily fits into the cereal-based cropping systems, due to its short duration (70-75 days).

The average productivity of the crop is still below 500 kg/ha. Major constraints in improving the productivity of black gram are susceptibility of commonly grown varieties to Mungbean Yellow Mosaic Disease (MYMD), dry root rot (*Macrophomina phaseolina*) and insect pests such as stem fly (*Ophiomyia phaseoli*) and bruchids (*Callasobruchus maculatus* and *C. chinensis*). Black gram lines, Mash1/1 and VM2164 were identified for high levels of MYMD resistance during field evaluations in disease hotspots in Odisha state, India. Population (F_4) derived from the cross between these two lines were evaluated under natural field conditions in Odisha in *rabi* season (November sowing) during 2017. Fifty MYMD resistant selections coupled with high yield and early maturity (65-70 days) were identified. MYMD was evaluated visually on a 1-6 scale (1- highly resistant and 6 - highly susceptible) [1]. The seed yield of Mash1/1 and VM2164 was recorded as 28.5 and 20.75 g/plant, respectively. However, the yield of the population varied from 10.2 - 77.5 g/plant. The selections derived from Mash1/1 and VM2164 were evaluated for yield contributing traits in Hyderabad, India, in *rabi* season (November sowing) during 2018. During the evaluation, severe infestation of stem fly was observed. Interestingly, both Mash 1/1 and VM2164 suffered least damage (10-13%). The population showed good variation for tolerance to stem fly.

Storage insect pests like bruchids cause considerable damage in black gram. Bruchid resistant line, VM2164 (late maturing; 90 days) was utilized in the breeding program, as a donor for this trait. The progenies from the 50 selected F_5 lines will be tested for bruchid resistance.

Incidence of dry root rot and infestation of stem fly on black gram grown as part of rice-based farming system have been observed. Up to 35-40% incidence of dry root rot and 35-45% damage due to stem fly were recorded during *kharif* season (June sowing) in Odisha.

The dry root rot pathogen was isolated from infected root of black gram from WorldVeg South Asia field, Hyderabad. Cultural, morphological and molecular characterizations of the pathogen was carried out. Molecular characterization was carried by 18S rRNA specifically ITS1 and ITS2 regions. The primers used were 5'-TCCGTAGGTGAACCTGCGG-3' for ITS1 and 5'-GCTGCGTTCTTCATCGATGC-3' for ITS 2 [2]. The sequencing analysis revealed that the fungal pathogen is *M. phaseolina.* Out of 41 black gram lines/varieties screened against dry root rot by paper towel method, CO-5 showed resistance reaction (Fig. 1). Populations developed by crossing CO-5 with Mash1/1 and VM2164 will be evaluated for MYMD and dry root resistance.

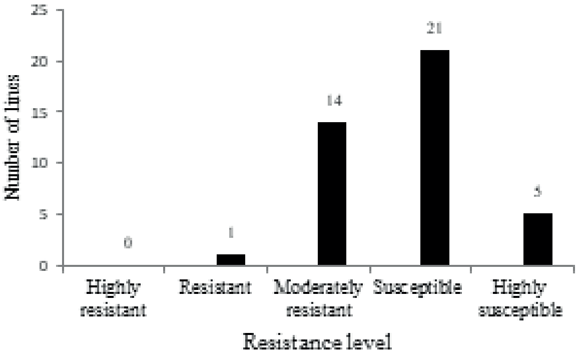

Figure 1. Resistant reaction of 41 blackgram lines/varieties against dry root rot disease

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Identification of QTLs controlling resistance to rust, aphid and weevil in wild peas by DArTseq SNP-based technology

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Keywords: pea, pest and disease resistance

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Pisum sativum is the cool season grain legume most cultivated in Europe and the second in the world [1]. However, its yield is still relatively unstable and low due to the limited adaptability to extreme environmental conditions of available cultivars and their susceptibility to diseases and pests. We studied in the past resistance to a number of fungal diseases and parasitic weeds.

Here we cover resistance to the biotrophic fungus *Uromyces pisi*, the causal agent of pea rust, and to the insect pests pea aphid (*Acyrthosiphon pisum*) and the pea weevil (*Bruchus pisorum*) [2] [3] [4]. Several studies have been performed, ranging from the screening of pea cultivars to the assessment of the mechanisms involved in the resistance. No complete resistance has been reported against these biotic stresses so far, but different levels of moderately incomplete resistance are available especially in the wild *Pisum* species. To unravel the genetic control of the resistance, a quantitative trait loci (QTL) analysis was performed using two recombinant inbred line (RIL F7:8) populations. The first one originated from a cross between two *P. fulvum* accessions, P660 and P651, while the second one originated from the cross between *P. sativum* ssp. *syriacum* accession P665 and *P. sativum* cv. Messire.

Both RILs populations were phenotyped for resistance under controlled and field conditions, and genotyped through Diversity Arrays Technology PL's DArTseq platform (Australia) [2]. The newly constructed integrated genetic linkage maps of wild peas includes high-quality DArT-Seq and SNPs derived-markers, as well as several previously mapped ”anchor” markers, which assembled in both cases in 7 Linkage Groups (LGs) and showed an average density lower than 2 markers cM^{-1}. Genomic regions involved in resistance to rust, aphid and weevil will be presented and critically discussed.

Acknowledgments: This work was supported by project AGL2017-82907-R.

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Sclerotinia sclerotiorum secrets an effector protein specifically interacts with and negates the inhibitory effect of plant Polygalacturonase-Inhibiting Protein (PGIP)

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Keywords: white mold, pathogenic mechanisms, fungal effectors

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Sclerotinia sclerotiorum causes the white mold disease of grain legume crops, as well as many other crops. The disease has tissue maceration as the prominent symptom. It uses polygalacturonases (PGs) to degrade plant cell wall in infection causing tissue maceration. Plants have developed polygalacturonase-inhibiting proteins (PGIPs) to limit fungal PG activity. The functions of PGs in fungal virulence and PGIPs in plant defense have been well documented. However, there have been no reports of pathogens' ability to counteract plant PGIPs. Here we show a small effector peptide (SsE1) secreted by *S. sclerotiorum* that specifically interacts with *Arabidopsis thaliana* PGIP1, as demonstrated using techniques of yeast two-hybrid, co-immunoprecipitation and bimolecular fluorescence complementation assays. Expression of *SsE1* is significantly induced during early stages of infection. Deletion of *SsE1,* as well as of *SsPG1,* resulted in significant reductions in virulence. In enzymatic assays using purified SsE1, SsPG1 and AtPGIP1 from heterologous expression in yeast cells, SsE1 exhibited no PG enzyme activity, but reduced effectiveness of AtPGIP1 in inhibiting SsPG1 activity in degradation of polygalacturonic acid. Expression of *SsE1*in *Arabidopsis thaliana* plant increased susceptibility to infection. Our results show that *S. sclerotiorum* secretes a special effector that interferes with plant PGIP, mitigating PGIP inhibitory effect and enhancing fungal PG activity in supporting the maceration effect of *Sclerotinia* infection. The effector SsE1 represents the first fungal effector targeting plant PGIPs.

Biotic and abiotic stress in chickpea-flax intercropping

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Intercropping can be defined as cultivating two or more crops in the same field with at least some temporal overlap. Potential benefits of intercropping include insurance against crop failure through diversification, better resource and niche utilization, weed suppression and disease management. Including a legume in an intercropping system can reduce the need to apply nitrogen fertilizer by fixing atmospheric nitrogen.

Chickpea is an important grain legume crop around the world because of its high nutrient value. However, chickpea production in the Canadian prairie is limited by two major constraints, one biotic (Ascochyta blight) and the other abiotic (timely moisture deficit to stimulate reproductive development). Intercropping chickpea with flax may have potential to address these two issues.

The biotic threat posed by Ascochyta blight, caused by *Ascochyta rabiei,* to chickpea is very significant. This disease can result in total crop failure. Current management of Ascochyta blight relies heavily on fungicides, in conjunction with partial genetic resistance, crop rotation and planting disease-free seeds [1]. All of these tools have limitations. Genetic resistance is incomplete. Both genetic resistance and fungicides can be overcome by pathogen evolution [2,3]. Indeed, all *A. rabiei* samples tested from the Saskatchewan 2018 growing season contained resistance to strobilurin (QoI inhibitor) fungicides, albeit, sometimes in combination with strobilurin-sensitive *A. rabiei*. Thus, additional disease management tools are needed.

Chickpea grows indeterminately and requires drought stress to induce maturity. Because of the short summer growing season in the Canadian prairies, delayed maturity can result in a crop that is still green and/or flowering when frost or snow commence. This can lead to a high percentage of green seed, in addition to other harvestibility problems. Because of the need for late-season moisture deficient, chickpea production is restricted to drier regions of the Canadian prairies.

Preliminary studies have been conducted on chickpea-flax intercropping at three locations in the Canadian prairie: Redvers, Indian Head and Swift Current. Swift Current is prime chickpea-growing area. Both Redvers and Indian typically have higher rainfall, increasing the risk associated with monocrop chickpea production.

Monocropped chickpea can experience higher disease severity, under moderate pressure, than chickpea intercropped with flax (Figure 1). Under high disease pressure, there can be both less disease and higher yield in intercropped chickpea as compared to the monocrop.

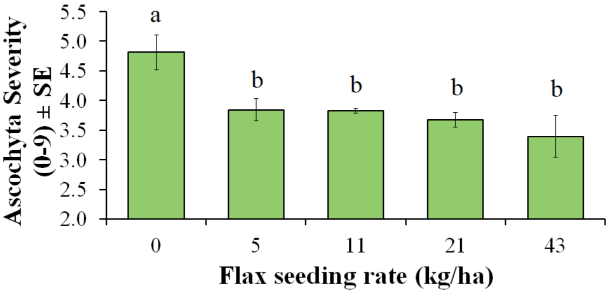

Figure 1. Ascochyta severity in chickpea intercropped with flax, or grown as a monocrop, in Redvers, Saskatchewan, Canada in 2018. Bars with the same letter are not significantly different ($p \leq 0.05$)

Preliminary studies suggest that intercropping flax with chickpea may be able to provide the abiotic stress needed to trigger chickpea maturity, thus, reducing percent green seed and increasing seed quality.

While intercropping remains relatively uncommon in large-scale Canadian agriculture, research out of the University of British Columbia (UBC) has shown significant advantages of pea-barley intercropping over monoculture [4]. Intercropping has been frequently practiced in other parts of the world. For example, in northwestern China, pea-maize intercrops have been found to increase yields over monocrops. Canadian farmers have started to tryout chickpea-flax intercropping. Thus, research is merited to explore the impacts of factors such as flax seeding rate, flax placement in either the same row as chickpea, or in alternate rows, the use of nitrogen fertilizer and abiotic and biotic stresses important to chickpea production. The results will be directly applicable to Canadian producers, and potentially relevant to other chickpea-growing regions worldwide.

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POSTER SESSIONS

Patterns of genetic diversity in a world collection of white lupin

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Keywords: crop evolution, landraces, *Lupinus albus*

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White lupin (*Lupinus albus* L.) is the main lupin crop in Western and Southern Europe, where it maximized the production of crude proteins per unit area in a comparison of several grain legume crops [1]. Various breeding programmes were recently set up in Europe, owing to the interest of lupin both as a non-GM feed protein source and as a component of healthy or vegetarian foods. The exploitation of white lupin genetic resources, which are limited to the primary gene pool, is hindered by lack of information on the structure and patterns of its genetic diversity.

The aims of this study were: (i) to investigate the structure of landrace genetic diversity across 3 diversity layers relative to variation among 9 major historical cropping regions, 83 landraces within cropping regions, and 283 genotypes within landraces; (ii) to explore patterns of genetic variation and possible diversity bottlenecks, by assessing the genetic diversity among 83 landrace accessions and 15 sweet-seed varieties or breeding lines belonging to 3 phenological types.

The study included 5 to 14 landraces from each of 9 major historical cropping regions, i.e., Italy, Egypt, Spain, Portugal, Turkey, Maghreb, Madeira-Canaries, Near East and East Africa (Figure 1). Each landrace was represented by 3-4 genotypes. The sweet-seed germplasm was represented by 6 French winter-type varieties, 5 French spring-type varieties, and 4 Mediterranean-type varieties or breeding lines from Italy, Chile and Morocco (each represented by 2 genotypes). Some 320 genotypes were characterized via genotyping-by-sequencing [2], using for analyses 6198 polymorphic SNP markers. A hierarchical analysis of molecular variance (AMOVA) estimated the variance components relative to the 3 landrace diversity layers. Nei's distances between landrace or improved accessions based on SNP frequency values were imputed in a non-metric multi-dimensional scaling analysis.

The AMOVA indicated the following ranking of variance components for landrace diversity: genotype variation within landrace > landrace variation within region > variation between historical cropping regions. This result, which was not expected for this predominantly self-pollinated crop, highlighted the importance of exploiting also within-landrace diversity for crop breeding.

The multi-dimensional scaling ordination of landrace germplasm displayed mainly a latitudinal gradient of genetic diversity. Also, it revealed outstanding variation among landraces from Near East, which agrees with the hypothesis of crop origin in this region.

Variety germplasm varied mainly according to its phenological type, and tended to be fairly distinct from landrace material. This latter result indicated that plant breeding exploited a small portion of the available genetic diversity, probably because of main focus on sweet-seed genetic resources (i.e., modern cultivars). This finding is similar to what reported for narrow-leaf lupin (*L. angustifolius*) [3], which, however, has much shorter domestication history than white lupin.

In conclusion, landrace genetic resources of white lupin await exploitation also because of their untapped diversity, besides their excellent yielding ability, regional adaptation [4] and tolerance to abiotic stresses [5].

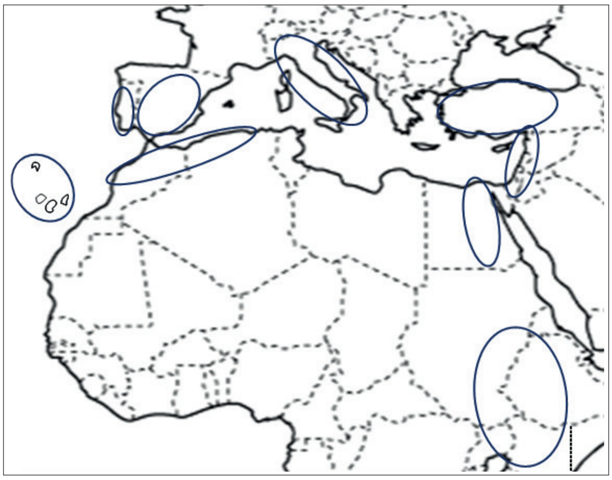

Figure 1. Regional landrace germplasm pools under study

Acknowledgments: This work was part of the project 'Legumes for the agriculture of tomorrow (LEGATO)', which received funding from EU's FP7 Programme under grant agreement No. 613551.

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Phytochemical variability studies of cowpea accessions (*Vigna unguiculata* (L.) Walp) from four agroecological zones and one unknown source in Ghana

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Keywords: cowpea accessions, agroecological zone, phytochemical traits

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A total of forty-two cowpea accessions were collected from the following four different agroecological zones of Ghana and one unknown source: Coastal Savanna (3 accessions), Sudan Savanna (3 accessions), Guinea Savanna (10 accessions), Deciduous Forest (8 accessions) and an Unknown source (18 accessions). These accessions were established in the field in single row plots. Pods were harvested and the seeds were pulverized and subjected to phytochemical analysis. The analysis was carried out to determine per cent protein content, per cent mineral content, polyphenol (phenols and flavonoids) content in mg/l and per cent inhibition of DPPH activity. The mineral elements were phosphorus, potassium, calcium, magnesium, nickel, iron, zinc magnesium and sodium. The phenols were 2,5-dihydroxybenzoic acid, caffeic acid, gallic acid, chlorogenic acid p-coumaric acid, syringic acid and vanillic acid and the flavonoids were quercetin and rutin and per cent inhibition of DPPH activity. The highest mean per cent crude protein value of 17.58±1.40% was recorded for the unknown source; the lowest mean value of 10.99±2.60% was recorded for Coastal Savanna. Unknown source recorded the highest mean values of 0.26±0.01% and 0.05±0.01% for nickel and iron, respectively. Sudan Savanna recorded the highest mean phosphorus (1.52±0.47%), potassium (0.86±0.09%) and calcium (0.14±0.02%) concentrations. Coastal Savanna recorded the highest values of 0.08±0.05% and 0.02±0.01% for sodium and magnesium contents, respectively. Both Coastal Savanna and Sudan Savanna scored the highest mean zinc content of 0.34±0.01% each. Guinea savanna and Deciduous Forest agroecological zones scored the lowest mean zinc concentration of 0.01±0.00% each. Coastal Savanna recorded the highest mean phenolic acid compounds and rutin concentration with mean values of 64.75±5.42 mg/l (2,5-dihydyroxylbenzoic acid), 84.75±6.36 mg/l (caffeic acid), 74.65±7.70 mg/l (gallic acid), 60.28±12.19 mg/l (chlorogenic acid), 23.17±10.45 mg/l (p-coumaric acid), 72.65±7.70 mg/l (syringic acid), 36.02±7.70 mg/l (vanillic acid) and 163.46±16.41 mg/l (rutin). Coastal Savanna scored the highest value of 233.81±10.64 mg/l for quercetin concentration whereas Guinea Savanna scored the lowest for inhibition (20.02±4.62%). Multiple regression analysis showed that phosphorus contributed 14.19%, quercetin contributed 11.96%, and combined flavonoids contributed 14.89% significantly ($P<0.05$) to variation with respect to free radical scavenging activity of extracts of the cowpea accessions studied. Pairwise correlation analysis among the phytochemical traits showed that there were 37 pairs of significant associations for accessions from unknown source, 25 pairs of significant associations for accessions from Sudan Savanna, 35 significant pairs of associations for accessions from Deciduous Forest and 27 pairs of significant associations for accessions from Guinea Savanna. Cluster hierarchical analysis grouped the 42 accessions into five different clusters. In conclusion the experiment showed that there was high variation among the 42 cowpea accessions studied with reference to the phytochemical traits studied; accessions from Guinea Savanna agroecological zone had the highest antioxidant activity; differences in agroecological zones is probably playing a role in variation in cowpea phytochemical traits. The cowpea accessions studied can be good source of mineral elements, polyphenols and antioxidant activity for both humans and livestock.

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Pod phenotypic variation in a set of snap beans collected in Europe

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Keywords: genetic diversity, quantitative traits, PCA, cluster analysis

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Common bean (*Phaseolus vulgaris* L.), particularly the group of snap beans, shows an extensive diversity in pod phenotypes. The aim of this work was to study the variation for pod traits in a set of snap bean accessions collected from european genebanks and seed companies in order to establish a snap bean panel including the maximum diversity and a minimum number of redundant lines.

A total of 233 bean lines were obtained in greenhouse by self-crossing of individual plants derived from landraces or cultivars collected in Europe. The lines were growth at Villaviciosa, Spain (43°2901N, 5°2611W; elevation 6.5 m) and phenotyped with help of Tomato Analyzer software [1] considering pod longitudinal dimensions [perimeter (P), area (A), width at mid height (Wm), maximum height (H), curved height (CH), index H/CH, color (L*, a* and b*)], cross sections [perimeter (Ps), area (As), width at mid height (Wms), maximum height (Hs), Index Hs/Wms), shape triangle (Ts), shape circular (Cs) and shape rectangular (Rs)] in 10 pods/line. To investigate the structure of the gathered diversity, the optimal number of cluster was investigated using the Elbow method and then a principal component analysis (PCA) was carried out. Tukey tests were used to investigate significant difference among the mains clusters. Statistical analyses were carried out using the mean phenotypic data and performed with the packages FactoMiner and FactoExtra in R software [2, 3].

The results revealed a wide phenotypic variation for all traits. The optimum number of cluster was 4 and, PCA revealed two main dimensions explaining 65.7 % of the variance (Figure 1). The variables maximum height (H), width at mid height (Wm), perimeter (P), area (A), and shape circulars (Cs) showed the greatest contribution to Dim1. The variables color dimension L* and b* as well as index H/CH and maximum height (H) were the ones with higher contribution to Dim2. The **cluster 1** includes 148 lines and they show the significant and lower values for longitudinal dimensions (P, A, H), cross section dimensions (Ps, As, Hs) and shape circular, indicating a circular cross section. This group included old French cultivars as '*Beurre de Rocquencourt*', '*Gloire de Saumur*' and '*La Victorie*' and well know cultivars as '*Tendergreen*', '*Cherokee trail of tears*' and '*Harvester*'. **Cluster 2** includes 7 lines, all them with purple color, intermediate longitudinal dimensions and, pear cross sections. **Cluster 3** contains 62 lines with intermediate longitudinal and cross section dimensions. This group has significant higher values to color dimension a* due to many of lines have pod with yellow color. **Cluster 4** has 16 lines showing the higher values for longitudinal dimensions (P, A, H), cross section dimensions (Ps, As, Hs) and share circular, indicating a flat cross section. This group includes old cultivars as '*Musica*', '*Garrafal Oro*' or '*Buenos Aires*'.

The results also show that some lines can be removed to maximize the phenotypic diversity in a putative snap bean core set, particularly in the cluster 1. Most lines include in cluster 1 have green and short pods with a circular cross section. Finally, the observed diversity offers the opportunity to investigate the architecture of the genetic control for pod traits through genome wide association studies and the adaptation to organic productions.

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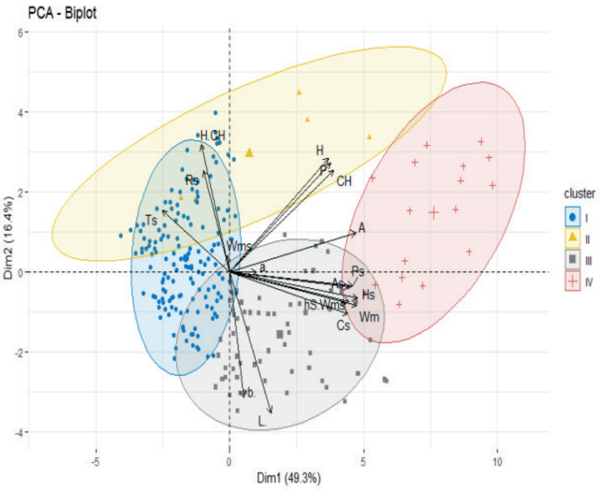

Figure 1. Biplot showing the distribution of the 233 bean lines considering the two main estimated dimensions revealed by PCA

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Phosphorus use efficiency in the recombinant [*accacia*×normal leaf] lines of the field pea (*Pisum sativum* L.) mapping population.

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Keywords: pea, phosphorus use efficiency

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During vegetation, pea is subjected to the climatic stresses, i.e. periodic fluctuations of the temperature and soil water content, as well as abiotic stresses related to the phosphorus and nitrogen content in the soil. The physiological efficiency of pea includes such features as: the root system construction, water, nitrogen and phosphorus efficiency in seed (biomass) plant formation, photosynthetically active light transmission in the field, leaf photosynthetic activity, leaf gas exchange efficiency, photoreceptor performance II and translocation and distribution of assimilates in the plant. Pea uses atmospheric nitrogen through its biological binding. In optimal conditions, this process secures the plant's nitrogen demand. However, under conditions of stress affecting photosynthesis (drought, phosphorus deficiency), nitrogen supply proves to be insufficient. Recognition of variance and co-variance between components of the physiological efficiency components will allow to avoid excessive use of mineral fertilization, as well as deficiencies in field conditions, and will allow to determine relationships with nitrogen use efficiency and yield.

In field experiments, variance and co-variance between components of phosphorus efficiency was evaluated among lines of the Polish mapping population [Wt10245×Wt11238] during the whole growth season under varied nitrogen nutrition in two years. To assess the correlation between seed yield and the efficiency of phosphorus utilization, field experiments were carried out in the different climatic and soil conditions (Wiatrowo, 2 locations, Przebędowo, one location, 2 replicates in each location). The soil analysis was carried out. They concerned forms of nutrients available for plants.

The Wt10245 parental line had lower value than Wt11238 of the nitrogen use efficiency, but higher phosphorus use efficiency PERg. The Wt10245 PERg was 17% lower than in 2016 (132 in 2016 mg/ mg, 109 mg/ mg in 2017).The yield from a plant in both seasons was the largest in Wiatrowo, in the optimal location, the smallest in Przebędowo. The yield in "optimal" and "weak" Wiatrowo and Przebędowo location was lower in 2017 than in the previous year for this population [Wt10245×Wt11238]. The pea yield and phosphorus utilization efficiency (PER_{gen}) were positively correlated in optimal and stress locations (Wiatrowo, optimal conditions r=0.66, stress conditions: Wiatrowo r=0.73, Przebędowo r=0.36).

Genotypes effect (G) and soil treatment effects (E) were significant for all characters in 2017, however not significant for %Nzia, NHI, NERgen and phosphorus components in 2016. Genotype-treatment (G-E) interactions were significant for all characters in 2017, except nitrogen and phosphorus content in vegetative mass ($\%N_{veg,}$ $\%P_{veg}$) and not significant for all characters in 2016, except pea yield. Noteworthy, relationships between GY and phosphorus efficiency were weaker in nitrogen-limited conditions in Przebędowo and stronger in nitrogen-limited Wiatrowo comparing with optimal Wiatrowo (optimal conditions r=0.66, stress conditions: Wi, r=0.73, P, r=0.36). This could be result of consideration the location No. 2 in Wiatrowo as a weak position (despite a slightly higher nitrogen content than in location No. 1) due to nitrogen deficiency, boron deficiency (micronutrient important for pea cultivation) and iron excess.

Both nitrogen (a building block of photosynthetic enzymes) and phosphorus (it participates in energy transfer) are one of the most important elements necessary for an efficient photosynthesis process. The effectiveness of the use of nitrogen and phosphorus in pea are parameters strongly correlated with the growth and productivity of plants. The results indicate that the importance of components of physiological efficiency for pea yielding decreases in the suboptimal conditions. Broad variability of all physiological traits gives a high probability QTL mapping with success.

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Evaluation of cowpea genotypes using selected mineral elements and protein content in grain

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Keywords: cowpea, genetic diversity, mineral, protein

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Cowpea is indigenous to the African continent and is grown for its leaves, immature pods and grain in different countries of the world. It is a legume crop with agood source of protein, minerals, vitamins, carbohydrates and antioxidants that are essential for human health, growth, and development. Nutritionally enhanced plant varieties provide considerable amounts of bioavailable nutrients useful to alleviate nutrient deficiency among rural and urban populations towards food and nutritional security. The objective of this study was to assess the variability in the concentration of mineral elements and protein content in the plant part of diverse cowpea genotyeps.

Field trials with 22 clones were established at the University of Limpopo research station and Roodeplaat sites using a randomized complete block design with three replications. The experimental plots consisted of four rows of 4 m long separated by 1m spacing. Agronomic management practices were followed at both locations. The seeds were oven dried at 80°C for 24 hours and the dried seeds were ground using a laboratory seed grinder and then sieved using a 0.5 mm sieve. Mineral content and protein analysis was conducted at the ARC Soil, Water and Climate analytical laboratory, Pretoria, South Africa using an Atomic Absorption Spectrophotometre (Spectra AA 300). Crude protein content (N × 6.25) was determined by the combustion method (Leco ®model, FP-528, St. Joseph, Ml). The data set was subjected to analysis of variance (ANOVA) using GenStat for Windows 17th edition (VSN, International Hempstead, UK) [1].

The univariate analysis revealed significant differences among the genotypes for the traits evaluated. PCA was used to visualize this data set in a two dimensional biplot (Figure 1) which revealed a wide genetic variation among the test cowpea genotypes (Figure 1) that could be exploited in selecting suitable parents when breeding for nutritional qualities and population developemnt. The present study indicated the genetic potential of the cowpea genotypes studied and their importance for use in the breeding programme aimed towards contributing in combating malnutrition, poverty and food insecurity in South Africa. Significant genetic diversity was observed among the genotypes in their genetic potential to absorb the mineral elements available from the soil and translocate them into the plant system for different physiological processes and functions [2, 3] and finally to the grain. This genetic variability would contribute to achieving food security and improved nutrition towards sustainable production and conservation of biodiversity of cowpea [3] (Badigannavar et al. 2015) and finally to the grain.

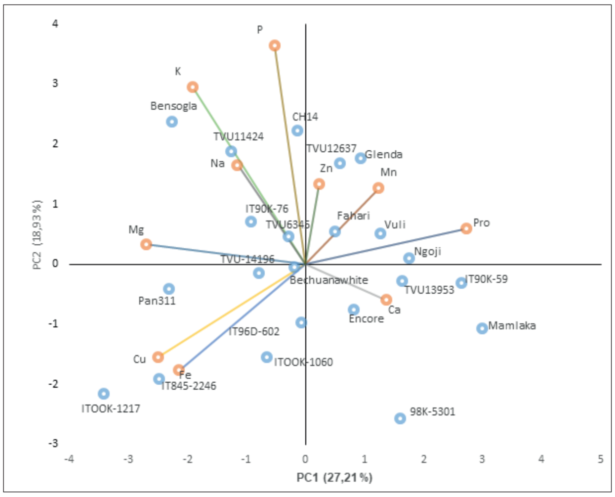

Figure 1

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Selection from *Desi* type chickpea landraces collected in west of Iran to introduce high yielding new varieties

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Keywords: *Desi* type chickpea, mass selection, seed yield, drylands

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Latest *Desi* type chickpea cultivar has introduced more than 40 years ago in Iran. During this period, *Desi* type chickpea and possibility of selection and introducing of new varieties has not paid attention. Mass selection is one of the breeding methods of self-pollinated crops. In this method, a large number of plants of similar phenotype are selected and their seeds are mixed together to constitue the new variety. Allard [1] suggested that plants selected by mass selection be subjected to progeny test. In such a case, poor progenies are rejected and remaining plants are mixed together to build the new population.This research project carried out in order to purification and evaluation of *Desi* type chickpea landraces from West Iran, Kurdistan, West and East Azerbaijan provinces through mass selection coupled with progeny test.

In 2012, the seeds of local varieties collected from the mentioned areas were purified physically. In the second year, evaluation of landraces and their purification has done at the field and considering maximum variation, 87 plants were selected for the next step. In the spring of next year, grain yield and other traits of genotypes have comprised with two check varieties, Pirouz and Kaka in an observational trial. In 2014, seventy superior lines with 2 checks has conducted and assessed in a 9 × 8 rectangular lattice design in three mentioned stations (Kurdistan, East Azerbaijan, and West Azerbaijan).

Based on the assumptions of fixed location and randomized genotype, the combined analysis of variance was accomplished. Chickpea lines varied significantly for many traits measured, particularly for weight of 100 seeds at 1% and for days to flowering and maturity, and grain yield at 5% probability levels.

Results suggested that, *Desi* type chickpeas in different locations have high genetic diversity and include various types of seed skin in different colors. Due to the fact that *Desi* type chickpeas are used internally to produce dhal or split, the color of the seed skin has no effect on marketability of this type of chickpea.

Therefore, in the final selection of promising cultivars, in addition to grain yield, plant height and seed size should be considered. According to the results of this experiment, there was a significant difference in the yield of seedlings of *Desi* chickpea lines collected from different locations. Based on the principal component analysis (PCA) method which used to categorize chickpea genotypes. Seed yield, one hundered seeds weight and plant height were the most important morphological traits in describing chickpea genotypes.

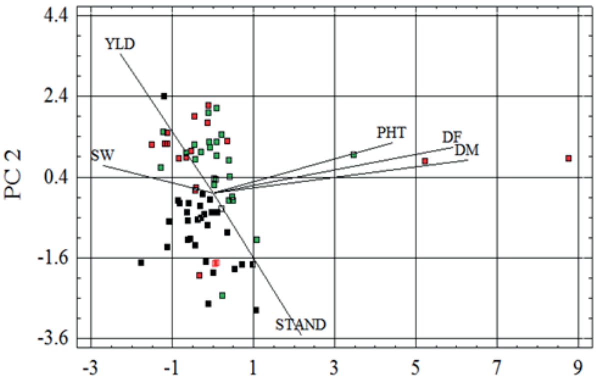

Figure 1. Principal component analysis (PCA) method was used to categorize chickpea genotypes

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Diversity of the composition of cyclitols and their galactosides in seeds of the genus *Lathyrus* (Leguminosae)

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Keywords: *Lathyrus*, seeds, bornesitol, lathyritol, carbohydrate

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Cyclitols called cycloalkane polyols or sugar alcohols (containing one hydroxyl group on each of three or more ring atoms of cycloalcane) have numerous pharmaceutical properties and are widespread in the plants. The most widespread cyclitols are hexitols, and among them *myo*-inositol and its isomers (*chiro*-, *scyllo*-inositol) or methylated ether derivatives (D-pinitol, D-ononitol, D-sequoitol). Cyclitols are involved in signal transduction, membrane biogenesis, cell wall formation, ion channel physiology, phosphate storage, osmoregulation, and belong to compatible solutes, accumulated in plant response to abiotic stresses [1]. Some cyclitols (*myo*-inositol, D-pinitol, D-*chiro*-inositol, *scyllo*-inositol) indicate healing-promoting properties and can be used for treatment of different disorders [2]. Beside *myo*-inositol, ubiquitous in all living organisms, legumes contain different inositols and their a-D-galactosides [3]. In seeds of *Lathyrus odorathus* unique cyclitol – D-bornesitol and its a-D-galactoside lathyritol was identified [4]. However, the diversity and content of this unique cyclitol and unique galactosyl cyclitol among genus *Lathyrus* remains to be discovered to find the possible new source for isolation and purification of both compounds and testing their biological activity.

Material and methods

Seeds of 16 species of *Lathyrus*, namely: *L. sativus* (23 accessions), *L. clymenum* (15), *L. ochrus* (13), *L. cicera* (11), *L. aphaca* (10), *L. tingitanus* (10), *L. hirsutus* (8), *L. sylvestris* (6), *L. annuus* (3), *L. angulatus* (2), *L. gorgoni* (2), *L. nissolia* (2), *L. latifolius*, *L. odorathus*, *L. pseudocicera* and *L. sphaericus* derived from genetic collection of Institute of Plant Genetics Polish Academy of Science in Poznań (Poland). The composition and content of soluble carbohydrates were analyzed by the high resolution gas chromatography (GC-FID) method as described earlier [5].

Results

Seeds of the investigated species contain common sugars (sucrose, galactinol, raffinose family oligosaccharides – RFOs, glucose, fructose and galactose), *myo*-inositol and its a-D-galactoside - di-galactosyl *myo*-inositol (DGMI), D-bornesitol and lathyritol. The last two compounds were not detected in four species – *L. clymenum*, *L. nissola*, *L. ochrus*, *L. sphaericus*. The correlation between *myo*-inositol (free and bound in its galactosides) and D-bornesitol (free and bound in lathyritol) was not found. Moreover, the levels of D-bornesitol (and lathyritol) did not correlate with the levels of RFOs. It means that accumulation of D-bornesitol and lathyritol in seeds did not affect accumulation of galactinol, DGMI and RFOs.

On the other hand, among studied *Lathyrus* species, some could be useful for both, the isolation of free D-bornesitol and/or lathyritol (for estimation of their biological activity) and assess of their physiological role in seeds and plants (**Fig. 1**).

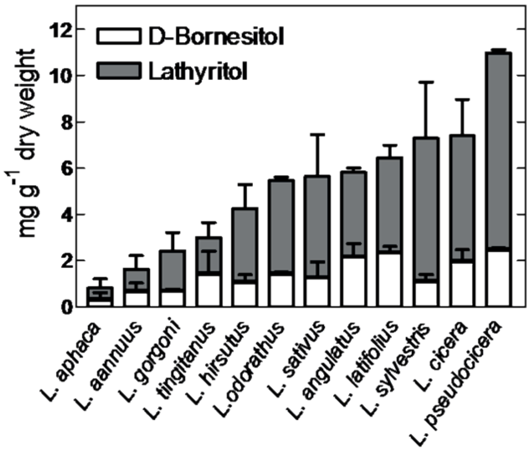

Figure 1. The concentration of d-bornesitol and lathyritol in seeds of differnt Lathyrus species. Values are means for accessions in each of species ± SD

Acknowledgments: This work was financially supported by Ministry of Science and Higher Education of Poland.

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Spearlike Bean Dreaming - The hundredth millennial of *Vigna lanceolata*, an Australian Aboriginal uncultivated legume crop

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Keywords: Australian Aboriginal farming, legume crop improvement, *Vigna lanceolata*

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During the last ice age, 70,000-50-000 years ago, modern man finished his travel out of Africa, along the Indian Ocean coastline to the Southeast Asia, then the landmass of Sundaland, and via Wallacea in Sahul, what today are the Australian continent, New Guinea and neighbouring islands. However, palynology dates first human activities around 100-120 millennia before present on the basis of numerous charcoal findings witnessing a large-scale use of fire-stick farming, an aeons-long practice of the Aboriginal Australians aimed at easier hunting and gathering, which resulted in significant ecological changes, including rapid and wide distribution of edible yam species [1].

The yam-like legume species *Vigna lanceolata* Benth., known in English as bush carrot, Maloga bean, native bean, parsnip bean, pencil yam or small yam, is the only member of this genus native solely to Australia. Its growth habit is prostrate, with a tap root and relatively short, elongated and moderately thin tuberous formations producing carrot- or pencil-like rhizomes, with smooth, long and creeping or vining stems, with trifoliolate leaves and spear-like leaflets of various width, with aerial yellow flowers on long peduncles, clustered and with successively maturing dehiscent pods and vetch-sized seeds and also with amphicarpic flowers, pods and seeds.

The specimens of more than 1800 accessions of *V. lanceolata* have been catalogued in nearly all Australian states and territories, out of which almost 91% are those collected in the Northern Territory, and are kept in various national herbaria. The remarkably wide ecogeographical range of *V. lanceolata* covers sharply contrasting habitats and landscapes in the whole northern portion of Australia, such as streams, rivers, creeks, channels, drains, lagoons, gulfs and coasts, rock holes, mine soils, sandy flats, downs and hills, bushlands, grasslands, rainforests, farms and paddocks and roadsides, highways, railways, airstrips and ports, with a notable exception of southern regions.

Anthropology and ethnology emphasize the role of the women as the cardinal lore-keepers of yam biogeography and most experienced searchers for and harvesters of its tubers. Digging yams with sticks is comparable to ploughing, turning over soil layers, enhancing its aeration and without destroying the plants, which all result in higher tuber yields in the following seasons. The underground fibrous fruits are baked within a heap of earth upon which the fire is lit and maintained until they are desirably juicy. The *V. lanceolata* tubers are rather rich in anti-inflammatory, anti-hypertensive, cholesterol-lowering and many other beneficial agents for human health.

The Aboriginal Australians treasure colossal knowledge on astronomy, biology, ecology, ethnobotany and mathematics. The cornerstone of their culture is the *Dreamtime*, a philosophical and religious concept. In some remote epoch, supreme progenitor beings descended from the sky and settled near the great rocky complex of Uluru. One of the most frequent shape-shifting depictions of these heavenly teachers is in the form of yam-like humanoids, with *V. lanceolata* named *Wapurtarli* by the Anmatyerre and Warumungu peoples and to which religious ceremonies are still celebrated [2]. An imagined line linking Alpha Arae and Theta Scorpii constitutes the Arrernte constellation of Yam Stick.

A phytolinguistic quest for the vernacular names denoting *V. lanceolata* in the available dictionaries and other lexical resources brought forth around 120 words in more than 80 extinct and living Australian Aboriginal languages and dialects, making a quarter of their total number. The morphological variability of the compiled popular names is incomparably wider in comparison to any other ethnolinguistic family of the world, primarily due to an equally incomparably longer development. The etymology of these names suggests possible roots, such as the Proto-Arandic **manaatyi* and its cognates in Wagaya and Waramungu and the Proto-Ngarna **jika*, within the Pama-Nyungan languages [3].

The Australian legume research community began to recognise the remarkable potential of *V. lanceolata*, already apprehended from time immemorial. The knowledge accumulated for tens of millennia is currently propelling *in situ* preservation and *ex situ* conservation, characterisation by molecular markers and breeding, with controlled hybridisation and designing the ideotypes of future cultivars, targeting the duration of growing season, grain yield components, tuber yield and chemical composition [4]. We are also privileged to observe a true resurrection of the Australian Aboriginal art, ignited by the immortal Emily Kame Kngwarreye, where dreaming this spear-like bean has already is a piece of eternity.

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GWAS in the USDA kabuli chickpea mini-core set for protein, fat and fiber

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Keywords: *Cicer arietinum*, seed quality, oil

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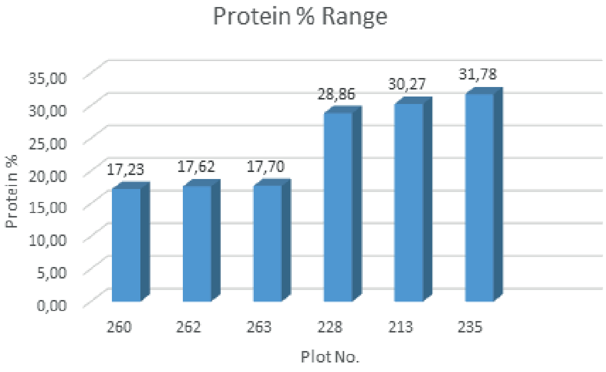
The USDA kabuli chickpea mini-core of 88 accessions was skim-sequenced (GBS) and 174K SNP polymorphisms were identified. The SNPs were filtered using no missing data criteria for the 88 kabuli lines resulting in a complete set of 36,850 markers across the eight chromosomes of the chickpea genome. The SNP numbers per chromosome were (1) 5,536; (2) 3,866; (3) 3,593; (4) 8,564; (5) 3,672; (6) 5,273; (7) 4,987 and (8) 1,359. A preliminary genome-wide marker-trait analysis using TASSEL was conducted to identify SNP markers for breeding for protein, fiber, fat, and starch % concentrations in the seed. The highest significant markers were found for seed protein concentration (*P*=7.96E-06), fiber (*P*=4.06E-07) and fat (*P*=9.83E-07) on chromosomes 1, 2 and 5 controlling 20, 23, 20% of the phenotypic variation, respectively. The most significant SNPs for starch were lower, (*P*=3.60E-5 and *P*=4.28E-05), on chromosomes 1 and 3 controlling 16% each of the variation, respectively. Validation of the SNP markers in a broader set of plant genetic resources will be needed to determine their usefulness in breeding for end use characteristics. Seed of the kabuli mini-core is available from USDA GRIN Global (https://npgsweb.ars-grin.gov/gringlobal/search.aspx) and the SNP data is available for download on the CSFL database (https://www.coolseasonfoodlegume.org/).



POSTER SESSION

1.10

Broadening soybeans variability by interspecies *Glycine max* × *Glycine soja* hybridization

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Keywords: *Glycine soja*, interspecies hybrid

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Wild soybean *Glycine soja* (Sieb. & Zucc) is a annual species that is native throughout China and parts of Korea, Japan and Russia and is an ancestor of cultivated species *Glycina max* (L.) Merrill) [1]. It has a higher genetic diversity than cultivated soybean [2]. Cosequently to this and to their good adaptability *G. soja* has become an important source of novel genes and alleles for the improvement of cultivated soybean: e.g. tolerance to salinity [3], SCN-resistance [4], resistance to disease [5] and even yield [6].

Soybean breeding in Poland is conducted in one of the northernmost environmental conditions (49°00'N - 54°50'N). For this reason there are very small number of genotypes wich are useful for breeding programs. To broadening genetics variability, *G. soja* genotype growing in natural conditions in latidude similar as Poland were used. Accesion PI 507825 (VIR 9040) of *G. soja* was obtained from Soybean Germplasm Collection, NPGS, Urbana, USA and had been collected over Amur river in Amur Region of the Russian Federation (Far East of Russia).

Interspecific crossing between two very early varieties (000 MG) Mavka and Annushka of *G. max* and genotypes PI 507825 have been made in 2015. In 2016, F_1 hybrid plants were identified on the basis of the following characteristics: vining stem, the color of the seed coat and seed size. The plants of F_2 generation were sown in 2017 on the field in ARS Dłoń. They were characterized by huge variability and have mostly vining stem and long vegetation period. From F_2 generation 99 plants were selected out and their 99 F_3 progenis were sown in 2018.

As in F_2 generation, most of F_3 plants were characterized by the wild type of *G. soja* - indeterminate type of growth, a large number of branches and small seeds. Proportion of plants with indetermined growth was higher than expected. Despite of very high temperatures in 2018, the majority of plant have very long vegetation period (160 days) but the first plants were harvested before 20 of August. Finally 201 plants were selected: 79 from 6 progenies of Annushka x PI507825 and 164 from 40 progenies of the Mavka x PI507825 cross combination.

The range of variability in F_3 generation of all traits was very high in both cross combinations. For cross Anushka x PI507825 the range of plant height was - 60 - 214 cm, number of branches 3 – 12, number of pods per plant – 2 – 437. The highest number seeds - 980 and the wieght of seed from plant – 104.9 g. The seeds of all plants had a small 100 seed weight - in average of 7.71 g. The plants harvested from Mavka x PI507825 cross were characterized by even greater variability in terms of the examined traits compared to the plants from the combination of Annushka x PI507825. The range of plant height and number of branches has been also high: 48 - 210 cm and 3 – 16 respectively. The highest number of seeds harvested from plant was 1431 and the weight of seed from plant – 119.3 g. In this cross combination 43 plants with the 100 seed weight as *G. max* were selected. This results confirm the previous year observations that in this progeny segregated more plants in the type of *G. max* parent.

Overall, the vast majority of F_3 plants were characterized by features of the wild *G. soja* parent: an indeterminate type and a very long vegetation period, and therefore was not collected. However, from the point of view of soybean breeding goals in Poland, the valuable single plants among plants from both cross combinations were selected: high plants, high first pod setting, plants with a large number of pods and seeds from the plant, and plants with a high 100 seeds weight. These results indicate also that the backcrossing to cultivated parent is necessary for improvement of agronomic traits.

Acknowledgments: This work was supported by Polish Ministry of Agriculture and Rural Development, Project No 43 of Basic Research for Biological Progress in Crop Production program.

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1.11

Exploiting wild germplasm to expand the genetic diversity and enhance the adaptive potential of domesticated chickpea

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Keywords: crop improvement, disease resistance, crop wild relatives

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Chickpea (*Cicer arietinum*) is an important legume crop, which is produced and consumed worldwide, with Australia being the second largest producer and the largest exporter of chickpea [1]. However, chickpea production is constrained by several biotic and abiotic stresses, as well as its particularly narrow genetic base [2].

In order to overcome these limitations and, ultimately, improve the currently stagnant yields of chickpea for Australian growers, wild relatives can be exploited. To this end, we are utilising a collection of wild *Cicer reticulatum* and *Cicer echinospermum* accessions, which are the direct progenitor and a sister species of chickpea, respectively [3].

With a view to expand the genetic diversity of chickpea, diverse wild genotypes have been crossed with an elite Australian chickpea variety, namely PBA HatTrick. Segregating populations derived from these crosses have been assessed in the field for various agronomically important traits, such as flowering time and growth habit. Genotyping of these populations will enable us to investigate the genetic basis of these valuable traits.

In addition to this, we have taken a more targeted approach to improving resistance to Ascochyta blight, a devastating fungal disease caused by *Ascochyta rabiei*. Screening of the collection has revealed some promising accessions that displayed resistance against a diverse set of isolates, hinting at possible sources of durable Ascochyta blight resistance. Mapping populations are being generated to elucidate the underlying QTLs associated with resistance.

Acknowledgments: This research is a *Grains Research and Development Corporation* investment (CUR00024).

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Genotyping by sequencing of cultivated lentil (*Lens culinaris*) highlights population structure in the Mediterranean gene pool shaped by geographic patterns and anthropic selection

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Keywords: *Lens culinaris*; genotyping-by-sequencing; population structure

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Cultivated lentil (*Lens culinaris* Medik.) is one of the oldest domesticated crops and one of the most important grain legumes worldwide. No genetic structure was defined within the Mediterranean gene pool, which holds large part of lentil biodiversity.

In this study, high-throughput genotyping by sequencing (GBS) was used to resolve the genetic structure of an *ex situ* lentil collection, mostly composed of Mediterranean landraces.

Sequencing of a 188-plex GBS library and bioinformatic treatment of data yielded 6,693 single nucleotide polymorphisms (SNPs). Analysis of non-redundant genotypes with non-parametric and parametric methods highlighted the occurrence of five highly differentiated genetic clusters (Figure 1). Patterns of variation within the Mediterranean gene pool could be related to specific geographical areas and phenotypic traits related to seed size. This indicates that post-domestication routes introducing cultivation in the Mediterranean Basin and anthropic selection were major forces shaping lentil population structure. The estimation of the fixation index F_{ST} at individual SNP loci allowed the identification of distinctive alleles across clusters, suggesting the possibility to set-up molecular keys assigning lentil germplasm to specific genetic groups.

In conclusion, GBS proved to be a powerful tool for the simultaneous discovery and genotyping of SNP polymorphisms in lentil. Overall, the results of this study provide insights on the lentil evolutionary history and are of major importance for lentil conservation genetics and breeding.

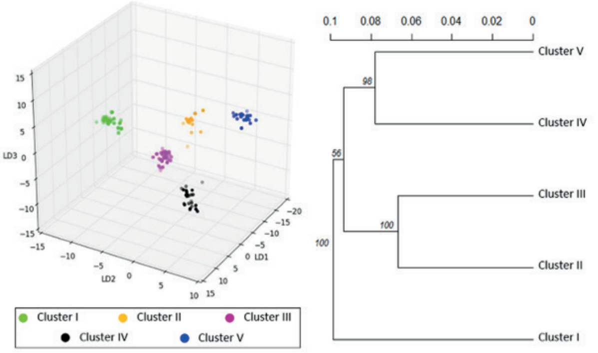

Figure 1. Analysis of genetic structure with non-parametric methods. (Left) Discriminant analysis of principal component (DAPC) applied to k-means clusters. (Right) Neighbour-joining dendrogram of k-means clusters, based on the Nei's genetic distance

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Gene pool white lupine - basis for new variety development in Bavaria

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Keywords: white lupine, breeding, anthracnose

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In Germany, the grain legume lupin is a source for native protein in human nutrition and feeding. Grain legumes are generally essential in crop rotations of organic farming due to nitrogen fixation, positive environmental effects and the preceding crop effect. In spite of public interest the acreage in Bavaria and whole Germany is still low (Germany 2018 about 23,000 ha of sweet lupines [1]). In order to increase the attractiveness of these crops, high-performance varieties with good resistance to current diseases must be available to the farmers. At present, there are hardly any breeding activities for grain legumes in Bavaria, with the exception of soybean, as there is no economic incentive for breeding companies.

The demand for regionally produced protein food and feed in Germany is increasing. Beside soybean, the lupine species are particularly suitable due to the good protein quality and the high yield potential. The white lupine (*Lupinus albus* L.) is well adapted to Bavarian soil and climatic conditions. The cultivation and yield stability in Germany is currently endangered by anthracnose in all species, but especially in the white lupine. Anthracnose (*Colletotrichum lupini*) is a seed-borne fungus that can cause total crop failure. No efficient pesticides are currently approved for Germany. In the case of blue lupine (*L. angustifolius* L.) an approach to develop resistant varieties by intensive screening of international genotypes in the combination with marker selection for anthracnose resistance was successful. There is still considerable need for research on white lupine [2; 3].

To promote the cultivation of this valuable crop in Germany and especially in Bavaria, breeding of white lupine must be supported. In a first step, a well-described gene pool with diverse genetic material will be set up in a research project started in 2018. This gene pool will be available to the Bavarian plant breeders and used for research at the LfL.

For this purpose, international varieties were collected and 16 of them were tested in a plot experiment at three locations in Bavaria in 2018 to evaluate the cultivation suitability under Bavarian conditions. The material was provided by breeders and dealers from Germany, France, Poland, the Czech Republic and Chile.

In addition to the varieties, genetic resources (gene bank material) will be evaluated to increase the genetic basis and thus the potential for breeding high yielding, disease-tolerant crops. A first field trial for anthracnose screening was established.

In 2018 the international varieties showed a relatively large range for time to flowering, maturity and grain yield. At the test sites, there was partially extreme drought stress due to extreme weather conditions of the year 2018 in Bavaria with a long-lasting drought period combined with above-average temperatures in the months April to August. Late flowering varieties hardly formed pods at one location due to the stress situation. Despite the exceptional warm weather, some varieties could not be threshed at the usual time in August, but only several weeks later. The experiment gave first indications of the suitability of these varieties for Bavaria (Southern Germany). Due to the drought and heat in 2018, the plants were hardly infested with anthracnose.

In order to implement marker-assisted breeding methods a molecular marker system has to be developed. Therefore, a genotyping by sequencing (GBS) approach will provide important information on the genetic diversity of the investigated gene pool.

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Phenotyping a pea collection for resistance to fungal diseases as a first step for Genome Wide Association

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Keywords: pea breeding, disease resistance, phenotyping

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Pea, *Pisum sativum*, is one of the major legume crops worldwide. It is an important source of proteins for both humans and animals [1]. In addition it has the ability to improve the soil economy of agricultural fields by fixing atmospheric nitrogen through symbiotic interactions with soilborne bacteria [1]. However, pea production is constrained by several biotic stress factors being particularly susceptible to fungal diseases such as powdery mildew, fusarium wilt powdery mildew or rust [2].

The use of resistant cultivars is currently the most efficient control approach. However, the existing resistance level introduced in legume cultivar is still insufficient to control these diseases. It is thus crucial to identify and exploit new sources of resistance to these diseases [2]. This requires the availability of large pea collections containing important genetic diversity and efficient methods to detect and transfer these traits to modern cultivars. The massive technological advances allow the genome wide study of genetic variations facilitating breeding programs. Therefore, phenotyping capabilities are currently the bottlenecks to breeding

This research, seeks to address the biotic stress components limiting pea production through the detailed phenotyping of a core collection of 324 *Pisum* spp. germplasm from worldwide origin for resistance to three of its main limitation namely powdery mildew, rust and fusarium wilt. This research aims to identify new sources of resistance to single or multiple pathogen(s) to be incorporated in our breeding program. As a prereqiusite step to the implementation of genome wide association studies to boost our breeding program, this research also pursue the detailed phenotyping of the pea accession in response to these diseases.

Phenotyping of plant response to diseases were performed under controlled condition to limit the effect of environmental factors. Fusarium wilt and rust were screened with whole plant assays while a cut leaf assay was used to screen powdery mildew susceptibility [3]. For each disease, plants were inoculated with standard methods as described in [3], [4], [5] two week after sowing. Each evaluation was performed in triplicate and the whole screenings wer repeated thrice independently. Pea responses to powdery mildew were determined by estimating the percentage of leaflet area covered by mycelia [3]. Pea responses to fusarium wilt were determined periodically by estimating the disease severity expressed as the percentage of leaves with symptoms per plants [4]. Estimation of pea responses to rust was performed periodically to determine the latent period and the infection frequency. The periodic data were then used to calculate the area under the disease progress curve (AUDPC) [5].

Large variation was detected among accessions in response to each disease. Resistance was detected not only in *P. sativum* spp. *sativum* but also in other *Pisum* species and subspecies including *P. fulvum* and *P. sativum* spp. *elatius* of worldwide origin. This reinforces the important role of wild accessions and landraces as reservoir of resistance trait for pea breeding. The incorporation of these accessions in pea breeding program will contribute to improve pea resistance level in the field. In addition, the detailed cahracterisation of this *Pisum* spp. core collection to these diseases will be implemental to refine resistance QTL more closely through GWAS which will ease the breeding process.

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1.15

Variability in resistance to mechanical loads and chemical composition of seeds for selected accessions of chickpea (*Cicer arietinum* L.)

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Keywords: chemical composition, chickpea collection, mechanical loads

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For one of the most important members of food legumes belong chickpea (*Cicer arietinum* L.). Chickpea also called garbanzo bean or Bengal gram is an Old World specie and is a member of the West Asian Neolithic crop assemblage, associated with the origin of agriculture in the Fertile Crescent some 10.000 years ago. It most probably originated in an area of present-day south-eastern Turkey and adjoining Syria [1]. Is traditionally grown in many parts of the world and like other pulse crop chickpea has multiple functions in the traditional farming system in many developing countries. An attempt was made to assess the reaction of seeds to mechanical loads, taking into account their geometry expressed as seeds thickness and 100 seed weight. Harvested seeds of each object were also used for estimation of protein and fat content as well as fatty acids composition. The research material comprised a seeds of 48 of chickpea accessions derived from an independent collection as well as from the Gene Bank in Gatersleben (Germany) and RICP Prague-Ruzyne (Czech Republic). Some of the accessions constituted chickpea of Kabuli type, characterized by white flowers and white or beige-coloured seeds, with thin seed coat. The remaining accessions belonged to the Desi type, having a pink or deep-red flowers, and a coloured (brown or dark-brown) and thick seed coat. Generally, the small-seeded accessions expressed by 100 seed weight were characteristic of the Desi type, with the lowest values 12.3 and 14.6 g, respectively concerning the seeds originating in Ethiopia and India, and the highest value (26.6 g) for accession derived from Turkey. The large-seeded accessions were typical of the Kabuli type, with the highest value of 100 seed weight above 50 g for obtained for the accession derived from Czech Republic. The obtained result allowed for selecting of chickpea accessions displaying a high resistance of seeds to static loading, from among Kabuli and Desi plants types. The average protein content for the Kabuli and Desi seed types were on the same level (14.6%), ranging for all accessions under analysis from 11.8% to 18.4%. The fat values ranged from 6.4% for Desi type No. 44 originated from Ethiopia to 13.7% for Kabuli type No. 26 of Turkey origin and the average content was slightly higher in the Kabuli seed type (8.73%) than in the Desi seed type (7.89%).

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Characterization of six Portuguese landraces of common bean (*Phaseolus vulgaris* L.)

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Common bean (*Phaseolus vulgaris* L.) is the most consumed grain legume all over the world. This legume is an important source of protein, dietary fiber and micronutrients such as iron, zinc and copper [1]. Like other legumes, common bean has the capacity to increase the fertility of the soil through its association with N-fixing bacteria and improve the P cycling, reducing inorganic fertilizers requirements [2]. There is a great diversity of common bean in Portugal, resulting from the adaptation and farmer's selection. Several regions of Portugal are still reach in landraces [3] being mostly Andean type [3]. The characterization and evaluation of these genotypes is essential as they are considered an important genetic resource in plant breeding [3] and can be useful to improve European cultivars.

The aim of this work was the characterization of six Portuguese common bean genotypes. Three determinate bush and three indeterminate common bean genotypes were characterized for six qualitative traits (colours of standard and wings, dry pod colour, pod beak orientation, seed shape, seed coat patterns) and six quantitative traits (days to flowering, pod width, pods/plant, seeds/pod, seeds/plant, 100 seeds weight) according to IBPGR descriptors (1982).

The field trial was carried out in North of Portugal (41.537796, -8.400919, 170 m) using four replicates per genotype. All flowers of indeterminate plants have standards pale-yellow with white wings. Two determinated plants presented purple-lilac flowers and one has white flowers. The days to flowering were higher in the indeterminate plants. The number of seeds/plant was the trait with the highest variability, ranging from 23 to 71 seeds/plant. The crude protein value ranged between 21.8% and 26.3%. Agronomic and phytochemical studies are ongoing aiming to increase the knowledge about these common bean landrances which is relevant for agrobiodiversity conservation.

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1.17

The role of the biopolymer coating of soybean seeds for yield improvement

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Keywords: soybean, seed-coating, biopolymer, agriculture, sawing, development, yield, crops

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Soybean (*Glycine max* (L.) Merrill) is one of the most important agricultural species. Cultivated in the Asian region for more than 5,000 years, over the past three centuries this legume has spread throughout the globe. Soybean seeds are a balanced source of high quality protein, fatty acids, vitamins, mineral components, so products from them are widely used in numerous national and dietary recipes, including vegetarian. In addition, soybean by-products are used as nutritional feed additives for livestock. The high nutritional value of soybean, its wastelessness and genetic lability - all this makes soybean a promising solution to the emerging problem of food deficiency as well as valuable material for breeding [1].

Under natural conditions, representatives of leguminous crops tend to enter into symbiosis with soil microflora, thereby enhancing their own growth, nutrient absorption and, as a result, yield. The interaction of these plants with nodule-nitrogen-fixing bacteria is a classic example of a mutually beneficial symbiotic association that enriches the soil with assimilable nitrogen compounds. In addition, some strains of symbiotic organisms come into opposition with natural fungal pathogens, insects and nematodes, thereby reducing the loss of plant biomass [2]. Such biological features of leguminous crops make bacterial symbiotic cultures a promising component for inclusion in seed coatings. Active attempts to use bacterial cultures for these purposes were made in the late 70s - early 80s of the twentieth century. To date, numerous bacterial cultures are actively used, including: nodulation-inducing *Bacillus cereus*, *Bradyrhizobium* sp., *Mesorhizobium* sp., *Rhizobium* sp.; *Bacillus megaterium*, *B. subtilis*, *Burkholderia* sp., *Gliocladiumvirens*, *Pseudomonas fluorescens*, *Serratiamarcesens*, *Trichoderma album*, *T. asperellum*, *T. lignorum*, *T. harzianum*, *T. viride* with fungicidal action; *Bacillus simple*, *B. megaterium*, *Sinarhizobiumfredii* – nematicidal; *Bacillus thuringiensis* – insecticidal [3, 4].

The purpose of this study was to determine the effect of biopolymer seed coating on soybean yield, as well as a comparison of the effect of coating with the influence of bacterial symbionts. Seven highly productive soybean varieties created by AgeSoya company were selected for the study. The choice of biopolymer mixtures was determined by several factors, among which: the softness of the impact on the seeds being processed, safety for the environment, complete biological decomposition in the soil without the risk of accumulation in it. As a result, three variants of biopolymer mixtures were tested. Commercial bacterial-containing product was used as a bacterial inoculum, applied at manufacturer's recommended working concentrations. Vegetation of treated seeds in open soil occurred from May to October 2018 in Podkarpackie Voivodeship (South-Eastern Poland).

The study showed that the influence of biopolymer compositions and bacterial culture caused the maximum increase in yield compared with the control for such varieties as Annushka (16.81% and 26.37% respectively), Atlanta (8.64% in both cases), Lajma (17.06% and 16.79%), Madlen (13.19% and 12.02%), Mavka (8.35% and 5.66%), Smuglyanka (10.91% and 17.66%), Violetta (29.81% and 28.81% respectively). The decrease in yield was not observed in any of the experiments. Visual inspection showed increased vegetative traits of plants obtained from treated seeds, as compared with control samples. Thus, the first trials in real field conditions have plainly showed that our research concept is right and proper moreover positive effect of the biopolymer coating of seeds on the preservation of their viability and following yield was clearly shown.

We are currently finishing development of the final biopolymer coating formula. Thanks to applied solutions in the field of nanotechnology and active bio components, the final coating will provide a high one-year increase, by approximately 40%, of the yield from selected soybeans varieties with an increased stable germination force owing to solutions that are know-how of the AgeSoya company.

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Sustainable stewardship of the landrace diversity in legumes

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Keywords: breeding, competition, crop stand uniformity

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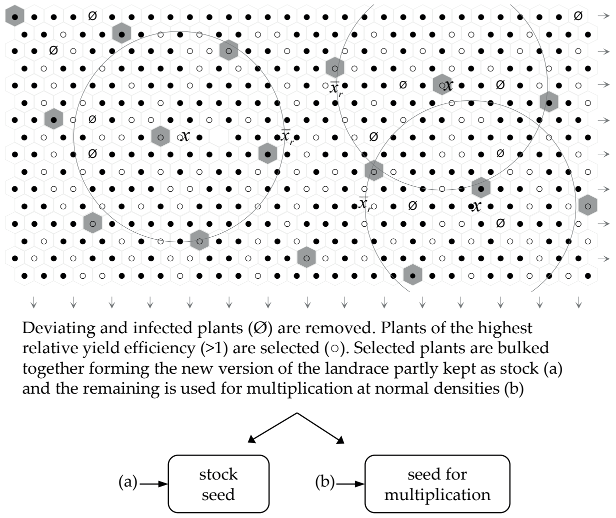
Landraces of legumes are preferably grown under low input conditions thanks to higher stability, i.e., regardless of the varying stresses, one or more genotypes will yield satisfactorily. Nevertheless, naturally evolving landraces may lose their identity and healthiness in the long term, due to contaminating and degrading forces. A pilot scheme is provided that entails the manipulation of the landrace diversity through bulk or mass selection, with the aim of establishing perpetual adaptation to an ever-changing environment [1,2].

Implicative key assumptions that led to the proposed conservation breeding procedure were: (i) Endogenous molecular mechanisms of new genetic variation expand the landrace heterogeneity adding desirable and deleterious mutations. (ii) Due to plant-to-plant dissimilarity within the crop stand, ineffective resource use is the outcome of the unbalanced growth of individuals. (iii) Intra-species competitive ability is inversely connected with yielding capacity, thus constituent genotypes of high-yield capacity are those of the 'weak-competitor' ideotype. (iv) Under intra-species competition strong competitor–low yielders prevail masking the yielding capacity of weak competitor–high yielders. (iv) Landrace evolution under inter-species competition may gradually lead to seed degradation from preferential and gradual proliferation of the low yielders at the expense of high yielders.

The procedure aims to replenish the seed, partly to be kept as stock, and the remaining to feed the multiplication rounds (Figure 1). About 2000 plants are grown at *nil*-competition in a honeycomb trial [3]. As an option, the stock seed is used as the check so as to facilitate detection and removal of the "off-type" plants. Seed yield of the remaining plants is recorded. Placing each individual plant in the center of a ring, its absolute yield, x, can be expressed in relation to the average yield of the ring (i.e., the moving circle replicate), resulting in the unitless that measures the relative plant yield efficiency devoid of the confounding effect of spatial heterogeneity. A number of 35-15% plants of the landrace type are selected, on the premise that their relative yield efficiency is above one, >1, and their seed is mixed. The procedure may represent an effective cultural practice to improve the landrace yield and health status concerning seed-borne or soil-borne diseases in the absence of certification systems, and such case studies have been made in lentil [4,5]. A new relative project is now under way (see Acknowledgment).

The scheme also offers the possibility to draw new varieties for registration. By considering only the top selected plants, a multigenotypic variety will result. Multigenotypic varieties may deserve more room henceforth due to the enormously varying environment. Moreover, and particularly for the self-pollinating species, by considering single-plant progeny lines, pedigree breeding may result in highly yielding pure-line varieties. Such case studies have been conducted in lentil [6] and common bean [7].

The honeycomb layout at *nil*-competition conditions including about 2000 plants. Delineated positions belong to check (optional)



Deviating and infected plants (Ø) are removed. Plants of the highest relative yield efficiency (>1) are selected (○). Selected plants are bulked together forming the new version of the landrace partly kept as stock (a) and the remaining is used for multiplication at normal densities (b)

Figure 1. A pilot scheme of the landrace bulk breeding at *nill*-competition leading to upgraded seed [1]

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Peas – a genetic resource for sustainable protein production in the Arctic?

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Keywords: *Pisum sativum*, climate change, phenotyping

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Peas (*Pisum sativum*) are an important source of plant protein for humans and animals. Pea has historically been an important cultivated crop in the Nordic countries, but has mainly been bred for the southern parts of the region. Today, much of the plant protein in the Nordic region is imported, and a substantial part of animal feed consists of soy. Domestic grain legume production in the Nordic countries is now slowly increasing, but the room for increase in traditional crop production areas is limited, due partly to crop rotation concerns. Climate changes expected in the near future will result in higher temperatures and longer growing season at high latitudes. This might open up for possibilities for pea production in Arctic and northern areas, and the need for cultivars more adapted to northern conditions is likely to increase.

At NordGen (The Nordic Genetic Resource Centre) - a common genebank for all the Nordic countries - a large number of Nordic pea accessions are conserved, including both cultivars, landraces and breeding material. The ongoing Nordic cooperation research project «Arctic peas» aims to identify germplasm of peas well adapted either for breeding or immediate cultivation in the Arctic/Nordic regions. The project evaluates important traits in 50 selected accessions from NordGen in field trials at four contrasting Nordic locations, at latitudes ranging from 55° to 69° N (Figure 1). The accessions are also screened with molecular markers for disease resistance. The project aims to identify plant accessions with traits and resistance genes suitable for future cultivation of peas in the Arctic/Nordic region, increase the knowledge and use of the Nordic pea accessions conserved at NordGen, and strengthen the collaboration between companies, organizations and researchers in the Nordic countries.

Figure 1. Map indicating the locations of field trials for trait evaluation of pea genetic resources in 4 countries: 1. Tromsø, Norway (69° 39′ N; 18° 54′ E), 2. Röbäcksdalen, Sweden (63° 49′ N; 20° 11′ E), 3. Jokioinen, Finland (60° 48′ N; 23° 28′ E) and 4. Taastrup, Denmark (55°67′ N; 12°30′ E)

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POSTER SESSION

1.20

Mycological profile of national soybean seeds varieties

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Regarding needs comprising usage of alternative protein sources in Europe, legume plants become a subject under investigation. Promising *Fabaceae* plants, rich in valuable protein, are national varieties of soybean. As a legume plant, soya positively influents crop rotation and relatively not require extraordinary weather or agrotechnical conditions. Soya plants are resistant to diseases and vermins, however, contamination by different microorganisms or fungi producing mycotoxins decreasing seed value, may occur [1, 2].

The main goal of monitoring study is to have an insight into the variability of the mycological and chemical composition of a soybean seeds varieties harvested over the last years.

Soybean seeds (*Glycine max* Merill) of 18 cultivars were analyzed from the mycological point of view. Analyzed data of soybean seeds were possessed from the crop harvested in different years (2015-2017). Additionally, seeds harvested in year 2017 were analyzed under fungi, mold and micotoxins contamination.

Aflatoxins and ochratoxin A were determined by HPLC method with fluorescence detection. Analysis of deoxynivalenol (DON), nivalenol (NIV), diacetoxyscirpenol (DAS), zearalenone (ZEN), toxin T2 and HT2 was performed by HPLC-MS/ MS. The samples were purified on Agilent Bond Elut Mycotoxin columns.

The study pointed out deoxynivalenol and zearalenone as mycotoxins most frequently occurring on soybean seeds (Table 1). The least frequency showed OTA (ochratoxin, in Abelina variety from 2016), NIV and DAS (nivalenol and diacetoxyscirpenol, in Mavka variety from 2016). AF (aflatoxin) was not present during monitoring investigation. In some cases micotoxin level exceed the applicable standards.

In analyzed material *Alternaria, Cladosporium* and *Penicillium* were found as most frequently occurring molds. Fungi were present in all the samples. A total number of fungi was in the range from <20 up to 10^3 in Brunensis, Silesia and Sirella varieties.

Summarized, cultivated in Poland soya seeds characterized by high fungi and yeast contamination. Among mycotoxins, generally deoxynivalenone, zearalenone and ochratoxin was found in the seeds. In some cases the levels of these mycotoxins were very high and exceed strongly applicable standards.

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Table 1. The content of most frequently present mycotoxins among studied soybean seeds varieties (ppb)

Soybean variety	DON	ZEN	Year of harvesting
Abelina	0	<0.20	2015
	<3.00	0.94	2016
	<3.00	**47.60**	2017
Aldana	4.42	0.68	2015
	0	0	2016
	<3.00	<0.20	2017
Aligator	3.78	0.70	2015
	244	**529**	2016
Annushka	0	0	2015
	<3.00	0	2016
Augusta	**19.8**	2.57	2015
	0	0	2016
	4.24	3.04	2017
Brunensis	**15.8**	5.84	2017
Erica	0	0	2016
	<3.00	<0.20	2017
Lissabon	3.09	<0.20	2017
Madlen	0	<0.20	2015
	<3.00	0	2016
Mavka	0	<0.20	2015
	178	**396**	2016
	0	0.46	2017
Merlin	0	0	2016
	<3.00	<0.20	2017
Naya	7.33	**19.3**	2017
Paradis	<3.00	0	2016
Petrina	<3.00	0.22	2016
	<3.00	<0.20	2017
Protina	<3.00	1.12	2017
Silesia	<3.00	0.68	2017
Sirelia	0	0.38	2017
Solena	<3.00	0.47	2017

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Potential of chickpea genotypes on yield and yield components under Mediterranean condition of Turkey

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Chickpea (*Cicer arietinum* L.) is adapted to environmental stresses such as drought, high temperatures and poor soils and may thus be an important food security crop for small holder farmers in the semi-arid tropics. This study aimed at evaluating the agronomic performance of some chickpea genotypes in Mediterranean condition of Turkey. For this purpose, a total of 17 chickpea genotypes with three controls *vis.* İnci, Seçkin and Hasanbey were used as material. The field experiment was conducted in winter season of 2016-17. Randomized complete block design with three replications was conducted at the Eastern Mediterranean Research Institute Adana province, Turkey. Result of the experiment revealed that genotypes were significantly differences some traits such as days to flowering, plant height, first pod height, 100 seed weight and seed yield. Results revealed that the performance of FLİP 09-13 C, FLİP 97-677 C, TB-2012/40 as well as F4 09 (X 05 TH 21-16189-12-4) genotypes were the best in case of 100 seed weight and seed yield among all investigated chickpea genotypes.

POSTER SESSION

1.22

Leaf epidermal micromorphology related to digestibility of forage plants

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Keywords: epidermis, micromorphology, *Trifolium*

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The presence of thichomes on vegetative organs of forage plants is one of the parameters responsible for limiting their digestibility, due to their difficult degradation. Trichomes also affect the hay drying rate, as well as plant's resistance to insects and pathogens. Presence of epidermal wax and thick cuticle affect accessibility of soft tissues to ruminal microbes and enzymes.

In this research we examined leaf epidermal characteristics of 18 wild-growing *Trifolium* L. species, some of which widely used as forages (*T. repens, T. pratense* and *T. hybridum*). Cross sections were made of the segments of median lateral leaflets from the middle part of the plants using cryostat, at temperature 20°C, at cutting intervals of 25 µm. The research was conducted using light and scanning electron microscopy. Special emphasis was put on the type, number and distribution of non-glandular and glandular trichomes and wax. All examined species, with an exception of *T. repens*, had epidermal wax platelets on leaves, and some of them had papillose epidermal cells. Non-glandular and glandular trichomes were present on leaf surfaces of most of the examined species. The species did not differ in trichome types, but only in indumentum density and trichome distribution pattern. Uniseriate non-glandular trichomes were composed of variable number of short basal cells and elongated terminal cell. In some species they had protuberances on their cell walls. They were more densely distributed abaxially and along the veins. Two types of capitate glandular trichomes were recorded, both composed of unicellular or multicellular stalk and multicellular secretory head. They were rare in all examined species, present mostly abaxially. Species from the section *Chronosemium*, together with *T. angulatum, T. hybridum, T. repens* and *T. pratense* had glabrous leaves, or leaves with rare trichomes on abaxail epidermis. The average number of non-glandular trichomes per mm^2 of the leaf surface ranged from 0 to 23 (in *T. striatum*).

The results showed that the species which were used as forages shared similar epidermal characteristics. They did not have trichomes on adaxial epidermis and their number on abaxial epidermis was relatively low, compared to other *Trifolium* species. Low trichome density, together with low wax content, contributes to higher digestibility of vegetative organs of these forage species.

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Comparative characteristics of Old World lupin chromosome variation by combinig BACs with novel oligo-based approach

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Keywords: lupins, cytogenetics, oligonucleotides

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Genus *Lupinus* as one of the most widly distributed, consists of more than 270 species, divided in two groups: New World lupins (NWL) and Old World lupins (OWL). In our research we are focused on OWL species, native to the Mediterranean region as well as North Africa. This group comprises12 annual herbaceous species, divided into 7 sections and characterized by high diversity in genome size, basic and total chromosome number [1].

According to previous research [2], OWL have experienced genome duplication and/or triplication during early stages of evolution (about 25 million years ago) as well as multiple chromosomal rearrangements.

The main focus of this research was to track structural changes within chromosome 6 of *Lupinus angustifolius* (Lang06) using four wild lupin species: *L. cryptanthus* (2n = 40), *L. cosentinii* (2n = 32*), L. micranthus* (2n = 52) and *L. pilosus* (2n = 42). Cytogenetic map [3], BAC library [4] and genome sequence [5] of *L. angustifolius* (2n = 40) have been used in this research.

A set of seven BAC clones, specific to Lang06, was used as probes for fluorescence *in situ* hybridization (FISH) in comparative mapping of studied species. All clones were locus-specific in *L. angustifolius* and *L. cryptanthus*, while in other species some still remained specific, but other generated dispersed signal/or were not detected at all. Although no differences were detected in closely related *L. cryptanthus*, each of the remaining three species endured different structural changes.

In order to perform mored detailed study of the complex structural rearrangements in wild species as compared to *L. angustifolius*, we decided to supplement our BAC-FISH results with the use of different probe type, designed to cover substantial chromosome regions. The utilization of thousands of oligonucleotides, bulked into pools and labeled with fluorescent dyes as FISH probes, proved to be very useful particularly in cross-species comparative analyses [6]. Therefore, we designed two sets of oligonucleotide probes, locus-specific to both arms of chromosome Lang06, using *L. angustifolius* genomic sequence. Each set consisted of 20 000 of short (45 nt) oligonucleotides covering one chromosome arm with approximate density of 1 probe/10 kb of genomic sequence. Results obtained from both BAC- and oligo-based FISH were not only compatible, but also complementary – coverage of larger chromosome region provided novel information about structural changes which occurred in studied species.

In further studies, additional sets of oligonucleotides for other chromosomes should facilitate better understanding of complex rearrangements among individual lupin chromosomes.

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Validation of QTL associated to pod and seed size in common bean using two nested RIL populations of common bean

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Keywords: morphological traits, bean genome, QTL mapping, QTL interactions

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Common bean (*Phaseolus vulgaris* L.) is an important legume worldwide which, depending on genotype, can be consumed as green pods or dry seeds after rehydration. The aim of this study was to identify QTL associated with seed and pod phenotype and evaluate the consistency of these QTL across different environments, studies and genetic backgrounds.

Two nested recombinant inbred populations obtained from the crosses Xana/Cornell 4924 (XC, 115 $F_{6:7}$ lines) [1], and Xana/BAT93 (XB, 145 $F_{6:7}$ lines) were used. Both populations were phenotyped for 6 pod traits (length, width, thickness, area, perimeter and number of seeds per pod), and 6 seed traits (length, width, thickness, area, perimeter and weight of 25 seeds). XC population was manually characterized in five seasons (2004, 2005, 2006, 2013 and 2014), while XB population was evaluated in two seasons (2015 and 2016) from image analysis of pods [2] and seeds [3]. Each season consisted of, at least, one replication per line arranged in a randomize complete-block design, where the replication consisted of 10 plants per line distributed in 1 m row plot. OneMap package of R software was used for linkage map construction [4]. A linkage map of 1390 cM was obtained in the XC population which includes 732 markers, most of them SNP markers obtained with the BARCBean6K_3 bean chip [5]. In the XB population a genetic linkage map of 1547 cM and 497 SNP markers was developed using genotyping by sequencing [6]. QTLNetwork 2.0 software was used to detect QTL with individual effects, epistasis and QTL–environment interactions [7].

A wide variation was observed in both RIL populations for all evaluated traits. For pod traits, 13 single-locus QTL were detected in the XC population (4 for pod length, 2 for pod width, 3 for pod thickness and 4 for number of seeds per pod) involving linkage groups (LG) Pv01, Pv02, Pv03, Pv07, Pv08, and Pv11. In the XB population, 18 single-locus QTL were detected for pod traits (4 for pod perimeter, 4 for pod area, 4 for pod width, 4 for pod length, and 2 for number of seeds) on LGs Pv01, Pv03, Pv05, Pv06, Pv07, Pv09, and Pv11.

Concerning seed traits, 21 single-locus QTL were detected in the XC population (5 for seed length, 6 for seed width, 6 for seed thickness and 4 for seed weight) on LG Pv02, Pv03, Pv05, Pv06, Pv07, Pv08, Pv10, and Pv11. In the XB population, 25 significant QTLs were detected for seed traits (6 for seed area, 5 for seed perimeter, 6 for seed length, 3 for seed width and 5 for seed weight) on LGs Pv01, Pv02, Pv03, Pv06, Pv07, Pv08, Pv09, Pv11.

Analysis of epistatic interaction between QTLs led to identified 20 and 27 significant epistatic interactions in the XC and XB populations, respectively. Overlap among identified QTL in the two populations was investigated considering physical positions of the underlying markers in the bean genome. Four overlapping regions for pod traits and eight for seed traits were identified on chromosome Pv01 (50.7-51.10 Mb), chromosome Pv02, (49.03-49.60 Mb), chromosome Pv07 (5.76 -6.94 Mb), and chromosome Pv11 (3.59 -4.52 Mb). Results showed the complex architecture of the genetic control of pod and seed phenotype and the usefulness of the genome in the integration and validation of QTLs detected in different genetic backgrounds.

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Genome-wide association studies of mineral content in common bean landraces

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Common bean (*Phaseolus vulgaris* L.) is a species of great interest worldwide. It is grown in Europe, Asia and Africa, where it presents similarities to Andean and Mesoamerican domestication centers or forms hybrids between both genepools [1]. In Croatia the production is based on landraces which display high levels of diversity and are important reservoirs of natural genetic variations [2].

On the basis of results of our research, 135 out of 174 accessions were assigned to 14 morpho-genetic groups originating from distinct domestication events: (1) Mesoamerican (‘Trešnjevac’, ‘Tetovac’, ‘Kukuruzar’, ‘Biser’), (2) Andean - indeterminate type (‘Sivi’, ‘Puter’, ‘Maslina’, ‘Dan i noć’, ‘Visoki Trešnjevac’), and (3) Andean - determinate type (‘Niski Trešnjevac’, ‘Dan i noć’, ‘Puter’, Bijeli’, ‘Zelenčec’). The rest of the accessions could represent putative hybrids among morpho-genetic groups including the hybrids among the gene pools.

Common bean is one of the most beneficial crop species while in diet it provides macro- and micronutrients. However, there is a substantial variability present in seed mineral content among genotypes. As reported by Palčić et al. (2018) [3] among investigated Croatian common bean landraces the highest nitrogen (N) content in seed dry weight was in ‘Visoki Trešnjevac’ (3.92%) while the lowest was in ‘Zelenčec’ (3.13%). Moreover, average N content of indeterminate types was significantly higher compared to determinate types. Phosphorus (P) values varied from 0.49% (‘Puter’) to 0.57% (‘Biser’) where determined P content in landraces of Andean gene pool was significantly lower compared to landraces of Mesoamerican gene pool. Significantly lower seed potassium content was determined in ‘Niski Trešnjevac’ (1.42%) and ‘Zelenčec’ (1.38%). Calcium content in all studied landraces varied from 0.34 (‘Kukuruzar’) to 0.42% (‘Biser’), with no significant differences among the researched landraces. Also, there were no significant differences in magnesium content among landraces. Magnesium content varied from 0.17 to 0.2% (‘Puter’), with the average of 0.18% (‘Biser’). Concerning the investigated micronutrients content, we can conclude that the highest iron content was found in landrace ‘Niski Trešnjevac’ while the lowest was in ‘Kukuruzar’. ‘Kukuruzar’ also had the lowest value of manganese (Mn) content (15.19 mg kg-1) and zinc (Zn) content (24.98 mg kg-1) while the highest Mn content was in ‘Biser’ (19.94 mg kg-1) whereas the highest Zn content was determined in ‘Visoki Trešnjevac’ landrace (27.84 mg kg-1).

Genotyping was carried out using microsatellite and DArTseq-based SNP markers to be used in genome-wide association study (GWAS). Linkage disequilibrium (LD) based GWAS has gained popularity particularly for screening a great number of accessions and to associate markers with desired traits in crops [4]. The unified mixed-model approach [5] allows the correction for population structure and family relatedness in order to remove false positives. Mixed model Q matrix was based on population structure obtained by model-based clustering method [6], while K matrix was constructed by calculating Loiselle’s kinship coefficients [7] in SPAGeDi [8]. Genome-wide scan for association between markers and nutrient content was performed using single marker approach as implemented in TASSEL [9] as well as multi-locus approach implemented in R package “mlmm” [10].

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Probing transcriptomes of divergent legumes via merging multiple, independent assemblies: *Cassia sturtii*, *Chamaecrista mimosoides* and *Senna obtusifolia*

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Keywords: transcriptome, Fabaceae, nodulation,

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The symbiotic interaction with rhizobia is a hallmark trait of Fabaceae family and confers a distinct adaptive advantage by facilitating atmosphering nitrogen fixation in specialized root nodule structures. However, while nodulation is characteristic of legumes, not all legumes are equally capable of nodulating. In general more than 80% of Mimosoideae and Papilionoideae legumes nodulate whereas less than 30% of the Caesalpinioideae species form nodules. Thus, this divergent offshoot represents a transition from non-nodulating to nodulating legumes and can help tease out the evolutionary origins of nodule formation (Doyle et al. 2011).

In our work we hypothesize that the differential distribution of nodulating species in some early-diverging legume taxa is reflected in divergent or convergent gene family changes throughout the clade. Moreover, we consider the possibility that differential loss rather than gain of several genes together with previously reported whole genome duplication (WGD) might have been the driving force that contributed to genomic changes related to the evolution of symbiotic nitogen fixation (for example, through altered affinities for microbial interactions). Therefore we aim to characterize gene families susceptible to evolutionary selection pressure in the early-diverging lineages of legumes. Transcriptomic and genomic data from closely related genera of Caesalpinioideae legumes, in particular *Chamaecrista* and *Senna*, and so called early diverging Papilionoideae as well as Mimosoideae genera *Desmanthus* and *Mimosa* will serve as basal research material in order to establish whether the changes have been gradual or punctuated. Comprehensive comparative analysis of several legume will be performed to indicate these potential determinants shared between Mimosoideae, Caesalpinioideae and Papilionoideae and cross correlate them with results of past inquiries (Brechenmacher et al. 2008).

Here we present preliminary data concerning transcriptome sequences of three legumes: *Cassia sturtii*, *Chamaecrista mimosoides* and *Senna obtusifolia*. A combined strategy of multiple, merged assemblies was deployed in order to obtain best coverage of different groups of putative transcripts. Firstly, the obtained raw data were filtered to remove low-quality reads (<Q10), trim primer, adaptor and low quality terminal residues. The pre-processed high-quality paired reads were assembled *de novo* using four methods: Trinity; SOAPdenovo-TRANS; VELVET/OASES pipeline at multiple values of k-mer length (20 - 70 bp, kroc et al. 2019). The individual assemblies were merged using Evidential Gene toolkit. Ortholog completeness of resulting curated assemblies was assessed using BUSCO v3 (Simao et al. 2015) with the Embryophyta reference set (embryophyta_odb9). The assembled transcripts were functionally annotated using Blast2GO Pro vs UniProt/SwissProt as well as using DAMMIT automated annotation pipeline.

Table 1. Raw data statistics (average over 2 replicates) and ortholog completeness of resulting transcriptomes:

Species name	Reads (Gbp)[a]	Reads (nb)[b]	GC (%)[c]	AT (%)[d]	Q30 (%)[e]	Ortholog completeness (BUSCOs %)
C. sturtii	18.6	123,224,964	46.7	53.3	94.6	95.8
C. mimosoides	20.1	133,288,485	45.9	54.1	94.6	94.6
S. obtusifolia	20.1	132,471,448	46.3	53.1	94.6	95.5

[a] Reads (Gbp): Total number of bases sequenced; [b] Reads (nb): Total number of reads. For Illumina paired-end sequencing, this value refers to the sum of read 1 and read 2; [c] GC(%): GC content; [d] AT(%): AT content; [e] Q30(%): Ratio of bases that have phred quality score of over 30.

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RNA-seq and RT-qPCR analysis reveal the involvement of precursor and mature miR169 in flower development and abscission in yellow lupine

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Plant miRNAs are small regulatory RNAs that recognize their target transcripts based on imperfect sequence complementarity. They repress expression of their target gene by guiding degradation and/or translational repression of the bound mRNA. MiRNAs are produced from single-stranded precursors that form hairpin structures, with the mature miRNAs sequence residing in one arm of the stems [1].

Yellow lupine (*Lupinus luteus* L.) is legume crop in which formation and development of flowers and seeds, is often associated with their abscission. In our previous analysis of transcriptomes of flowers, flower pedicels and pods of *L. luteus* dropped or maintained on plant among differentially expressed unigenes (DEGs) we identified the precursor of miR169 [2].

In present study, by using small RNA-seq, transcriptome and degradome sequencing and RT-qPCR analysis we identified mature miR169. We also analysed the expression profiles of both pre-miR169 and mature miRNA during development of flower on upper and lower part of raceme, in flower pedicels and after application of growth regulators.

As the result, we identified 5 isoforms of mature miR169 that could originate from the pre-miR169. We also identified the miR169 target genes. All mature isoforms, like pre-miR169, are strongly accumulated in pedicels of flowers undergoing abscission. Their expression is also up-regulated in upper flowers, usually dropped, and after application of stress-related growth regulators.

Presented data indicate that miR169 is involved in regulation of flower development and abscission in yellow lupine and can also be used as the molecular indicator of negative organ condition.

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2.6

Genetic variability study and kasp marker mapping analysis revealed a new haplotype determining pale hilum *Hc* in *Vicia faba*

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Phenotyping and genotyping of the Tunisian faba bean populations by comparison to two local commercial varieties (Badï and Bachaar) and world diversity collection, revealed significant diversity. Consequently, three individuals carrying pale hilum were identified within two Tunisian populations. Several studies mentioned that pale hilum trait was in linkage with low vicine-convicine gene (VC-) that reduces 20 to 30 fold these two anti-nutritional factors. Thus, vicine-covicine were quantified in the three pale hilum Tunisian individuals as putative candidate for VC- gene, Badï and Bachaar varieties and other pale hilum accessions from different origins and compared to the check varieties Disco and Melodie carrying pale hilum and VC-. Results showed that the pale hilum trait in the Tunisian individuals is not associated with zero vicine-convicine. However, some of them were distinguished by their significant lower vicine-convicine levels compared to the Tunisian and international commercial cultivars. A separate genetic mapping study using a segregating biparental population developed previously at NIAB (UK) was carried out. This analysis allowed the identification of new kasp marker linked to pale hilum traits segregating in the population. Genotyping of the F2 generation and plant and seed phenotyping over F2:3 and F3:4 generations were conducted. The Mendelian factors Hc (Hilum color) controlling hilum color were located in linkage groups 2. The mapped marker for Hc allowed the identification of a new haplotype within the Tunisian individuals that differ from the European pale hilum cultivars.

Metabolite profiling of pea (*Pisum sativum* L.) seeds during accelerated ageing revealed accumulation of sugar alcohols and sugar acids

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During seed storage, natural metabolic and non-metabolic discrete processes occur, leading to seeds deterioration, decrease of their vigor and loss of viability. In legume seeds, galactinol and raffinose family oligosaccharides (RFOs), accumulated during seed maturation, seem to participate in seed longevity *via*: *i*) maintain cytoplasm in glassy state [1], *ii*) scavenging hydroxyl radicals [2], *iii*) retain the integrity of cellular membranes through interaction with the phospholipids headgroups, replacing water during cells dehydration. However, during both natural and accelerated ageing of seeds, a degradation of RFOs occurs, leading to an increase in the levels of reducing monosaccharides [3, 4], which can accelerate both production of reactive oxygen species and non-enzymatic modification of proteins and enzymes through Amadori and Maillard reactions [5]. It can be expected that during seed storage/ageing significant changes occur not only in sugars but also in whole metabolome. To improve our understanding of pea seed storability, the metabolite profiling of embryonic axes and cotyledons of accelerated aged pea seeds by GC-MS method was analyzed.

Material and methods

Seeds of pea cultivars differing in RFOs content (11-12% of dry weight, DW - cv. Kelwedon Wonder and Telefon, and 7% - cv. Hubal and Venus) were exposed for accelerated ageing (AA-test) at 37°C and 75% RH. Electrolyte leakage, seed viability, seedlings length/fresh weight, and polar metabolic profiles (in embryonic axes and cotyledons, by GC-MS method [6]) were analyzed after 0, 1, 2, 3 and 4 weeks of seed ageing. Moreover, for targeted analyzes of RFOs, a GC-FID method was used [4].

Results

The water content in embryonic axes and cotyledons of dry seeds increased from 4.7-6.4 to 11-12% after 28 days of accelerated ageing. A gradual decrease in seeds viability (except of cv. Venus) and vigor (all cultivars) was observed along with seed ageing. Among 37 identified polar metabolites, RFOs, sucrose, galactinol and *myo*-inositol were the major compounds. Total soluble sugars shared 92-94% of total polar metabolites. Embryonic axes of dry pea seeds contained higher amounts of RFOs than cotyledons (19-24 and 7-12% of DW, respectively). The significant ($P<0.05$) changes in the concentration of some sugars, phosphate and malate were detected. The concentration of RFOs transiently (up to 14th day of AA-test) increased in cotyledons (all cultivars) and embryonic axes (cv. Hubal and Kelwedon Wonder) and thereafter significantly decreased in embryonic axes (except of cv. Venus, resistant to ageing). The degradation of RFOs was not associated with appropriate increase in mono-saccharides and sucrose. Therefore, the accumulation of sugar alcohols (sorbitol, galactitol) and sugars acids (gluconic and galactonic acid) in embryonic axes (and at lesser extent in cotyledons), detected along with seed ageing (**Fig. 1**), can be explained as a part of defense mechanisms leading to decrease of reducing sugars, released from oligosaccharides, and/or activation of ascorbate synthesis [7].

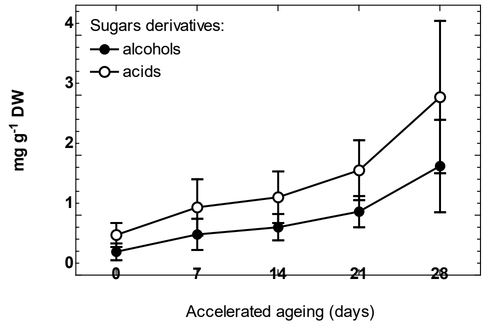

Figure 1. Accumulation of sugar alcohols (sorbitol and galactitol) and sugars acids (galactonic and gluconic acid) in embryonic axes of pea seeds during accelerated ageing. Values are means for 4 cultivars ± SD

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Effect of sucrose and asparagine on transcriptome of embryo axes of white lupin (*Lupinus albus* L.) and Andean lupin (*Lupinus mutabilis* Sweet)

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Keywords: autophagy, degradation of autophagic bodies, sugar starvation

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Lupin embryo axes are very useful objects which are used in research of regulation of metabolism of germinating seeds. By simple modification of trophy conditions of *in vitro* culture of isolated embryo axes it is possible to study the effects of variety of nutritional and regulatory substances on the metabolism [1]. In our research we focused on the role of sucrose and asparagine (a central amino acid in lupin seed metabolism) in a course of autophagy. We found that sugar starvation enhances autophagy in cells of lupin isolated embryo axes, but one of the final stages of autophagy, namely the decomposition of autophagic bodies inside vacuole, is clearly inhibited by asparagine [2]. Trying to describe the mechanism of asparagine action in the decomposition of autophagic bodies we performed transcriptomic analysis of lupin embryo axes which were isolated from imbibed seeds and cultured for 96 h *in vitro* on medium with 60 mM sucrose, without the sugar, and on both above mentioned media supplemented with 35 mM asparagine. From such embryo axes RNA was isolated and transcriptomic libraries were prepared. The quality of the libraries was verified by Sanger sequencing method. After positive qualification of the libraries we have performed large-scale transcriptomic sequencing using Illumina HiSeq Next Generation Sequencing technology (NGS). The obtained sequence reads were aligned to reference transcriptome and counted in the aim to find differentially expressed genes.

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Genome-wide association study for seed quality traits in chickpea

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Keywords: chickpea, seed quality, GWAS

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Chickpeas are an important source of nutrition for the world's population. Chickpea protein, which is more bioavailable than other pulses can range from 18-28% total dry weight basis. Its oil content is relatively high, ranging from 2.7-6.48%. This can be problematic for millers as the high oil content causes the flour to be sticky and susceptible to rancidity [1].

Global demand for high quality chickpeas and chickpea products is growing [1]. Canadian chickpea production is expected to increase as a result. Breeding efforts for Canadian varieties have mainly focused on agronomic traits with little attention paid to quality traits. Seed protein and oil content are important traits, and quantitatively inherited [2].

Genome-wide association studies (GWAS) have been employed in breeding populations to aid marker assisted selection (MAS) and genomic selection (GS) applications [3]. To improve seed protein and oil content in chickpea an understanding of the genetic basis of the seed quality and nutrition related traits is crucial.

GWAS is a popular association mapping technique that uses genome-wide markers to identify marker-trait associations. Association mapping relies on linkage disequilibrium (LD) and historic recombination events across the genome. Mapping panels are composed of diverse individuals with wide allelic variation, allowing GWAS to capture as much trait variation as possible. Combined with dense marker coverage as GWAS offer high power, and high resolution mapping [3].

Though often performed within diversity panels, performing association mapping within breeding program populatons leads to results that more applicable to the program which can expedite implementation of markers into the selection process [3].

A panel of 184 diverse chickpea accessions including materials developed at the Canadian breeding program were grown during the summers of 2017 and 2018. The panel is composed of 50 desi types and 134 kabuli type lines. The population was grown at two locations each year, with three replications per location. The panel was phenotyped for total crude protein, oil content and flour colour. Over 60 K single nucleotide polymorphisms (SNP) derived from whole genome sequences were used to analyze genetic diversity and population structure of the 184 chickpea accessions. Population structure analysis revealed several distinct groups, mainly composed of either desi or kabuli type accessions. QTLs and candidate genes associated with total protein and oil content will be identified through genome-wide association analysis.

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2.10

Characterization of microsatellites revealed by transcriptome sequencing of *Trifolium resupinatum*

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Persian clover *Trifolium resupinatum* L. is an annual clover used as fodder and hay, native of Southern Europe, the Mediterranean and Southwest Asia. It is an important hay crop in cold regions of Iran, Afghanistan, and other Asia countries with cold winters. It grows to 60 cm high when cultivated and may produce more than 85 ton/ha of green fodder and more than 11 ton/ha of dry matter when used in appropriate rotation. Being tolerant to low temperatures and drought stress period, this fodder crop is of outmost interest in a global climate-changing world.

Roots and aerial parts of three genotypes with different potential for biomass were used for transcriptome analysis. "De novo" and reference based analysis of the transcriptome of roots and aerial part of the plant were conducted to obtain and annotate a global "de novo" reference transcriptome for this species. A total of 1,9x107 100-bp pair-end reads were assembled with Trinity into 214,364 contigs, with a N50 contig of 1050 bp. The transcriptomes of roots and aerial parts of Persian clover were mapped against the genes models catalogue of *Medicago truncatula* Mt4.0v1 and *Trifolium repens* V2 (www.phytozome.net). In general the 70-80 % of the fragments mapped against the genome sequences of these two legumes. This new genomic resource was proved valuable to identifiy and develop new molecular tools such as microsatellite markers, that will to assist *T. resupinatum* breeding and for comparative genomic studies with other species of clovers.

Genetic variability and heritability for yield and yield components in chickpea

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Keywords: phenotypic and genotypic coefficient of variation, chickpea, heritability

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Chickpea (*Cicer arietinum* L.) is one of the important legumes widely grown for dietary proteins in semi-arid Mediterranean climatic conditions. To evaluate the genetic diversity with improved heat and drought tolerance capacity in chickpea, thirty-four selected chickpea genotypes were tested under three different field-growing conditions (rain fed winter sowing, irrigated-late sowing and rain fed-late sowing) in 2014-2015 and 2015-2016 growing seasons. A factorial experiment in randomized complete block design with 3 reps was conducted at the Eastern Mediterranean Research Institute Adana, Turkey. The objective of this research was to evaluate the magnitude of genetic variability and heritability for yield and contributing characters of 34 chickpea genotypes. Heritability in broad sense ranged from 0,31- 68,41% in winter sowing condition; 7,47-66,35% in irrigated- late sowing condition as well as7,87-66,35% in rain fed-late sowing condition. The traits such as hundred seed weight in winter sowing condition, flowering day, plant height and hundred seed weight in irrigated- late sowing condition as well as first pod height and hundred seed weight in rain fed-late sowing condition had higher genotypic coefficient of variancethan phenotypic coefficient of variance values, which indicated the lower effect of environment.

3.1

Opportunities and strategies for integration of molecular markers into an applied pulse crop breeding program

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Pulse crops including pea, lentil and chickpea are important crops across the northern tier states and the upper Midwest region including North Dakota and eastern Montana (Mon-Dak) lead the nation in production, accounting for about 80 percent of total US pulse production. The Pulse Breeding Program at North Dakota State University continues to develop new high-yielding varieties of pea, lentil and chickpea with increased resistance to pest and diseases and superior quality traits, best suited for landscapes and climate of the Mon-Dak region. The breeding program uses the conventional breeding method and a network of controlled environment and field experiments to develop elite breeding lines. While breeders have successfully used the conventional procedure to develop better varieties, making this process faster and more efficient is a perennial challenge for plant breeders. The emergence of cost-effective high-throughput genotyping and modern sequencing technology are revolutionizing plant breeding. The plummeting costs of genotyping are allowing crop breeding programs to routinely genotype breeding lines with gene-based and genome-wide DNA markers at a large scale. We aim to test and integrate marker-assisted selection to increase the accuracy of selection for large-effect genes conferring disease resistance. For traits with complex genetic architecture, we aim to systematically evaluate the efficacy of genomic selection for seed yield and quality traits. Our exploration and implementation of molecular markers for pulse breeding would spur interest to other pulse breeding programs and will provide a big step forward for integration of genomic selection in a public breeding program.

Soybean light use efficiency upon sufficient soil moisture

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Keywords: soybean, light use efficiency, day and night dynamics

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Even though improved photosynthetic efficiency has played only a minor role in the increases in crop yields in recent decades, improved photosynthesis is expected to be a major driver of yield increases in the future [1]. Genetic improvements in the conversion efficiency of radiation into biomass is now a promising strategy to meet projected changes in the demand for agricultural crops [2]. The aim of this study to evaluate light use efficiency (LUE) of soybean plant leaves from instantaneous measurements over day and night. The measurements of photosynthesis were obtained using a photosynthesis and transpiration rate monitor PTM-48A («Bio Instruments SRL», Republic of Moldova) taking continuously over 24 h periods. This device has been previously successfully used by other authors [3,4 and others].

The measurements were carried out during typical summer days on soybean plants (cultivar Amelina) in 10 l pots under conditions of sufficient water supply, with a FTSW (fraction of transpirable soil water) of no less than 0.7. All data were collected from measurements on fully developed leaves. Gas exchange measurements (96 time points at 15 min intervals throughout the day and night) were conducted on 20-23.07.2018 (67-71 days after planting) on fully developed sun-lit leaves (specific leaf dry weight 45 $g\ m^{-2}$).

The following parameters were measured: photosynthetically active radiation, PAR, ($\mu mol\ quanta\ m^{-2}\ s^{-1}$), real assimilation ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$), total respiration ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$), CO_2-exchange (real assimilation minus respiration, $\mu mol\ CO_2\ m^{-2}\ s^{-1}$).

The slope of light response curve for evaluating quantum efficiency [5] is highest in early morning (PAR less than 100 $\mu mol\ quanta\ m^{-2}\ s^{-1}$). Maximal quantum efficiency (~1,5 $\mu mol\ CO_2\ m^{-2}\ s^{-1}$ per 20 $\mu mol\ quanta\ m^{-2}\ s^{-1}$) was 0.075, i.e. absorption of one CO_2 molecule needed 13 quanta PAR. This value for maximum quantum efficiency compare with the following published data for soybean: 0.068-0.054, or 15-19 *quanta* PAR per absorbed CO_2 molecule [6].

CO_2-exchange dynamics (see Figure 1) are especially important for plant productivity. The pattern observed here consists of two parts: day (sunlight) and night. At night (from 21 in the evening to 5 in the early morning) there is no photosynthesis and CO_2 is emitted due to respiration (up to -0.3 $g\ CO_2\ m^{-2}\ hour^{-1}$). The period of sunlight (from 6 in the early morning to 20 in the evening) characterized by high rates of CO_2-exchange (up to 5.0 $g\ CO_2\ m^{-2}\ hour^{-1}$). From continuous measurements of CO_2-exchange (20-23.07.2018) we estimated daily CO_2-assimilation, 27.80±0,16 $g\ CO_2\ m^{-2}\ day^{-1}$. Total solar (Q) daily radiation was 22.25±0.52 $MJ\ m^{-2}\ day^{-1}$. In view of several definitions of LUE [7], LUE was determined as the amount of CO_2-assimilation in relation to amount of total solar daily radiation and was established to be 1.25±0.04 $g\ CO_2\ MJ^{-1}$. Our value corresponds to known constant LUE for soybean: 1.3 *g dry matter* MJ^{-1} [8].

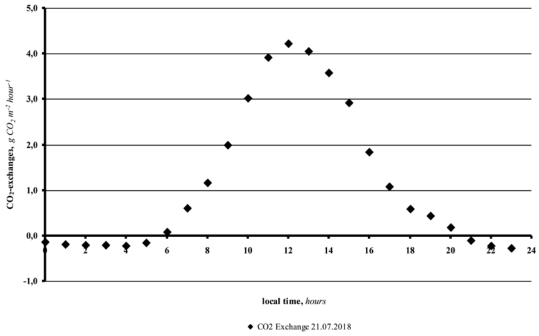

Figure 1. Day and night dynamics of soybean leaf CO_2-exchange (21.07.2018)

Conclusion. For well-watered soybean plants cv. 'Amelina' the maximum quantum efficiency of photosynthesis equal 0.075, i.e., the absorption of one CO_2 molecule needed in 13 quanta PAR. Over a long period (24 hours) soybean leaf LUE was 1.25±0,04 $g\ CO_2\ MJ^{-1}$.

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Possibilities of plant breeding to powdery mildew resistance – case study “Peas” – *Erysiphe pisi*

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Keywords: pea, powdery mildew, resistance

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Powdery mildew (*Erysiphe pisi* DC., *Ascomycetes*) is a specialized form of *E. pisi* f. sp. *pisi* adapted to pathogenesis on pea (*Pisum sativum* L.) only. Seed yield losses of 10 – 60 % caused by powdery mildew in the warmer and drier conditions were reported in peas.

Pea genetic resources with resistance to powdery mildew were detected in Peruvian peas populations and otherwise in commercial cultivar of wrinkle seeded pea *Stratagem* derived from Mexican population of Mexique 4. The resistance to powdery mildew is controlled by recessive genes *er – 1* and *er – 2*). Gene *er-1* can bring about full resistance while gene *er – 2* provides only leaf resistance. Both genes are inherited independently to each other. The crosses of susceptible and resistant plants result in F_1 generation all susceptible ones and segregation of F_2 into susceptible and resistant plants give a good fit to 3: 1 ratio. Disadvantages of cited sources of resistance were low yield potential, low TSW value and high sensitivity to all other pea diseases [1]. Qualitatively different resistance sources mainly with higher yield potential, TSW value, tolerance to complex of root pathogens and all other diseases (viruses included) are necessary for successful powdery mildew breeding realisation. This issue is recently the object of intensive activities of breeder teams. The first pea lines of *afila* type close to those characteristics are acquiring. During the years 2010-2018, several lines of field pea were developed by crossing. The Canadian variety Melfort and the American variety Franklin were used to create these resistant materials that were used and included in the gene pool collection. 36 new lines and 13 commercial varieties were compared under field conditions. Due to the large occurrence of deadly flies, statistically significant differences in the yield of individual genotypes were found, which confirms the literary sources about the connection of the disease and its impact on the amount and quality of the yield. Resistant lines to the paddles were resistant to other pathogens such as brown scab and virus complexes. The yields of varieties and lines are shown in the columns in Figure 1. Here, the yield differences between resistant lines and sensitive varieties, which in the years when there is not a strong occurrence of dumplings, are clearly visible. The highest yield from the 13 registered varieties in the Czech Republic reached Abarth, which is on the list of recommended varieties (LRV) and it is the only variety of field peas resistant to mildew. Its average yield of three reps was 3.35 kg / 10 m^2. The average yield of 36 breeding lines from Agritec was 3.05 kg / 10 m2. The highest yields showed AGT 2018.10, 4.09 kg / m^2, AGT 2018.7 (4.20 kg / 10 m^2) and AGT 2018.9 (Beate x (Kamelot x Franklin)) with 4.81 kg / 10 m^2. There has been great progress in garden pea breeding. Six varieties (Cetris, Dragon, Morriss, Twinset, Johan and Cedrik) have been registered in the Czech Republic with resistance to powdery mildew and other fungal and viral pathogens.

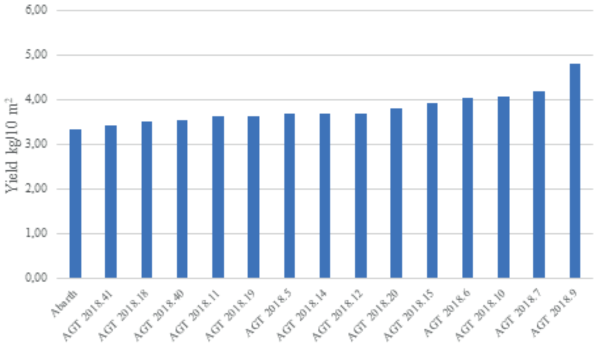

Figure 1. Average yield of lines (kg/10 m^2)

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The variability of the chlorophyll fluorescence parameters, the chlorophyll content, the leaf water potential and their relationship with seed yield of narrow-leafed lupin (*Lupinus angustifolius* L.)

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Keywords: chlorophyll fluorescence, chlorophyll content, water capacity of leaves, seed yield, narrow-leaved lupin

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Narrow-leaved lupin (*Lupinus angustifolius* L.) and other lupins are characterized by a high potential of seed yield production, especially with reference to protein content. However, final seed yield depends on a selection of appropriate cultivars as weel as environmental and agricultural factors. Furthermore, plant productivity of lupins is affected by the intensity of the photosynthesis process. Each limitation of the intensity of this process can contribute to a decrease of seed yield size and quality [1]. Physiological indicators which determine the efficiency of the physiological state are rarely used as selection criteria in breeding programs.

The aim of this study was to assess the variability of the physiological indicators (the chlorophyll fluorescence parameters, the chlorophyll content and the leaf water potential) and their correlation with seed yield in narrow-leafed lupin cultivars at the flowering and green leaf phases.

Field experiments with selected 30 lupin cultivars were conducted at two locations in the Wielopolska region in Poland with three replications in 2016-2018. The chlorophyll fluorescence parameters, the chlorophyll content and the leaf water potential measurements were made in field conditions at three phases of plant growth and development (from the beginning till the full flowering phase, from the full till the end of flowering phase and at the green leaf phase, before plant maturity). All measurements were made using the mobile fluorimeter HANDY PEA (Handy Plant Efficiency Analyser, Hansatech Instruments Ltd., King's Lynn, Norfolk, UK), the SPAD 502 Plus leaf chlorophyll meter (Konica Minolta, Wrocław, Poland) and the PMS leaf hygrometer (Geomor-Technic Ltd., Szczecin, Poland). On the basis of the measured values, the parameters characterizing the energy conversion in photosystem II (PSII) were calculated using the fluorescent JIP test [2]. Seed yield was assessed on the basis of representative sample of plants from each plot. The varialibity of the physiological indicators for each cultivar of lupin over measurement terms was illustrated graphically. The results of the measurements were analyzed statistically, independently for each year using the analysis of variance (ANOVA) under a linear model for randomized complete block design [3]. The analysis of correlation were applied in order to identify relationships between seed yield and the physiological indicators using the MS EXCEL 2016 program.

Measurements of photochemical activity of PSII were differentiated for all measurement terms and research years. Generally they showed decrease, indicating poorer condition of leaves of narrow-leafed lupin before plant maturity. Based on statistical analysis, the five chlorophyll fluorescence parameters (Fv/Fm, psi0, P.I.csm, ETo/CS, ETo/RC) were selected for further studies. The chlorophyll content was the highest from the beginning till the full flowering phase (average SPAD value 58,10) and it was the lowest at the green leaf phase (average SPAD value 45,71) in both locations and measurement years. The leaf water potential was also the highest from the beginning till the full flowering phase (average 5,89 bar) and it was the lowest at the green leaf phase (average 2,96 bar) in both locations and measurement years. The correlation between selected physiological indicators and seed yield were differentiated for all measurement terms and research years. The results of this study suggests the direction of further research aimed at the elaboration of breeding method for seed yield using the field equipment.

In conclusion, the fluorimeter is a quick, precise and non-destructive tool, widely used in investiganting damage caused in the photosynthesis plant system by various types of stress [4]. The chlorophyll meter is a simple, portable and diagnostic tool that measures the relative chlorophyll concentration of leaves [5] on the basis of leaves greeness index compared with the traditional destructive methods. Furthermore, the leaf hygrometer could be used to manage a crop irrigation and to adapt an irrigation to cultivars.

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Breeding methods applied to faba bean for enhancing sustainable yield production

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Keywords: breeding methods, recurrent selection, pedigree method, synthetic cultivars

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Faba bean (*Vicia faba* L.) is one of the oldest crops grown in the Fertile Crescent. Presently faba bean is one of the most important grain legumes in East Asia, East and North Africa and Middle East and is classified as the fourth most widely grown cool season legume [1]. The crop is grown across a wide agro-geographical region [2] in more than of 38 different diversified cropping systems. It remains an important crop because of its high-yield potential, nutrition-dense grains, its high protein content and its role as forage crop [3]. Faba bean (*Vicia faba* L.) is a partially allogamous crop with outcrossing rates ranging from 3 to 83% [4] Breeding faba bean methods are either based on self pollination which may lead to independant pollinated cultivars or on open pollination leading to dependant pollinated cultivars.

The purpose of this research is to compare the performance of self pollination breeding methods as Pedigree method (PSM) and open pollination breeding methods as recurrent selection (RSM) and Synthetic development (SYN) and to determine which traits explain the major variability.

Eleven parental lines were used to develop 24 self pollinated lines using pedigree selection method, 35 open pollinated lines using recurrent selection method and nine syntheitc populations from 2012 to 2016. The different developed lines were evaluated in two cropping seasons 2014/2015 winter and 2016/2017 winter) in alpha design with two replications. Grain yield (GY) biological yield (BY), days to flowering (DFLR), days to maturity (DMAT), branches per plant, pods per plant (PNPLT), seeds per pod (SNPP), seeds per plant (SNPLT), hundred seed weight (HSW) were recorded for all developed lines. Significant differences among genotypes were observed for GY, BY, DFLR, DMAT, HSW and SNP in 2014/2015 and 2016/2017 seasons. Signficant differences among the breeding methods.

Synthetic cultivars had higher yield than lines developed by recurrent selection method and those recurrent lines had higher yield than the lines obtained by pedigree selection method. The major reason might be due to the exploiting of heterosis and heterogeneity in faba bean synthetic varieties [5]. The lines with superior yield as compared to the checks are presented in the Table 1.

Multivariate anaylsis indicates that most of the variation among different lines developed by different methods is due to variation in biological and grain yield.

open pollinated cultivars were found more appropriate to increase the yield in farmers field and Faba bean open pollinated cultivars thus can contribute to pollinator and biodiversity protection – and this potential might be considered by breeders to more harmonize agriculture and biodiversity protection.

Table 1: Mean value of all traits for all selected lines by different methods, average of parent lines and best check and standard error

Lines	BY (Kg/ha)		GY (Kg/ha)	
	2014/15	**2016/17**	**2014/15**	**2016/17**
	Pedigree Lines			
PE32	5276	5663	2758	2679
PE33	5276	5766	3100	2787
PE39	5139	4852	2832	2311
PE40	6113	5548	3045	2352
	Recurrent Lines			
R-6	4861	7924	3020	3406
R-9	6116	5766	2850	2353
R-14	6528	5620	3104	2496
R-16	6807	6006	2993	2371
R-22	7362	5240	3575	2671
R-25	6667	6282	3296	2669
R-28	6665	6491	2869	2751
R-29	7083	6470	3114	2582
R-45	6391	5664	3180	2715
R-47	6111	5452	3711	2363
R-53	6520	-	2958	-
	Synthetic Lines			
Syn3	4862	7971	3007	3349
Syn4	6947	6863	3437	2809
Syn5	8057	6997	3144	3067
Syn6	7493	7652	3433	2843
Syn7	--	7018	--	2993
Syn8	6667	6409	3340	3232
Syn9	9167	6740	3465	2881
Syn10	7084	6862	3388	2623
Syn11	7221	6184	3488	3523
Best check means	7778	4815	2614	2267
SE	195.8	243.8	83.56	97.87

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Future faba bean for food and feed – a research and breeding initiative on faba bean (*Vicia faba*) in Sweden

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European plant protein consumption is today highly dependent on soybean import [1]. In Sweden, grain legumes are only grown on a minor part, at most 3%, of the total arable land and the majority (80%) is used for feed purposes. However, the trend of using locally produced fresh/green or dried pulses in food is increasing and the potential for increased production and consumption of these crops in Sweden is therefore large. To make use of this potential, investments in breeding of grain legumes suitable for a Swedish agricultural context to handle current challenges are necessary. This will contribute to a sustainable and secure production of food and feed in Sweden.

This project will initiate a breeding program on faba bean (Figure 1) and develop state-of-the-art techniques targeting traits of importance for successful country-wide production for both food and feed. It will also through molecular and genetic research dissect specific traits of interest for the development of novel healthy and tasty faba bean food products. New faba bean varieties adapted for food products will add economic value for the farmer through the diversification of this crop from being used mainly for feed as at present in Sweden, while new feed varieties should aim at increased acreages for feed production.

Faba bean has been a target of plant breeding in Swedish companies during 1930-1990 when seed size and earliness were the traits in focus. A breeding program for faba bean today will include targeting traits like yield by addressing some of the bottlenecks (ex; earliness and concentrated flower period), plant protection (ex; resistance to root rot; *Phytophtora pisi* and chocolate spot disease; *Botrytis fabae*), seed size, and quality (ex; protein content, good taste, a balanced amino acid composition, low levels of anti-nutritional compounds such as tannins and convicin/vicin.

Central to the execution of this project will be assembly of a faba bean germplasm panel from available genetic resources from diverse parts of the world [2] with properties relevant to a Swedish context. This germplasm panel will be phenotyped in field and genotyped to develop statistical models for the development of molecular breeding tools. The development of such tools and increased knowledge on the genetic regulation of specific traits in this crop should contribute to paving the way for realising the potential of faba bean in the future [3].

The ultimate goal with this project is to meet the Swedish demand for protein-based food and feed products by enabling domestic production of high-quality protein crops. The project is collaboration between academy and industry.

Figure 1. Colored flowering genotype of faba bean.
(*Photo: Å. Grimberg*)

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Gibberellic acid enhances crossing success rate in lentil

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Keywords: gibberellic acid, intra- and inter specific crossing, breeding, pre-breeding

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Lentil (*Lens culinaris* Medik.) is an important food legume that could help to sustain farming systems thanks to its ability to fix N in soils. Its nutritionally-rich seeds are consumed as staple food and as source of proteins in vegetarian dishes as well as in salads thus contributing to secure foods in developing countries and to provide healthy diet worldwide. Enhancing lentil production is needed in many parts of the world. Developing high yielding varieties with resistance to biotic and abiotic stresses and improved grain quality could help to achieve this goal. Intra- and interspecific crossing is key step for breeding and pre-breeding research programs aiming at developing improved varieties. However, due to the small size, fragility and cleistogamous nature of the flowers, the crossing success rate in lentil is often low. To overcome this, we used gibberellic acid (GA_3) (75 ppm) in intra- (*Lens culinaris* Medik. X *Lens culinaris* Medik.) and interspecific (*Lens culinaris* Medik. X *Lens orientalis*) crossing blocks. A total of 260 and 380 intra- and interspecific individual crosses, respectively, were made. Interestingly, immediate spraying of GA_3 into pollinated flowers increased the crossing success rate from 13.07 % and 3.15 % (in blocks without GA_3) to 23.2 % and 9.37 % for intra- and interspecific crosses, respectively. The obtained results show that the application of GA_3 could help to improve the success rate of crossing in lentil in the perspective of its efficient use for breeding and pre-breeding purposes. Indeed, GA_3 plays an important role in many plant growth and development processes

Single seed descent under extended photoperiod as a simple, rapid and efficient breeding method for accelerated genetic gain in lentil

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Keywords: speed breeding, SSD method, extended photoperiod, genetic gain

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Lentil (*Lens culinaris* Medik.) is an important grain legume for food and sustainable diversification of cereal-based cropping systems. Its grains, nutritionally rich in protein, iron and zinc, are consumed as staple food in developing countries as well as in vegetarian dishes and salads worldwide. Improved varieties are among the key solutions for enhancing crop productivity. However, conventional plant breeding commonly used, especially in developing countries, to develop varieties is a time and resources consuming process. Furthermore, resistance/tolerance to biotic and abiotic constraints are under continuous evolution. Thus, there is a need to speed crop improvement and delivery of adapted varieties. We tested a simple, rapid and efficient breeding protocol based on single seed descent method under extended photoperiod to accelerate genetic gain in lentil. An F$_2$ population of 280 individuals from a single cross between an elite line with rust resistance (F00-24) and an adapted Moroccan landrace (MGB1027) was advanced. The population was grown at high density under an extended photoperiod of 22 hours light/25.5 °C and 2 hours dark/15 °C in a growth chamber. This protocol accelerated the growth with plants taking 31 and 39 days after sowing for the first flowers and pods. Pods at physiological maturity were harvested 64 days after sowing. The harvested F$_3$ seeds were oven-dried (30-35°C for 2 days) and planted using the above-described protocol to obtain F$_4$ seeds and thus speed up the generation turnover. This protocol will allow 4-5 generations per year instead of only one or two in conventional greenhouse-based methods. After F$_6$ generation first selection tests will start, and afterwards selected lines will go through comparative yield trials to identify promising lines for variety development. This simple but efficient method will contribute to speed breeding and variety release. An additional potential use of this protocol is the rapid generation of recombinant inbred line populations (RILs) and other genetic resources for genetic and molecular studies.

Identification of SNPs associated with agronomic traits in lentil using bulk segregant analysis in advanced backcrossed mapping population

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Keywords: SNPs, BSA, association, agronomic traits, lentil

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Wild relatives in lentil have been shown as wonderful repository of an array of useful genes, hitherto not found in the cultivated species. However, these precious genetic resources have not been used widely due to their poor yield and close association of undesirable traits with desirable traits (linkage drag). Advanced backcross-QTL analysis has been has been identified as a potential solution for the discovery and transfer of valuable QTLs from unadapted wild relatives into elite breeding lines in a single process. Therefore a $BC_2 F_2$ mapping population comprising 835 individuals was developed from cross made between cultivated species (IPL 220; *L culinaris*) and a wild species (ILWL 118, *L orientalis*) and data recorded for agronomic traits on 487 individuals of this population . Bulk sergeant analysis was used to identify the SNP markers associated with agronomic traits. DNA of bulks along with corresponding parents were used for SNP genotyping following double digestion restriction site DNA sequencing method. It resulted in identification SNPs associated with plant height, pods/plant, flowering time, 100-seed weight, primary branches, pods/cluster, and internode length. The SNPs possessing nucleotide sequences are being analyzed for identification of functional genes underlying these traits. These SNPs will be used to genotype the BC_2F_2 population for QTL mapping.

State and outlook for white lupin breeding in Russia

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White lupin has the highest productivity potential among grain legumes under the Russian conditions (4.5-5.0 t/ha) and its seed quality is close to the soybean one (protein content is 36-38%, oil content is 8-10%). Amino acids composition of white lupin seeds has high nutritive value: there are 20% of glutamine, 11% of asparagine acids, 40.6% of essential amino acids, 4.7% of them is lysine [1]. As well white lupin ripens earlier, is more energy saving and has higher yield than soya.

The main areas of white lupin breeding are as follows: optimal growth season (110-120 days), increasing of grain productivity potential, complex resistance to fusaria and anthracnose, drought resistance, lower alkaloid and fiber content in seed, higher content of protein, oil, lysine.

At present the early ripening, fusaria resistant var. Dega takes the main cultivation area for lupin in Russia. 2004 it' was registered in the State List of Breeding Achievements in Russia. Late var. Alyi parus has high grain and green mass, is the State List since 2012, early drought resistant varieties Mitchurinsky and Piligrim are in the State List since 2017 and 2019 respectively.

Cluster analysis of recent white lupin varieties and promising lines bred in the Russian Lupin Research Institute allowed to highlight three samples' groups which differ in vegetation period, seed and green mass yield (Figure 1). Samples of the first group are early maturing (their growth season is 110-112 days), seed and green mass yield is 4.1-4.4 t/ha and 58-61 t/ha respectively. The growth season of the samples from the second group is in average 5 days longer, seed and green mass yield is 4.4-4.8 t/ha and 63-67 t/ha respectively. The late var. Alyi parus (120 days) is in the single cluster. Its seed yield was as varieties of the second group had and its green mass yield was over 70 t/ha [2].

The correlation analysis of productivity elements of non-determinant and determinant white lupin lines showed high positive relationship between seed productivity and green mass of a plant (r = 0.90 … 0.95), as well as medium relationship between seed weight and harvest index per a plant (r = 0.44 … 0.57) [3]. Thereby increase of the total plant green mass is the condition to increase yield potential of new varieties of white lupin if the harvest index will be saved as 45-50%.

Mutant and transgressive lines are bred; their green mass is 1.5-2 times higher than the standard var. Dega has, their potential seed yield is 6-7 t/ha.

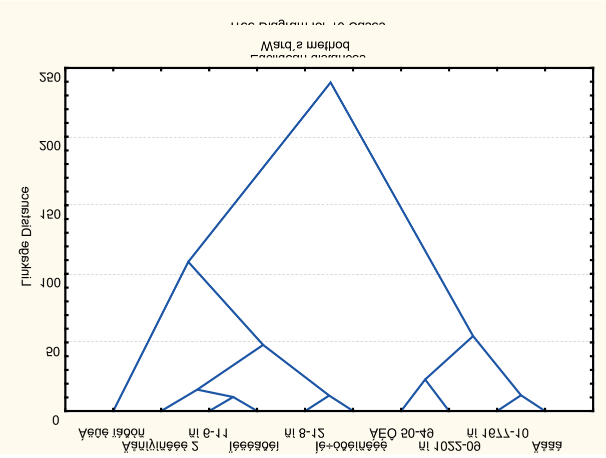

Figure 1. The distribution of white lupine samples for yield and growing season

Sources of high protein content (40-41%) have been picked out from the collection/ There are late Egyptian alkaloid lines k-3665, k-3667, k-3670, and Ethiopian lines k-486 k-495. Their protein content is 3-4% higher than the standard and they are included in hybridization.

Breeding for anthracnose and fusaria resistance is made on special infectious backgrounds. There white lupin hybrid and mutant lines with increased resistance to these dangerous diseases.

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Nodule development, root traits and productivity of lentil genotypes

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Keywords: Lens, varieties, *Rhizobhium leguminosarum*

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Lentil is a significant pulse throughout the Mediterranean basin that lately has gain significant interest as a component of Mediterranean diet. Symbiosis between lentil plants and nitrogen-fixing soil bacteria (*Rhizobium leguminosarum*) is considered a significant factor for the development and productivity of lentils [1].

The aim of the research was to study the development of nodules in lentil, to investigate the existence of genetic variation in the number of nodules and other characteristics of the root and their correlation with productivity.

The genetic material used was three Greek varieties (Thessalia, Dimitra, Samos), four French (Rosana, Anicia, Santa, Flora) and two Swiss landraces (Kleine Schwarze, Spaths Albinise klein) which were planted in pots. No fertilization or plant protection procedures were applied during the experimentation. Five samplings of the root system were taken in the following stages: 1st, 4th, 8th composite leaf, beginning of inflorescence and full bloom. Number of nodules, length of the root system (on first three growth stages), volume and dry weight of the roots (full bloom) were measured. Seed yield, above ground biomass, nitrogen of above ground biomass and the harvest index (HI) were measured at the stage of full seed maturity. Nitrogen fixation was also calculated. The measurements of the above-mentioned characteristics in every growth stage were taken from 16-20 individual plants per variety. A technique for preparing roots for electronic scanning and measurement of root traits was optimized using the programs Matlab and Image J.

Significant variability was observed between varieties in terms of number of nodules and their growth rate. The number of nodules (full bloom stage) ranged from 136 to 281, while the varieties Anicia and Rosana ranked in the top. Greek varieties showed a rapid increase in the number of nodules, occupying the three first places up to the stage of the 8th composite leaf, without nevertheless continuing at the same pace. The average nodule growth rate between vegetative stages was 51% (1st to 4th composite leaf), 56% (4th to 8th composite leaf), 355% (8th composite leaf to emergence of inflorescence) and 30% (emergence of inflorescence to full bloom). All varieties followed the above pattern of nodule growth except for Dimitra, which showed a 361% nodule increase between 4th and 8th composite leaf stage. Significant differences were observed among varieties regarding the length of root system and its growth rate. At the 8th composite leaf stage, root length ranged from 29,3 to 245,1 cm with Dimitra variety occupying the first place. The average root growth rate between vegetative stages was 23% (1st to 4th composite leaf) and 90% (4th to 8th composite leaf). High correlation was recorded between the length of the root system and number of nodules (R=0.963*).

Varieties differed for seed yield. Samos had the highest seed yield, followed by Dimitra. A positive correlation between the number of nodules and seed yield was observed at all vegetative stages. The correlation coefficient at the full bloom stage was R=0,3.

Variety Samos exhibited the highest nitrogen fixation value, but there was no significant difference with the other varieties. A significant correlation between N derived from nitrogen fixation with the above ground N (R=0.808*) and the seed N (R=0.838*) was recorded.

In conclusion, remarkable genotypic variability was observed for the number and growth rate of nodules along with the extend and root growth rate, highlighting two main types of varieties in relation to the above characteristics. The positive correlation observed between the number of nodules and seed yield should be further studied as a potential breeding target for the modern lentil breeding programs.

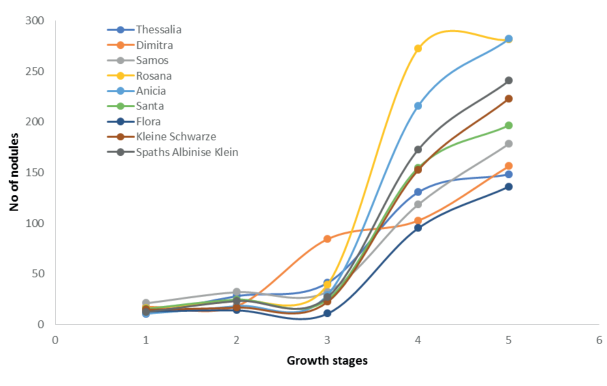

Figure 1. No of nodules/variety/growth stage

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Validation and implementation of soybean molecular markers for early maturity and growth determination genes

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Soybean (*Glycine max*) is the major grain legume crop cultivated worldwide. Soybean seed is recognized as a source of protein for animal feed and human consumption as well as a base for vegetable oil production rich in healthy beneficial mono- and poliunsaturated fats. Soybean is grown in 94 countries, however the crop areas are located predominantly in low- and mid- latitudes. Poland (49°00'N - 54°50'N) is one of the northernmost countries performing soybean breeding programs.

As soybean is a typical short day plant, genotypes transferred from lower latitudes are fairly unadapted to spring 15-hour photoperiod and their flowering time does not match the constraints of vegetation period in Poland. Thankfully, high variablity in genetic control of photoperiod response and flowering regulation pathways has been revealed in soybean, enabling selection of highly photoneutral germplasm. Numerous early maturity loci has been identified, including *E1* and *E2* [1], *E3* [2], *E4* [3], *E5* [4], *E6* [5], *E7* [6], *E8* [7], *E9* [8], *E10* [9] i *J* [10]. Molecular mechanisms were resolved for 7 loci: *E1-E4*, *E9*, *E10* and *J*. *E5* gene was revealed to be misidentified, whereas genes underlying *E6*, *E7* and *E8* loci remain unknown. To enable tracking of desired maturity alleles in untapped germplasm, numerous molecular markers were developed. These include functional mutations for *E1-E4* and *E9* loci as well as some other gene-based (*E10*) or simple sequence repeat (*E7*) polymorhisms.

The second important agronomic trait of soybean is growth habit, which is regulated by two unlinked genes, named *Dt1* and *Dt2* [11]. The most adapted to Polish conditions is semi-determinate growth type conferred by a dominant *Dt2* allele. Candidate genes for both loci were revealed and molecular markers are available.

In the present study we tested the applicability of molecular markers tagging early maturity *(E1*, *E2*, *E3*, *E4*, *E7*, *E9* and *E10*) and growth determination (*Dt1* and *Dt2*) genes for tracking domesticated alleles in Polish soybean marker-assisted selection program. Optimization of diagnostic procedures was performed for 36 markers (7 for *Dt1*, 5 for *Dt2*, *E4* and *E9*, 4 for *E2* and *E3*, 3 for *E10*, 2 for *E1*, and 1 for *E7*). 33 markers could be resolved by typical laboratory methods (PCR, restriction enzyme cleavage, agarose gel electrophoresis), whereas 3 markers required amplicon sequencing. Markers differed in detection methods: 13 markers carried insertion/ deletion (INDEL) or simple sequence repeats (SSR) and were visualized by PCR product length polymorphism; 12 markers were resolved by a cleaved amplified polymorphic sequence (CAPS) method involving restriction enzyme digestion of the PCR amplicons; 8 markers were subjected to derived-CAPS (dCAPS) procedure based on the introduction of restriction site in the polymorphic locus by a semi-complementary primer.

Optimization was effective for all markers except *Dt2* which amplified unspecific sequence. Domesticated vs wild allele identification was possible for all *Dt1*, *E1*, *E2*, *E3*, *E4*, *E9* and *E10* markers tested. Determination of *Dt2* genotype by SSR markers was impossible because every of these markers yielded at least 4 different alleles. However, some information on *Dt2* alleles could be retrieved by sequencing of the first intron of Glyma18g50910.1 gene. *E7* marker was revealed to have six alleles, however expected phenotype is known only for four of them. To summarize, from 36 markers tested, as many as 31 were positively validated. Currently, own germplasm resources as well those received from external collections (150 accessions) are being genotyped with the use of these markers.

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Exploitation of genes variability to improve biological nitrogen fixation efficiency in red clover (*Trifolium pratense* L.)

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Red clover (*Trifolium pratense L.*) belongs to important leguminous forage with high nutritive quality to feed livestock, good yield potential and with high ecological value. Cultivation of red clover is suitable on moist and acidic, low fertile soils. It is often grown in highland and foothills in a temperate climate as mono or mixed culture with grass. Red clover has relatively small genome size 418 Mbp [1] and ploidy level could be diploid ($2n = 2x = 14$) or tetraploid ($2n = 4x = 28$).

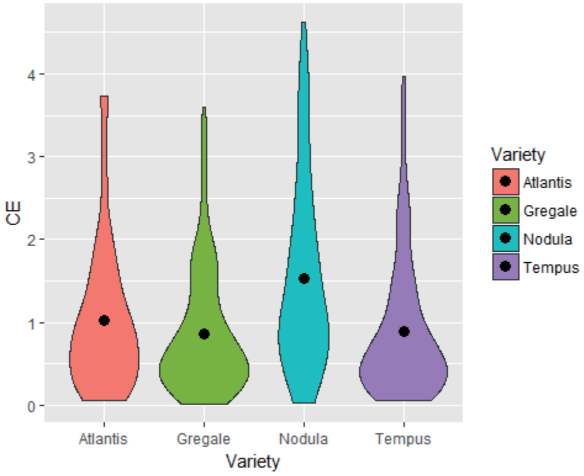

Figure 1. Inter- and Intra-variation of four tetraploid variety (Atlantis, Gregale, Nodula, Tempus) CE is concentration of ethylene from ARA experiments which correspond to BNF efficiency; black point is mean of 102 meassurments for each variety

The important ecological and economic value of red clover is also due to biological nitrogen fixation (BNF). Forage legumes are able to fix a few hundred kgs N/ha per year [2] and efficiency of BNF is influenced by the plant genotype, symbiotic bacteria, and environmental factors. The symbiotic process of BNF is driven by many plant genes in cooperation with genes of symbiotic bacteria [3]. However, plant selection for this trait may be highly effective because high heritability was demonstrated for legumes species [4]. In order BNF efficiency in red clover is influenced by many major and minor genes and red clover is self-sterile species which makes breeding difficult [5] we exploit the potential of the recent molecular technique to establish BNF efficiency selection tools.

Our goal was to analyze red clover BNF genes variability and design molecular tools for the selection of highly effective breeding material. Two acetylene reduction assay (ARA) [6] experiments were performed to assess intra- and inter-cultivar variations. Plants were cultivated in a nitrogen-free nutrient solution. In the first experiment, 648 plants of 4 diploids and 4 tetraploid variety were tested in 2017. Next year 408 plants of tetraploid variety were grown and tested in the second experiment (*Figure 1.*). Selected plants with contrast phenotype high and low BNF efficiency were selected for target sequencing of candidate genes. Two candidate genes panels of red clover homologous sequences were designed. The first panel contained 95 kbp sequences of 17 candidate BNF genes that are known from a other leguminous species (CLE12, CRE1, DMI3, DNF2, EFD, ERN, LYK3, NFP, NIN, NSP1, NSP2, PEN3, PHO2, PNO1, RDN1, SKL1, SUNN). The second panel contained 100 kbp sequences of 70 candidate BNF genes that are overexpressed in nodule according to the transcriptomic analysis of *Medicago truncatula* [7]. The sequences were captured to libraries using SeqCap (Roche) target enrichment tools. Each panel was applied to 25 highly effective BNF genotypes and 25 BNF inefficient genotypes. Target enrichment libraries were sequenced in PE mode with 150 bp reads using Nextseq500 (Illumina) sequencer. Sequence data processing and genotype variants calling were performed using bioinformatic BBMap pipeline [8].

Results reveal a couple of polymorphisms associated with higher BNF efficiency in red clover which can be genotype using high throughput microarray selection platform. Selected sequence fragments with SNP markers of thousands of plants are printed to solid state microarray surface and are hybridized with an allelic variant of the markers. Genotypic analysis of large numbers of plants particularly allows negative selection which decreases the number of BNF inefficient plants in the breeding population. This progressive system is currently implemented to the breeding process of red clover in local breeder in Czech Republic.

Acknowledgments: This work was supported by Technology Agency of the Czech Republic (grant no. TH02010351).

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Semi-determinate stem growth in soybean: agronomic and seed effects in early maturity populations

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Keywords: soybean, *Dt2* stem termination, grain yield

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Soybean shoot architecture is a key characteristic determining agronomic performance and adaptability to particular environments or production systems. Most of southern (late maturity) soybean cultivars have a determinate stem growth (*dt1/dt1*) where elongation of the main stem is stopping at the onset of flowering, and a terminal raceme of flowers/pods is developing. In contrast, most northern (early maturity) soybeans have an indeterminate stem growth (*Dt1/Dt1*) with stem elongation and flowering taking place simultaneously producing longer stems with more and longer internodes. Indeterminate stem growth combined with later maturity bears the disadvantage of lodging in many environments. Recently, the locus *Dt2* has been characterized causing a terminal flower raceme and semi-determinate stem growth in a *Dt1/Dt1* genetic background. The dominant allele for semi-determinacy (*Dt2*) has been described as a gain-of-function mutation [1], which might be of relevance to modify plant architecture particularly in early maturity soybeans.

Lines from bi-parental populations (F_3-derived lines in succeeding generations with 259 and 188 lines in two different crosses) segregating for the *Dt2* vs. *dt2* phenotype were tested in single-row plots across multiple environments in the east of Austria (years: 2014, 2015, 2016, 2018; locations: Gross Enzersdorf near Vienna, Tulln an der Donau). The central part of each single row (about 1 m^2) was harvested for yield determination. Near-infrared reflectance spectroscopy (NIRS) was used to determine seed protein, oil and sucrose content.

Semi-determinate stem growth was clearly recognizable at the flowering stage due to the formation of a terminal raceme of flowers. Subsequently, at maturity a terminal bunch of pods was visible. The difference in plant architecture between *dt2* and *Dt2* stem termination is clear in Figure 1 illustrating pod distribution along the main stem. Semi-determinate stems had a larger number of pods towards the distal end of the stem and a lower number of nodes bearing pods than indeterminate stems. Moreover, internode length was shorter and stem diameter was larger in semi-determinate stems as compared to indeterminate ones.

The effects of stem termination were also confirmed by ANOVA results of quantitative characters. Semi-determinate stem growth lines had a 10-16 cm shorter plant height, 5 days earlier maturity, lower oil and sucrose content, higher protein content and a 5-15 g lower thousand-seed weight than indeterminate lines. Grain yield differences between *Dt2* and *dt2* lines were significant in 4 out of 6 environments, and yield in *Dt2* lines was 5-7% higher than in *dt2* lines.

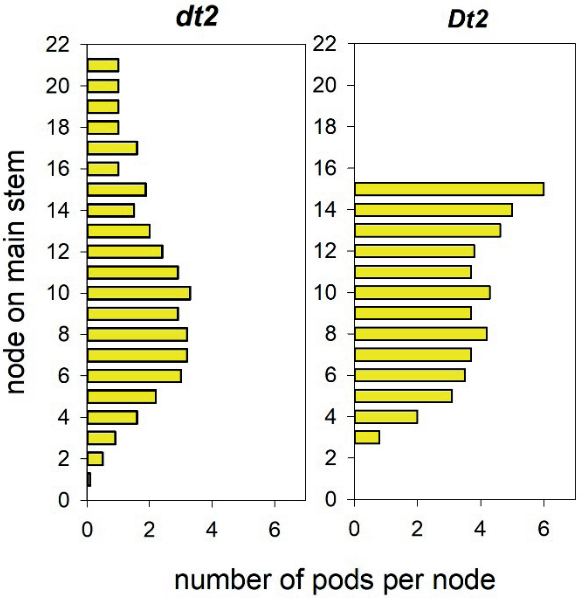

Figure 1. Distribution of pods across nodes on the main stem in indeterminate (*dt2*) *vs.* semi-determinate (*Dt2*) soybeans (averaged across n=10 plants each)

The results indicate a clear effect of variation in stem determination on agronomic and seed quality characters in early maturity backgrounds of soybean. The semi-determinate stem growth habit might be particularly interesting in soybean breeding for higher yielding environments [2].

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Nitrogen acquisition and allocation in lupin and its effects on the following cereal crop in agricultural conditions

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Keywords: *Lupinus albus*, N supply service, N_2 fixation

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White lupin (*Lupinus albus*), a grain legume crop, has multiple interesting characteristics: high grain protein content (33 to 47 % of protein) [1], low needs of fertilizers in particular nitrogen (N) and beneficial pre crop effect due to N supply service. However, in France farmers are reluctant to cultivate lupin mainly because of its low and uneven yields and the variability of its pre crop effect. Moreover there is a lack of local references obtained in agricultural conditions [2] which hinders anticipating and exploiting the services of such crops through adapted cropping system management.

The present study aims at characterising lupin performances in agricultural conditions and its effect on the following crop (wheat here).

A network of 15 fields was studied in the Pays de la Loire region, west of France for two couples of years (c1 and c2). Each field was cultivated with lupin in year n (2014-15 for c1 and 2015-16 for c2) and followed by wheat in year n+1 (2015-16 for c1 and 2016-17 for c2). Ten lupin fields (five of winter lupin and five of spring lupin) were studied during c1 and five (winter lupin) during c2. After lupin, all fields received a winter wheat crop (*Triticum aestivum*). Wheat fields were divided into fertilized and non-fertilized plots to better evaluate the N supply service provided by lupin. Fields were managed according to farmer's regular cropping practices during the whole study.

Soil inorganic N content was measured at sowing, after winter and at harvest. Yield components and N shoot (grains and straw) content were determined for both lupin and wheat at harvest. In addition, N derived from the air (%Ndfa) was estimated for lupin using the natural abundance method. Nitrogen harvest index (NHI) was computed as the ratio between N grains and N shoot.

Over the 2 cropping years, lupin grain yield showed a high variability (CV=45%) ranging from 1.3 to 4.9 t/ha with an average of 3.2 t/ha. Yield of winter lupin was higher and more stable (3.6 t/ha, CV=39%) than that of spring lupin (2.5 t/ha, CV=52%). Grain protein content was 36% on average with no difference between winter and spring lupin. Protein content has a lower variability than yield (CV=12%). Ndfa ranged between 34 and 89% with an average of 67% but was lower for spring lupin (53%). In line with previous study [3], biological nitrogen fixation (%Ndfa) of lupin was high even for high soil N content at sowing which yet ranged between 76 and 208 kgN/ha. The amount of N_2 fixed was significantly correlated to aboveground biomass (p-value=0.017*, r^2=0.39). The main factors explaining the growth variability within the network were frost and plant density for winter lupin and the date of sowing and weeds for spring lupin.

Lupin accumulated on average 267 kgN/ha of which 54 to 90% was located in grains. Thus, 10 to 46% of N contained in lupin shoot parts was restored to the field which corresponds to 85 kgN/ha on average, to which we may add N roots. Soil inorganic N at lupin harvest was high: on average 81 kgN/ha and reached 140 kg/ha at wheat sowing.

Regarding wheat performances after lupin, we observed a high variability of wheat grain yield ranging from 3.1 to 9.9 with a mean of 6.3 t/ha (CV=29%). This yield variability was even higher for non fertilized plots (CV=34%). However mean yield without N fertilization after lupin was high (5.6 t/ha) and represented 80% of the yield obtained by farmers with N fertilization (7 t/ha, CV=24%). The amount of N applied by farmers reached on average 195 kgN/ha.

With regard to the N supply provided by lupin to wheat, first results do not show any correlation between soil inorganic N content at lupin harvest and wheat grain yield or protein content. However, N shoot of wheat was significantly correlated (p-value=0.019*, r^2=0.44) to Ndfa/NHI ratio.

This study highlighted several interesting characteristics of lupin which are favourable to a significant accumulation of N in the following crop: i) high biological N fixation independent of soil N content at sowing ii) high soil inorganic N at harvest iii) high N content of residus. In that respect and in order to enable lupin crop to express its potential as a protein rich crop and as a N supplier for the following crop, efforts must be made to provide favourable conditions to lupin to fix N. A particular attention should be paid for biotic and abiotic factors that influence lupin growth and thus the amount of N from residues and the amount of N_2 fixed.

Acknowledgments: This work was carried within the project LEGITIMES (Legume Insertion in Territories to Induce Main ecosystem services) and supported by the French National Research Agency (ANR-13-AGRO-0004).

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Testing of prebreeding lines of narrow-leafed lupin (*Lupinus angustifolius* L.) for weed suppressing and intercropping strategies

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The focus of the joint research project ‚LupiBreed‘ was the genetical improvement of narrow-leafed lupin with regard to disease resistance, grain yield potential, seed composition, weed suppression and intercropping. In comparison to other crops lupins have a very low competitiveness against weeds [1]. The competitiveness can be improved by higher seed densities, lower row distances and the choice of variety [2] as well as intercropping [3]. For testing the weed tolerance and the suitability for intercropping, eight prebreeding lines of narrow-leafed lupin (*Lupinus angustifolius* L.) and two varieties [cv. Boregine (branched type) and cv. Boruta (terminated type)] as reference were examined in field trials in the years 2016 and 2017 at the Thünen-Institute of Organic Farming in northern Germany. Performance of the prebreeding lines, which were developed through artificially induced mutation at Julius Kühn Institute, was assessed against the reference varieties and between growth types of the prebreeding lines (2 terminated, 5 branched, 1 intermediate). Competitiveness against weeds was simulated at different levels of weed pressure by comparing yields under weed-free conditions, in competition with site-specific weeds and with a mixture of so called “artificial weeds“ consisting of rapeseed (*Brassica napus*), buckwheat (*Fagopyrum esculentum*) and phacelia (*Phacelia tanacetifolia*) at a lower and higher seed density (60 and 120 seeds per m²).

Suitability of narrow-leafed lupin for intercropping was tested in additive mixtures with spring wheat (*Triticum aestivum*), oat (*Avena sativa*) and false flax (*Camelina sativa*) as partners at two reduced seed densities (wheat and oat: 60 and 120, false flax: 200 and 400 seeds per m²) compared to sole cropping seed densities. Narrow-leafed lupin was sown at 100% sole cropping seed density (100 except Boruta with 130 seeds per m²).

The aim was to identify prebreeding lines with good competitiveness against weeds, which also yield highly as sole and mixed crops, and to specify a suitable mixed cropping system for narrow-leafed lupin. For that purpose, yields of above ground biomass and grain yield were surveyed separately for lupins, partners and natural weeds three times throughout the growing season (stem elongation, flowering and ripeness) and relative total grain yields in the mixtures were evaluated.

Variety-specific differences in weed tolerance were found at each sampling and were in addition dependent on weed abundance at final harvest (ripeness). However, weed suppression potential of varieties and prebreeding lines only differed at ripeness sampling and those differences were irrespective of weed abundance.

Competitiveness of prebreeding lines was not generally superior to comparative varieties in sole and mixed cropping systems. Branched and terminated prebreeding lines varied when sole cropped concerning better weed suppression of branched types, but not regarding lupin yields. In intercropping systems branched and terminated prebreeding lines did not show those differences.

There were positive effects of intercropped narrow-leafed lupins on weed suppression (Fig. 1) and total yield. A higher seed density of the partners resulted in better weed suppression (Fig. 1), but did not affect total yields. In addition, prebreeding lines with a good suitability for mixed cropping systems were identified.

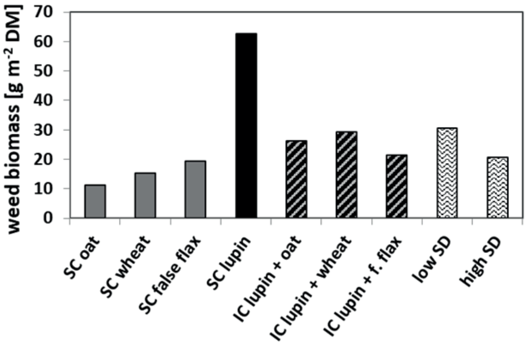

Figure 1. Site-specific weed biomass at harvest time [g m^{-2} DM] in the mean of the years 2016 and 2017 of sole cropped (SC) and intercropped (IC) lupins with low and high seed density (SD) of the partners averaged over all varieties resp. prebreeding lines

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Soybean based intercrops effects on soil mineral nitrogen pool for the following crop

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A recent report from the FAO on the State of the world's biodiversity for food and agriculture highlighted the importance of biodiversity for food production as providing more resilience and reducing need for external inputs [1]. European cropping systems tend to be simplified, for example an entire French region shifted from mixed farming systems to simple cropping systems with only few crops [2]. Diversification of cropping systems seems necessary to reduce the environmental impact of crop production but many barriers exist including lack of knowledge on the crop or fear of low competitiveness of leguminous crops against weeds [3]. To help overcome these barriers, we propose to use intercropping as a facilitator for the introduction of a new crop in cropping systems. Indeed, mixing a second crop to the crop to be introduced should improve stability of crop production and increase resilience to stresses. Also, intercropping can facilitate weed control and provide other services to the main crop and enhance global production per unit of area [4].

A common interrogation concerning the introduction of a new crop is the impact of that crop on the following one. It is recognized that leguminous crops, such as soybean, may have a positive impact on the following cereal with N pre-crop effect leading to a reduction of the N fertilizer use [5]. This effect may come from the lower C:N ratios of legume residues leading to a reduced immobilization of soil mineral N during mineralization [5].

Nonetheless, the impact of intercropping on subsequent soil mineral N pool is still poorly understood with regard to the species intercropped with the legume.

An experiment has been set up near Angers, France, to study the pre-crop effect of various soybean based intercrops (IC) on soil mineral N pool for the following wheat. Soybean was intercropped with sunflower, sorghum, lentil and buckwheat in a substitutive design (50:50), in alternate rows. The five crops were sown in sole crops (SC) as control treatments. Each treatment was replicated in three completely randomized blocks. Dry matter (DM) sampling was done at maturity to assess grain and straw yields. Four weeks later, wheat was sown on every plot. Total soil mineral nitrogen content was determined on the 0-90cm soil layer, after intercrop harvest, before wheat sowing and before and after winter.

At soybean harvest, soil mineral N in the 0-90cm layer ranged from 12.72(±5.58) kg/ha for soybean-sunflower IC, to 25.90(±5.92) kg/ha for soybean SC. Nevertheless, no significant difference was found between treatments suggesting that all crops were able to catch most of mineral N available in the soil during their growth.

At wheat sowing, lowest value of soil mineral N was for sorghum SC as pre-crop with 16.23(±3.34) kg/ha and the highest was for soybean SC with 52.44(±2.88) kg/ha. Soil mineral N after soybean-buckwheat IC and soybean-sorghum IC was significantly lower than after soybean SC.

When looking at the effect of soybean both in SC and IC on soil mineral N at wheat sowing, a linear relationship linking soybean straw DM at harvest and total mineral N in the 0-90 soil layer at wheat sowing was highlighted ($R^2=0.71$; $p<0.001$). This suggested that early mineral N availability for the next crop was directly linked to soybean's growth and DM accumulation in the field.

Results of soil mineral N at beginning and end of winter will both be available to bring more insight on the evolution of soil mineral N pool after soybeans IC.

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The effect of long-term tillage systems on yield and yield components of white lupine (*Lupinus albus* L.)

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Among Fabaceae, lupine is an important crop in Poland. It is an excellent source of protein. Seeds of lupine can be used as feed for animals or green forage, which can be also turned in to silage. The cultivation of legumes crop is recommended because of many benefits, but costs of production can above the income. The use of reduced soil tillage systems in lupine cultivation may increase profitability of production. The reduced soil tillage systems makes sense only if it leads to lower production costs without sacrificing yield.

The aim of the presented study was to evaluate effects of conventional, reduced and no-tillage systems on yield and yield components of white lupine cv. Butan.

The field experiment was conducted on white lupine cultivar Butan at the Złotniki Research Station in the Wielkopolska region (52°29’ N, 16°49’ E, Poland) in the years 2016-2018, as a one-factorial design with four replications. The factor was soil tillage system: conventional tillage, reduced tillage and no-tillage. The trials was carried out as a stationary experiment and at the same location for each year on the grey-brown podzolic soil (pH = 4.8 measured in 1 M KCL; 1.3% organic matter; 50-110 mg P·kg^{-1}, 115-195 mg K·kg^{-1}) in 4-year crop spacing in the rotation. Every year sowing dates of white lupine depended on weather conditions. The following tillage systems were applied continuously since 1997 (before 1997 conventional tillage was used). Each year before white lupine was sown, the straw of the previous crop (winter wheat) was removed from all plots. The conventional tillage consisted of tilling with a disk harrow (2.5 m wide) at a depth of 8 cm and it was done after harvest of the pre-crop. Then in August fertilization was applied. The autumn ploughing was performed to a depth of 30 cm with a 3-furrow reversible plough, in the third week of October. Each year of research in the spring, one week before sowing of white lupine, the pre-sowing tillage was performed with a field cultivator, followed by harrowing and rolling to a depth of 8 cm. The reduced tillage was based on the application of glyphosate herbicide (3.0 l·ha^{-1}) to control perennial weeds and volunteers plant. In reduced tillage, herbicide application was made in August. Before winter, in the third week of October, tillage operation was performed with only a stubble cultivator (2.5 m wide). In the reduced tillage, one week before sowing (in the spring) the pre-sowing tillage cultivation was performed with a field cultivator, followed by harrowing and rolling to a depth of 8 cm. The no-tillage consisted in the application of glyphosate herbicide (3.0 l·ha^{-1}) and fertilization. It was done after the harvest of winter wheat in August. In the spring, the no-tillage involved also the application of glyphosate herbicide (3.0 l·ha^{-1}) and sowing directly into the stubble of the pre-crop. Each experimental year the fertilization was uniform for all the tillage systems and amounted to 80 kg P·ha^{-1} and 100 kg K·ha^{-1}. The seeds before sowing, were dressed with fungicide containing tiuram and carboxin in dose 350 ml per 100 kg seeds and 700 ml water. During the vegetation period the weeds were controlled with herbicide at the rate of 1.5 l·ha^{-1} (linuron). The white lupine was desiccated, ten days before harvest by diquat (1.5 l·ha^{-1})**.** Annually in August, from the plot of 11.49 m^2 (7.6 × 1.5) lupine was harvested, using a 1.5 m wide Wintersteiger classic plot combine. The seed yield per 1 ha, was recalculated allowing for a standardized moisture 15%. The following traits of white lupine were assessed: plant density per square meter before harvest (4 frames with dimensions of 0.25 m^2), stem height in cm (measured on 15 randomly collected whole lupine plants before harvest), mass of plants in grams (the same 15 plant samples), the number of pods per plant (the same 15 plants), the number of seeds per plant (the same 15 plants), the number of seeds per pod (the same 15 plants) and 1000-seeds weight.

The yielding of white lupine depended on the weather conditions during vegetation period the most. The highest yield was observed in 2017 (3.87 t·ha^{-1}) and the lowest in 2018 (1.26 t·ha^{-1}). The highest yield was found in conventional tillage (2.82 t·ha^{-1}) and the lowest in no-tillage (2.38 t·ha^{-1}). There was not significant difference between conventional and reduced soil tillage system.

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The more research effort, the higher rate of gain for yield, a trend shown by the world pulses production data in the past four decades

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This report presents a trend implying that more research effort could lead to a higher rate of gain for yield among 11 pulse crop groups during the past four decades. The pulse production data are from one of the United Nation's databases, FAOSTAT, and the research effort is represented by the number of articles retrieved in Google Scholar searches using the scientific names of the pulse crops. The total world production of 11 pulse crops almost doubled (increased by 92.6%) in the past 40 years, from 42.5 million tonnes (MT) in 1977 to 81.8 MT in 2016. Since the harvested acreage only increased by 32.4%, the increased productivity is mainly due to an increased yield of the crops. In the same period the grand average yield of all pulses increased by 55% with great differences among the crops, from 9.5% for Bambara bean to 88.5% for lentil. From the nearly two million articles retrieved by Google Scholar searches using the scientific names of pulse crops, it was found that the rate of gain for yield is positively correlated to research effort with correlation coefficients around 0.41 for three different search options. These results indicate that research effort is positively enhancing pulse crop productivity. Today, agriculture is facing a multifaceted challenge of growing world population, shrinking arable land area, unpredictable crop productivity-suppressing weather patterns, and diminishing water and other natural resources. As increasing crop yield remains the best option, more research investment is needed to achieve the goal of producing sufficient crop to feed the world's growing population.

Critical success factors for the establishment of value chains for field beans and field peas in Germany

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Keywords: *Pisum sativum*, *Vicia faba*, value chains

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The knowledge transfer network for cultivation and utilisation of field peas and field beans in Germany (DemoNetErBo) aims at expanding the acreage of these crops sustainably. It consists of 75 conventional as well as organic farms growing field beans or field peas and demonstrating best practice examples for their usage, mainly as feed, but also as food. The network is focusing on the demonstration, development and optimization of value chains with grain legumes, with the overall aim to meet the growing need for regionally produced non-GMO protein crops.

To support and optimise the usage of field beans and field peas for animal feed and human food, critical success factors influencing the establishment of value chains were identified from cultivation through processing to marketing. Knowing these factors is vital for all actors being involved in these value chains to overcome obstacles and to transfer promoting factors and solutions into their own value chain.

For this purpose, guided interviews with experts at different levels in diverse value chains were conducted. Data and parameters were collected concerning factors being currently hampering or promoting the development and establishment of legume value chains. Those critical success factors were grouped and ranked according to the frequency of their denomination.

These factors are in part influencing each other, and are differing in conventional and organic systems, respectively.

One main argument for farmers to grow field peas or field beans, but also for feed mills to use these crops, is the price. While in organic farming producer prices are on a high and constant level, in conventional farming prices are rather low and not satisfactory for the farmers, since the prices for regionally produced grain legumes are higher than for imported soy extraction meal from overseas. Therefore, feed mills are using field beans and field peas rarely for their feed mixtures. An adequate payment should be provided for regionally produced, GMO-free feed.

In organic farming, regionally produced feed is rare, and the demand cannot be satisfied. There is much more feed mill capacity to process additional regional grown grain legumes. Plants for processing legume-cereal mixtures could improve the availability of grain legumes, as cropping in mixtures with cereals is popular in organic farming, but not all feed mills are accepting mixtures until now.

For the usage as human food, grain legumes are currently occupying just a small niche in Germany. Plants for processing grains to hulled seeds, flakes or flour are missing, which is in turn hampering the development of new products and their availability for the consumers. But for the emergence of new processing plants, companies want to be sure about the full utilization of the machines, depending on a steady supply with constant quality. On the other hand, a constant demand, and thereby, reliability for planning for the farmers is vital for expanding the acreage for field peas and field beans.

Another significant aspect, which is hampering the cultivation of field peas and beans in organic as well as conventional farming, is the variability in yield. Farmers feel that the yield variability is higher compared to other crops. Current studies have shown, though, that the risk of cultivation is not higher for grain legumes than it is for other spring grown crops e.g. spring cereals or winter rape [1]. Here, an improved consultation should transfer those facts to the farmers, and further on, develop and optimize cultivation systems along with the farmers to ensure grain yield. Progress in plant breeding is another way to improve yield stability.

By knowing the critical success factors, actors along the value chains can identify challenges and opportunities at each level of the value chain and develop individual optimization and solution strategies. Common approaches are aspired for storage, logistics, and bundling quantities as well as for purchase conditions.

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Sustainable biogas production using biomass from mixed culture of corn and legume

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The aim of the present work is to assess the influence of the number of individuals in mixed crops on the production of biomass, and to determine the optimal number of individuals per hectare.

Modern and competitive agriculture places emphasis on the economic appreciation of commodities grown. Nevertheless, technology and agro-technical processes meeting the requirements for sustainable agriculture, nature-friendly agriculture, or climate- and environment-friendly practices are starting to be implemented in agricultural systems.

For instance, corn, that is grown today in more countries than any other crop, has a wide range of uses (food, feed and industry). The disadvantage is that its cultivation is associated with soil degradation. Attempts to improve agrotechnical practices in maize cultivation are very intense now, especially with regard to soil protection. One direction for a possible change is ongoing research on the possibility of growing corn in a mixed culture system. At present, mixed culture (MC) is understood as a system of growing two or more crops simultaneously on one plot. Most commonly, the mixed culture is composed of plants of the family Fabaceae and Poaceae [1].

The pre-selected crops (corn - *Zea mays*, FAO 270 - white lupine - *Lupinus albus*, Zulika variety and White sweet clover- Melilotus albus, Meba variety) were planted in 2018 in order to obtain material for the production of silage utilizable in the laboratory biogas station. The combination of corn and legumes plants was sown in the same term. Mixed culture sowing was carried out by a thin-row system with 37.5 cm of inter-row spacing, alternating between two rows of corn and two rows of leguminous plants (Figure 1).

Figure 1 Schematic illustration of the organization of the maize and lupine stand in the alternation of two and two rows (Jandová, 2017)

There were prepared five variants of the experiment with different plant density. The sowing rate of each crop in MC was V1-75,000; V2-80,000; V3-85,000; V4-90,000; V5-95,000 individuals/ha, the total number of individuals (corn + white lupine, corn + white sweet clover) MC was 150,000 (V1); 160,000 (V2); 170,000 (V3); 180,000 (V4); 190,000 (V5) per ha. The determination of biomass yield of individual crops from each variant in the mixed-cropping system was assessed in accordance with the method by Loučka et al. [2]. Therefore, it is necessary to present the data thoroughly and in broader context. The results of the production of dry (100% dry matter) above-ground biomass (kg/ha): a) from individual variants of MC of corn and white lupine: V3> V2> V1> V4> V5 b) MC of corn and white sweet clover V3> V2> V1> V4> V5. In both combinations of mixed culture, optimal number of individuals with the respect to biomas production was 1700,00 per ha in 2018. Higer counts reduced biomass production per ha probably due to exceeding threshold when synergistic relationship changes to competition. It resulted in biomass production decrease. Contrarily, a lower sowing rate has caused the MC was less likely to use the available nutrients in soil. The presented differences can play a decisive role in choosing the number of individuals of mixed culture per hectare. Mixed culture has the potential to use natural resources such as soil, water, sun and nutrients more efficiently than crops grown individually. An indisputable advantage in the cultivation of mixed crops is the re-inclusion of plants of the family *Fabaceae*, which were the cornerstone of the sowing processes of the 18th century from the historical point of view. The significance of leguminous crops in today's crop rotation can not be neglected, and their cultivation in a mixed crop system is a tool for their use in agro-technical practices of sustainable agriculture.

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The use of natural polymers in soybean seed coating

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Natural polymers (biopolymers) constitute the largest and renewable source of raw materials available to human. They are biodegradable and the products of their degradation are not toxic. Therefore, products based on these polymers are ecologically friendly at all stages of manufacturing and use. Using such products in agriculture does not raise any concerns about residues or degradation products in the crop plants or the soil. Moreover, earlier studies have shown that natural polymers show specific bioactivity against a wide range of plant pathogens and stimulate growth of plants. They also show both antimicrobial and antiviral efficacy.

The ongoing BIOSOYCOAT project is aimed at development of a coating for legume seeds protecting against adverse weather conditions at the time of sowing (such as high humidity and low temperature) and against diseases caused by soil-borne fungal pathogens. The basis for the two-layer coatings are biopolymers.

The study was conducted on soybean cultivars provided by Scientific Research Center of Soya Development "AgeSoya". The selected cultivars belong to maturity groups ranging from 00 to 0000 and they are suitable for cultivation in our country. Seven cultivars were subjected to evaluation of resistance carried out under controlled conditions using pure cultures of fungi. Then, three of them, differing with the level of resistance, were selected for further reseach including testing effects of newly developed seed coatings.

However, first, the properties of the new seed coating formulations were examined in the laboratories of the Institute of Biopolymers and Chemical Fibres. The biodegradation tests which were carried out in accordance with a research procedure based on the Polish ISO standard (PN ISO 11266:1997). Within this task, the dynamics of biological deterioration of membranes under the influence of microorganisms naturally occurring in the soil was investigated. The degree of biodegradation of test samples was assessed by weight loss of the coating under controlled temperature and humidity conditions. Ecotoxicity tests were carried out according to our own test procedure, based on the PN-EN ISO standard (PN EN ISO 4833-1:2013). Then, the strength parameters for selected polymer membranes constituting the coating were assessed according to PN-ISO standards (PN EN ISO 4593:1999; PN EN ISO 527-3 1998). The rate of water vapor permeation through the membranes and contact angle by the sessile drop method were examined along with dynamics of the processes. The contact angle has been determined in accordance with the European Pharmacopoeia 8.0 p.2.9.45.

The results of ecotoxicity tests have shown that membranes prepared with encapsulated have no detrimental effect on soil microflora and organic compounds released from the coatings into the soil environment in the process of biological degradation; they can be an additional source of minerals required for nutrition of microorganisms.

The measurements of strength parameters have shown that two-layer coatings are mechanicaly resistant hence they may protect the seeds from mechanical damage during transport and storage. Other measurments have shown that after a certain time bilayer membranes absorb water, which is closed in their structure while the film has the ability to transmit water vapor. These properties of the membranes are important for maintaining viability of seeds in storage and ensuring high germination rate.

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Fodder and symbiotic potentialities of the legume *Sulla carnosa* in its natural biotope (Sebkha ElKelbia)

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Salinity leads to several physiological stresses in plants and consequently few plants can tolerate significant salinity levels in their root medium for any length of time. Of all the worlds' species, about 1% are considered halophytes [1], which are defined by Flowers and Colmer [2] as able to complete their lifecycles under saline conditions corresponding to at least 200 mM of NaCl in the root medium.

Wild legumes (herbs, shrubs or trees) play a critical role in natural ecosystems, agriculture, and agroforestry, where their ability to fix nitrogen makes them excellent colonizers of low-N environments, and hence an economic and environmentally friendly species. The field natural nodulation of the Tunisian *Sulla carnosa*, its symbiotic-efficiency and fodder production potentiality in its saline biotope (sebkha d'El kelbia, fig 1) were investigated in this study. A greenhouse experiment was conducted on plants transferred from sebkha with their soil in pots to explore the maximum potentialities of biomass production and nitrogen fixation of this legume when water is not a limiting factor (natural soil salinity was maintained in greenhouse). Obtained results from field and greenhouse studies demonstrated that Sulla carnosa can be a good candidate for saline agriculture regarding its important ability to grow, produce biomass and fix nitrogen under high level of salinity (about 150 mM NaCl). This legume protects its photosynthetic and symbiotic organs against their overload with sodium by an important uptake of potassium and accumulation of Na in the roots. Sulla carnosa can play a goal role in the sustainable development in a region traditionally considered marginal.

Further studies are in progress aiming to explore the genotypic variability of *Sulla carnosa* response to salinity using other provenance (5 regions of the arid and semi-arid area of Tunisia). A special interest is granted to isolation and purification of an efficient strain of *Rhizobia* for hydroponic and Lab experiments.

Figure 1. Sulla carnosa in association with halophytes and glycophytes in the Sebkha d'El Kelbia

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Site suitability analysis for soybean and lupin cultivation in Sweden

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Sweden is highly depending on imported plant-based proteins, as only 2% of the total arable land is cultivated with such crops. In other hand, the society's demand for domestic produced proteins is increasing, especially regarding organically produced protein crops. Lupin and soybean could be innovative solutions to reduce the Swedish produced protein gap, while offering a sustainable agronomic and economic alternative to organic farmers.

In recent years, attempts have been made to study the feasibility of soybean (*Glycine max*) cultivation in Sweden, showing that early maturing soybean cultivars could cope with the growing conditions even up to 59°N with up to 2.4 t ha grain yield and a typical protein content of 39-41% [1]. Further, it has been demonstrated that narrow-leafed lupin (*Lupinus angustifolius*) can grow at latitudes of 60° N [2]. Despite these advancements, there is a lack of scientific evidence for cultivar selection, crop management and productivity to support the development and realization of commercial soybean and lupin production in Sweden. Moreover, the cultivation of these crops was never done in contrasting Swedish pedoclimatic zones, in which differences in growing seasons and day length conditions constrain the choice of crops. Considering these knowledge gaps, there is a need to identify areas where both crops – and cultivars – could be produced. The suitability mapping with respect to temperature and soil requirements, calibration of crop growth models to Swedish conditions based on field experiments and productivity mapping will advance the knowledge on growth, N input and production potential of soybean and lupin across Sweden.

The objective of the site-suitability analysis is to indicate the regional pedoclimatic suitability for lupin and soybean in Sweden.

Indicators such as photoperiod, thermal sum (degrees day accumulation) during cropping season, soil aptitude and occurrence of lethal temperatures during the cropping season will be included. This suitability analysis will be plotted on high-resolution maps derived from CORINE Land Cover inventory for the whole Sweden considering arable areas only. Weather data – daily observations – will be gathered from Lantmet weather stations and the Swedish Meteorological and Hydrological Institute (SMHI). Soil maps will be obtained from the Geological Survey of Sweden (SGU). The crop-specific parameters such as minimum temperature for seed germination and phenophases (thermal time between different phenological stages) were obtained from scientific literature and experiments previously conducted in Sweden. For germination, we assumed a threshold of 10°C soil temperature, a basal temperature of 7°C for vegetative development and optimal development temperature between 28 and 35°C.

Preliminary assessments indicate a latitudinal gradient of cultivation suitability for both crops, in which the southern regions are more adequate for lupin and soybean cultivation than the central and northern regions. This is evidenced by the Fig. 1, showing the distribution of different annual temperature sum (in degrees day) regions in Sweden [3]. Only regions with more than 1100 degrees day would offer minimal conditions for soybean and lupin cultivation.

The suitability analysis was divided in two main components, namely germination and length of the cropping cycle.

For the first component, the determining factor is soil temperature, which is determined by solar radiation, air temperature and soil texture. A later germination caused by low soil temperatures in spring, implies delay in the emergence and a shorter growing season.

In the other hand, as a second component, a lower air temperature during the cropping season reduces the accumulation of degrees-day, extending the cropping cycle. In the northern regions, both components cause a delay in the establishment and development of the crops, resulting in the crop entering fall without reaching the physiological maturity.

Further effects such as photoperiod sensitivity need to be tested at species and cultivar level, so as the degrees day requirements for specific phenophases. Especially for soybean, maturity groups (MG) 000 and 0000 (also called high-latitude cold regions-HCR) need to be targeted for testing. Further tasks also involve the parameterization, calibration and evaluation of process-based models for a more detailed site-suitability analysis.

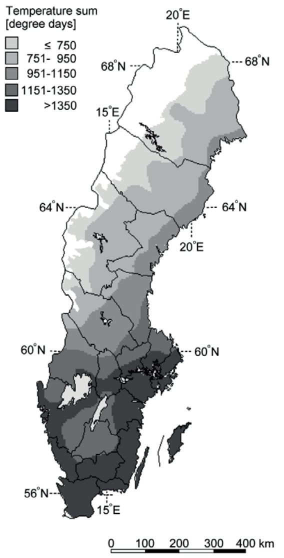

Figure 1. Map of Sweden showing the distribution of different temperature sum regions (according Stendahl et al, 2010). Site suitability for soybean and lupin cultivation in Sweden is primarily associated to higher temperature sums

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Biorefining legumes: a life cycle assessment of peas (*Pisum sativum* L.) distilled for neutral spirit and high-protein co-products

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Keywords: *Pisum sativum* L., pea, distilling, neutral spirit, life cycle analysis

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Neutral spirit (alcohol) production using peas (*Pisum sativum* L., a pulse crop) provides a novel pilot study for the up-scaling of pulses in agri-food- and feed-systems. This is due to the large-scale production and potential profitability of the main product and high protein co-product called 'pot-ale', which is a liquid 'waste' containing carbohydrate, spent-yeast, protein and minerals. We demonstrate that the alcohol production from pulse starch has considerable global mitigation potential in terms of climate-change offset and nutrient-loss reduction.

We undertook attributional and expanded boundary life cycle assessment (LCA) of gin production from wheat and peas. Allocation of system burdens between gin and animal feed co-products indicated that gin produced from peas had a smaller environmental footprint than gin produced from wheat across 12 of 14 environmental impact categories. Global warming, acidification and terrestrial eutrophication burdens of pea were all less than that of wheat gin. However, land occupation was approximately two times greater for pea gin compared wheat gin, due mainly to the comparatively low pea (starch) yields.

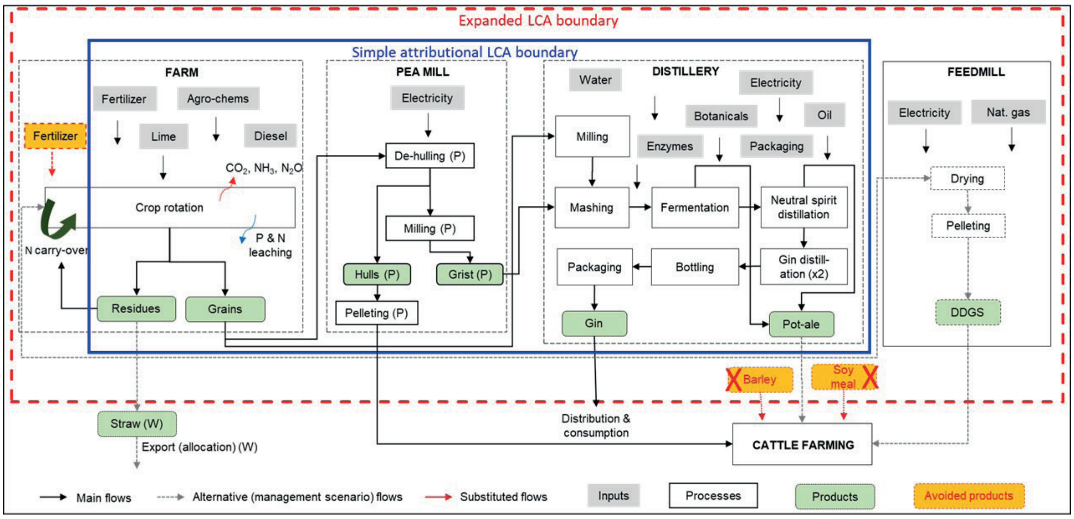

Figure 1. Main processes and inputs accounted for the within simple attributional and expanded LCA boundaries. Flows show processes for wheat-(W) or pea-(P) gin, including substitution of soybean meal and barley for cattle-feed with pea hulls and dried distillers' grains with solubles (DDGS) isolated from pot-ale. Pot-ale may alternatively be treated as a "waste" in simple attributional LCA or considered to replace fertilisers following land spreading within expanded boundary LCA

Animal feed substitution using co-products increased the calculated environmental advantage of pea gin overall, owing to larger amounts of protein contained in co-products. Enhanced soybean meal substitution from use of peas in alcohol production could reduce Europe's protein deficit whilst potentially avoiding deforestation in Latin America, leading to net avoidance of CO_2 eq. for every L gin (or neutral spirit) produced.

Land areas potentially spared from soybean meal production partially offset the greater direct land requirement for pea gin. However, full consequential LCA should be applied to account for the detailed farm and landscape changes (*e.g.* cropping sequence, potential cultivation in Ecological Focus Areas) associated with increased cultivation of legumes within conventional (cereal dominated) rotations.

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Complementation and facilitation for N-fixation and N-yield in faba bean-wheat mixtures

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Keywords: winter faba bean winter wheat mixture, facilitation, symbiosis

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Growing faba bean and a cereal as mixture without N fertilization should lead to positive mixing effects. We asked whether our novel winter faba beans may display genetic differences for N-related complementation and facilitation when mixing them with winter wheat [1; 2; 3; 4]. N=8 winter faba bean lines were assessed in pure stand and in substitutive mixtures with the winter wheat cv. Genius in 2Y and 2L (r=4) near Göttingen. Based on δ15N, symbiotic nitrogen fixation was determined and percent nitrogen derived from air (%Ndfa) and N yield per ha was deduced. The eight faba beans significantly differed for %NdfA (averages across pure stand and mixtures), and in mixture the beans increased their %Ndfa significantly over pure stand (86.7% > 77.9%). As well, the beans differed for their total N yield (grain + shoot) at maturity (averages across pure stand and mixtures; P=10%). N-yield of the mixtures was significantly lower than N-yield of faba bean pure stand (179 < 217 kg N per ha), yet, it was markedly higher than the average of bean sole crop and wheat sole crop (179 >> 133 kg per ha). In monoculture, beans differed significantly for total N yield, but not so for N yield of their mixtures. We thus could show breeding options for pure stand and for mixtures, and convincingly demonstrate complementation and facilitation of %Ndfa and of N yield of winter faba beans in mixtures with winter wheat.

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Rhizobium genotypes associated with high levels of biological nitrogen fixation by grain legumes in the British Isles with particular emphasis on a long-term field trial in the east of Scotland

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Commercially grown grain legumes in the British Isles, peas (*Pisum sativum* L.) and faba beans (*Vicia faba* L.), do not require any nitrogen (N) fertiliser (current UK Government and UK pulse growers – PGRO recommendations), as they can provide their own N needs *via* biological N fixation (BNF). They are nodulated spontaneously in UK soils by native *Rhizobium leguminosarum* bv. *viciae* (*Rlv*), therefore, the need for rhizobial inoculants is generally ignored. However, these crops are characterised by yield instability, and one reason for this may be that not all rhizobial strains are equal *i.e.* there is a potential to apply high-performing rhizobial strains as inoculants to boost BNF and grain yield. Thus, we examined the capacity of peas and faba beans for BNF under Maritime-Atlantic climates and characterised their symbiotic rhizobia genotypes.

Using the ^{15}N natural abundance technique we found that faba bean BNF ranged from 100 to 350 kg ha^{-1} y^{-1} over the 4 y field-scale experimental rotation in East Scotland. The residual N left after grain harvest ranged from 30–90 kg ha^{-1} y^{-1}. Also, more than 80% of plant-N was derived from BNF (*i.e.* "%Ndfa") for pea and faba bean crops locationed across the British Isles.

A wide diversity of *Rhizobium leguminosarum* bv. *viciae* genotypes were isolated, and 145 representative strains were screened for their ability to promote growth of pea. Sequencing of "core-" (*rrs, recA* and *atpD*), nodulating- (*nodAD*) and nitrogen fixation- (*nifDH*) genes showed that *nif* genes appear as a good predictor of BNF capacity. However, these are not the only determinants, as some poor-performing strains on peas had similar *nifDH* genotypes to high-performing ones. The genomes of the potentially elite strains are currently being sequenced, as it is possible that deeper analysis of their whole genome might reveal additional and more reliable genetic markers for symbiotic performance [1, 2].

This study has provided strong evidence naturally occurring soil rhizobia are able to provide peas and faba (through BNF) with sufficient N to support high grain yields in some locations throughout the British Isles. Some isolates could be developed into commercial inoculants to help maintain and stabilise pea and faba bean yields.

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Fostering organic cultivation of grain legumes; a multi-scale feasibility study for soybean and lupin production in Sweden

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The project presented in the following has started in 2019 for the duration of four years, addressing four specific objectives:

I. To identify the most suitable areas for the production of lupin and soybean by accounting for the current biophysical and socioeconomic context.

II. To test economically and environmentally sustainable cropping systems focusing on crop yield and quality as well as on N balance, weed, disease and pest management.

III. To determine the impact at farm level of the adoption of the new lupin and soybean managements.

IV. To estimate consumer willingness-to-pay for organically produced lupin and soybean-based products.

The project is a broad interdisciplinary system research approach involving agronomists, biologists, weed scientists, entomologists, microbiologists and economists.

Extending and growing a more diverse set of grain legumes is an opportunity to reduce the dependence on imported proteins as well as for diversifying agro-ecosystems. Lupine and soybean could be innovative solutions while offering a sustainable agronomic and economic alternative to organic farmers. Both species feature interesting characteristics for fodder production and for human consumption. In recent years, attempts have been made to study the feasibility of soybean cultivation in Sweden, showing that early maturing soybean cultivars could cope with the growing conditions even up to 59° N with up to 2.4 t ha^{-1} grain yield and a typical protein content of 39-41%. Further, it has been demonstrated that narrow-leafed lupine can grow at latitudes of 60° N. Despite these advancements, there is a lack of scientific evidence for cultivar selection, crop management and productivity to support the development and realization of commercial soybean and lupine production in Sweden. Moreover, the cultivation of these crops was never done in contrasting Scandinavian pedoclimatic zones, in which differences in growing season- and day length are major constraints.

The project aims to assess the biophysical and socioeconomic production potential for soybean and lupines in Sweden, as well as the development and testing of economically and environmentally sustainable crop management strategies for these crops. The pedoclimatic suitability will be assessed in a spatial modelling approach. Here, we combine soil and climate data for comparison with the pedoclimatic needs of lupine and soybean cultivars and the production of site suitability maps. Based on these site suitability maps, we will design and test crop management strategies for the most promising cultivation regions in Sweden. The design and analysis of the crop management strategies will pay special attention on monitoring, prevention and control of weeds, insect pests and fungal diseases as well as on nitrogen (N) balance and carry over effects on subsequent crops. Furthermore, this study will assess consumer willingness-to-pay for fresh milk – a product which heavily relies on protein fodder as an input and thus a measure of a potential price for organic and regionally produced products based on these legumes – by means of a stated preference study (discrete choice experiment). The impacts of various soybean and lupine management and price scenarios will be assessed at farm level in terms of impact on farmer's revenues.

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4.15

The yielding of soybean (*Glycine max* (L.) Merr.) cultivated in condition of long-term reduced soil tillage systems

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During the last years in Poland the interest of soybean cultivation is developing. According to PIORIN data in 2010 there was only 48 ha of certificated soybean seeds production but in 2018 already 6 109 ha. Reduce the cost of cultivation involves searching of possibility to improve reduced tillage systems. In Poland conventional tillage systems is common the most, but the different variants of reduced soil tillage systems are often used in the largest farms.

The aim of the presented study was to evaluate effects of conventional, reduced and no-tillage systems on yield and yield components of soybean cv. Merlin.

The field experiment with soybean cultivar Merlin was conducted at the Złotniki Research Station in the Wielkopolska region (52°29’ N, 16°49’ E, Poland). The study was carried out in the years 2016-2018, as a one-factorial design with four replications. The factor consisted of soil tillage system: conventional tillage, reduced tillage and no-tillage. The study was conducted as a stationary experiment and at the same location for each year on the grey-brown podzolic soil (pH = 4.8 measured in 1 M KCL; 1.3% organic matter; 50-110 mg· P kg^{-1}, 115-195 mg· K kg^{-1}), in 4-crop rotation. Sowing dates depended on weather conditions. The following tillage systems were applied continuously since 1997 (before 1997 conventional tillage was used). After harvest of the previous crop, the conventional tillage consisted of tilling with a disk harrow (2.5 m wide) to a depth of 8 cm and fertilization (in August). In the third week of October the autumn ploughing was performed to a depth of 30 cm with a 3-furrow reversible plough. In the spring, one week before sowing the pre-sowing tillage was performed with a field cultivator, followed by harrowing and rolling to a depth of 8 cm. The reduced tillage in August involved the application of glyphosate herbicide (3.0 l·ha^{-1}) to control perennial weeds and volunteers plants. In the third week of October only a stubble cultivator (2.5 m wide) was used. In the spring, one week before sowing the pre-sowing tillage operation was performed with a field cultivator, followed by harrowing and rolling to a depth of 8 cm. The no-tillage systems (after the forecrop harvest) consisted in the application of glyphosate herbicide (3.0 l·ha^{-1}) and fertilization. In the spring, the no-tillage system involved also the application of glyphosate herbicide (3.0 l·ha^{-1}) and sowing directly into the stubble of the previous crop. Fertilization was uniform for all the tillage systems and each experimental year (80 kg P·ha^{-1}, 100 kg K·ha^{-1}). During the growing season the weeds were controlled with herbicide (linuron) at the rate of 1.5 l·ha^{-1}. Soybean was harvested from the plot of 11.49 m^2 (7.6 × 1.5) using a 1.5 m wide Wintersteiger classic plot combine. Seed yield was recalculated on standardized 15% seed moisture. The following soybean parameters were assessed: plant density per square meter before harvest (4 frames with dimensions of 0.25 m^2), stem height in cm (measured on 15 randomly collected whole plants several days before harvest), mass of plants in grams (the same 15 plant samples), the number of pods per plant (the same 15 plants), the number of seeds per plant (the same 15 plants), the number of seeds per pod (the same 15 plants) and 1000-seeds weight.

There were considerable differences in the conditions of soybean growth and development in individual years of the research. The weather conditions during vegetation period, had the biggest influence on soybean yielding. The highest yield was observed in 2017 (3.41 t·ha^{-1}) and the lowest in 2018 (0.84 t·ha^{-1}). It turn out that the highest yield was recorded in conventional tillage (2.37 t·ha^{-1}) and the lowest in no-tillage (2.16 t·ha^{-1}). There was not significant difference between soil tillage systems.

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Effects of inoculation with PGPB and/or AMF as biofertilizers in cowpea (*Vigna unguiculata* (L.) Walp) yield and protein content under two watering regimes

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The production of food to sustain a growing human population allied to the global water scarcity, is enforcing the importance of sustainable agriculture and plant production. Drought-tolerant crops, such as cowpea, are of great interest for sustainable agriculture, due its tolerance to low water availability, high temperatures and soils with low fertility. These traits make this species widely cultivated in the semi-arid tropical regions, providing a cheap source of rich vegetable protein for human consumption [1].

A common approach to improve legume productivity in water stress conditions has been the reliance in effective plant growth promoting bacteria (PGPB), including rhizobia, and Arbuscular Mycorrhizal Fungi (AMF) as biofertilizers through inoculation. The tripartite symbiosis of legume–rhizobia–AMF can improve nodulation and biological N_2 fixation. These microorganisms when applied to the seeds increases nutrient cycling and suppress pathogens, among other beneficial processes [2].

After previous studies in greenhouse conditions, strains of PGPB were selected from different Portuguese soils (Pereira *et al.* unpublished data). The aim of the study was to evaluate the effects of these PGPB and AMF in cowpea yield and protein content under two watering regimes.

A field trial was carried out with the cowpea genotype CP553, performing the following treatments: T1= PGPB mix (*Pseudomonas* sp., *Bradyrhizobium elkanii* and *Burkholderia xenovorans*) inoculated through seed coating, T2= PGBB mix + AMF inoculated through seed coating and the commercial AMF conventionally inoculated in the soil, and T3= control, without inoculation of microorganisms. All treatments were submitted to two watering regimes: i) deficit irrigation (DI) - plants were irrigated only during the phenological stage of flowering, with a volume of water applied to satisfy crop water needs and ii) rainfed (R). Each plot had $4.8m^2$ and three replicates, with 18 plots in total, and a seed rate of $6.25m^2$.

After seed ripeness, fifteen plants from each treatment were characterized by: Pod and Grain Weight per plant (PGW), Grain Weight per plant (GW), Number of Pods per plant (NP), Number of Grains per plant (NG), Grain Yield (GY), and crude protein content [PB (%)].

The results showed an increase in the yield variables studied for the plants inoculated with PGPB and PGPB + AMF, being the differences statistically significant ($p<0.005$) when compared to the non-inoculated plants (regardless of watering regime). PGPB+AMF under DI treatment presented the highest values for all of the yield variables studied. It was similarly noticeable that plants inoculated with PGPB or PGPB + AMF under R presented higher values of production than the control plants under DI. Increased yield values between plants under DI and plants under R conditions, regardless of the inoculation treatment, and statistical significant difference ($p<0.005$) were observed.

Relatively to cowpea protein content, the results showed that inoculation treatments have influence on the PB (%), once statistically significant differences ($p<0.05$) we found between the control and all the inoculation treatments both under R and DI.

The results obtained with this work suggest that the use of microorganisms as biofertilizers is a sustainable organic alternative to chemical fertilizers once they are easy to use, can increase crop yield and protein content, even in non-irrigation conditions, and are environment friendly. When allied to watering at the flowering phenological stage, the yield boost is even higher.

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4.17

Impact of the preceding cultivation during the winter or no cultivation on fresh pod yield and soil-N availability in an organic bean crop grown in open field

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Keywords: crop rotation, organic farming, conventional farming, common bean

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In a study conducted at the Agricultural University of Athens, common bean (*Phaseolus vulgaris* L.) was cultivated during spring-summer 2018 in plots treated differently during the preceding winter. In particular, during autumn-winter 2017-2018, the experimental plots were: a) not cultivated (fallow), b) cultivated with organic faba bean that was incorporated to the soil as green manure, c) cultivated with organic broccoli, or d) cultivated with conventional broccoli. In variations (a), (b) and (c), organic farming practices were applied in the common bean crop, while in variation (d) the common bean crop was treated according to conventional farming practices. In each of the four variations applied in autumn-winter 2018-2019, two different variations of common bean were applied, particularly inoculation of the seed with *Rhizobum tropici sp*. before sowing, or no inoculation. The seeds of faba bean were inoculated with *Rhizobium leguminosarum bv. viciae* before sowing in the plots destined to accommodate common bean inoculated with *R. tropici* as subsequent crop, while they were not inoculated, in the plots destined to accommodate non-inoculated common bean. The aim of this work was to contribute to establishment of sustainable crop rotation schemes for organic vegetable production under mild-winter climatic conditions that optimize N availability to crops and maximize yield. The organically treated plots were fertilized using ship manure and patent kali, while the conventional plots were fertilized with inorganic fertilizers. Faba bean at the 50% flowering stage, plant residues of broccoli, and the wild flora in the fallow plots were incorporated into the soil through rotary tillage. The %Ndfa in the plant tissues of legumes was determined by assessing the differences in the natural abundance of ^{15}N [1]. During the experiment, the mineral-N levels (N-NO$_3$, and N-NH$_4$) in the soil were monitored.

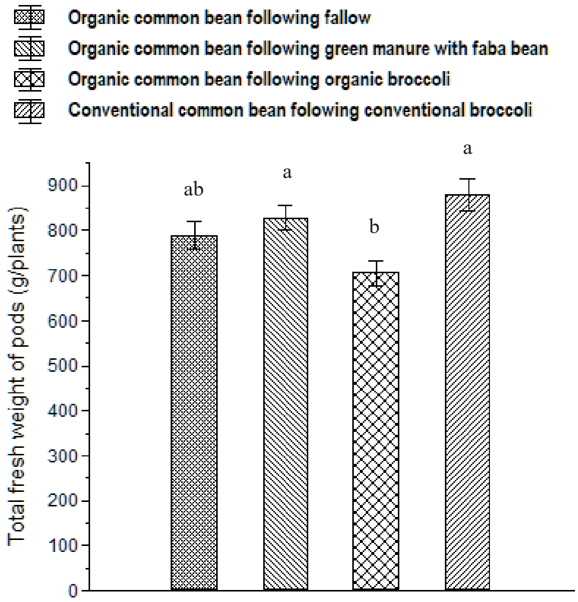

Figure 1. Total fresh pod weight of common bean

During the winter-autumn cultivation period, there were no differences in yield of broccoli cultivated under organic or conventional farming systems. The yield of common bean was significantly lower in the plots cultivated organically following organic broccoli, compared to that obtained from conventionally cultivated common bean following conventional broccoli *(Figure 1)*. However, the yield from organic common bean was similar to that obtained from conventional common bean, when the organic common bean followed incorporation of faba bean to the soil as green manure, or fallow, during the autumn-winter period. Furthermore, organic common bean after organic broccoli resulted in significantly lower yield also in comparison with organic common bean after soil incorporation of faba bean to the soil as green manure. Nevertheless, the yield was not influenced by inoculation of common bean with *Rhizobium tropici sp*. and this was in agreement with the absence of any differences in root nodulation between inoculated and non-inoculated bean plants. The levels of mineral N in the soil after termination of the common bean crop at crop termination (end of July 2018) and the impact of the treatments on biological nitrogen fixation by bean will be presented and discussed.

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How are legume crops valued in Europe? Insights from the analysis of several value chains case studies in the H2020 LegValue Project

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Keywords: value chains, socio-economic analysis, Europe.

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The goal of H2020 LegValue Project is to pave the road to develop sustainable and competitive legume-based farming systems and agri-feed and food chains in the EU. Using a list of thirty value chains reflecting the market diversity, it aims to demonstrate the added value of various legumes value chains and to provide a range of solutions to improve the economic interest of each actor involved in the value chains to use legumes.

The poster presents and discusses the main results of a stakeholders survey conducted in twenty seven legumes value chains located in ten European countries (Denmark, France, Germany, Italy, Latvia, Lithuania, Portugal, Switzerland, The Netherlands and UK). More precisely, it focuses on the typology of value chain obtained from the data survey analysis. Indeed, the analyzed case studies have been classified into four distinct clusters of legumes value chains by taking into account the diversity of legumes species (pea, fababean, soybean, chickpea, lentil, alfalfa, lupin, mixed species), of outlets (food, feed and non-food), of farming systems (organic and conventional), of stakeholders perceptions about value chains functioning, and of coordination between stakeholders (Figure 1). We show the main characteristics of each cluster of value chain, as well as its strengths and weaknesses. It allows not only to better know how legumes are currently valued in Europe, but also to discuss on how coordination between stakeholders could be improved in the value chains for a better legume valorization.

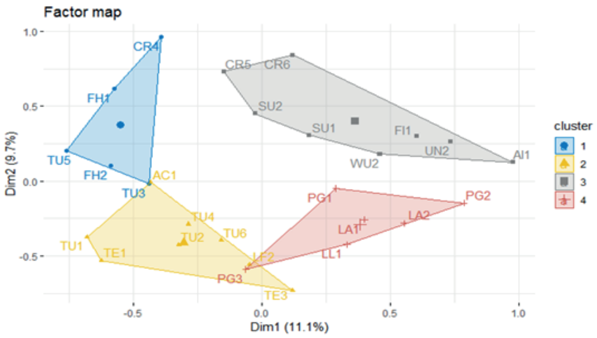

Figure 1. The classification into 4 clusters of the legumes value chains

The first result of our analysis is that the case studies of our sample are divided into four clusters mainly characterized by the outlet and the legumes species they deal with. The clusters in blue and in red gather case studies with value chains dedicated both to food and feed. These value chains deal with pea or fababean, which are the two most produced legumes in Europe. The yellow cluster includes case studies with value chains most often dedicated to feed and the associated legumes species are diverse. Two other commonalities in the cluster are the exportation activities and the lack of upstream contract in these value chains. Finally, the red cluster includes case studies with value chains most often dedicated to food and the associated legumes species are pulses and soybean.

Second, it is interesting to point that the two blue and red clusters with case studies associated to pea and fababean value are graphically opposite clusters in the figure. This means that there are clearly two distinct types of pea and fababean value chains from our case studies sample, according to some well-identified criteria. The most significant distinguishing criterium between the two clusters is the stakeholders' perceptions on the functioning of the value chains. While these perceptions are globally positive for the value chains in the blue cluster, they are negative for those in the red cluster. We then assume that the two types of value chains associated to each cluster are different in their functioning principles, which we explore more in depth by analyzing stakeholders strategies and coordination, and institutional context surrounding the value chains. Highlighting the main functioning principles of these two types of value chains enables us to discuss on the optimal conditions for the European pea and fababean value chains to develop.

Third, another stimulating result is that the yellow and grey clusters can also be seen as graphically opposite in the Figure 1. As the most significant distinguishing criterium between them is about the outlet (feed for the yellow cluster and food for the grey cluster), we assume that these two types of value chain are characterized by different functioning principles according to the outlet they are dedicated to. Here again, we analyze in depth the stakeholders' strategies and coordination as well as the institutional context surrounding the value chains, with a dual intention. First, we bring out the main differences between the two types of value chains in terms of functioning principles. Second, we discuss on the optimal conditions for these types of value chains to develop.

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POSTER SESSION

4.19

The effect of legumes as forecrops on productivity of rotation (legumes-winter triticale-winter rape) with nitrogen fertilization

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Keywords: pea, lupine, spring barley, winter rape, winter triticale, crop rotation

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After 90’s the free market economy caused changes in the agrarian structure and resulted in simplified crop rotation. In some regions of Poland, the share of cereals in the crop structure increased to over 80%. Similar situation concerns many countries in Europe. In consequence of these changes, post-harvest residues were no longer entered into soil, which reduced the inflow of organic matter. For this reason environment-friendly agricultural policy of the European Union recommended legumes cultivation. The role of legumes is very important in crop rotation because of nitrogen fixation, improve soil physical conditions, reduce erosion from water and wind as well as increase soil organic matter. These all benefits can increase yielding of crops, which are cultivated after legumes.

The aim of this study was evaluation of influence legumes as forecrops on productivity of winter triticale and winter rape in rotation.

The study was conducted as a field experiment with four replications as a split-plot design, over a 4-year period from 2012 to 2015,. The trial was carried out at the Experimental Station in Gorzyń (52°33’53 N, 15°53’42 E; Poland). The details connected with the crop rotation contained Table 1. The experiment included two factors. The first factor were forecrops with following levels: yellow lupine (indeterminate cv. Mister), yellow lupine (determinate cv. Perkoz), narrow-leaved lupine (indeterminate cv. Zeus), narrow-leaved lupine (determinate cv. Regent), white lupine (indeterminate cv. Butan), pea cv. Tarchalska (PEA) and spring barley cv. Rubinek (SB). The second factor was nitrogen fertilization of winter triticale (WT) and winter rape (WR): 0 kg N·ha^{-1} (control), 60 kg N·ha^{-1}, 120 kg N·ha^{-1} (60 + 60) and 180 kg N·ha^{-1} (60 + 60 + 60).

The trial was carried out on grey-brown podzolic soil under ordinary growing conditions. Sowing and harvest dates depended on species and weather conditions. The recommended sowing standards for seeds capable of germination were: 100 seeds per 1 m^2 for lupine indeterminate cultivars and pea, 115 seeds per 1m^2 for lupine determinate cultivars, 400 grain per 1 m^2 for SB and WT, 45 seeds per 1m^2 for WR. The area of forecrops plots was 70.6 m^2. The soil was ploughed and harrowed. Phosphorus and potassium fertilization were uniform for all crops in rotation and each year in autumn it was applied in dose 80 kg P·ha^{-1} and 100 kg K·ha^{-1}. There was no nitrogen applied under legume plants. A rate of 60 kg N·ha^{-1} was applied once in spring, before sowing SB. After harvesting forecrops and carrying out of necessary post-harvest and pre-sowing tillage treatments, each of forecrops plots was divided into smaller plots because of second factor. The area of each plot for WT and WR was 14.5 m^2. WT nitrogen fertilization rates (NH_4NO_3; N 34%) were as follows: 60 kg N·ha^{-1} (early spring, before vegetation starting); 120 kg N·ha^{-1} (the second rate was applied during straw shooting phase); 180 kg N·ha^{-1} (the third rate was applied during earing). Nitrogen fertilization rates (NH_4NO_3; N 34%) for WR were as follows: 60 kg N·ha^{-1} early spring (before vegetation starting); 120 kg N·ha^{-1} (the second rate was applied during stem elongation); 180 kg N·ha^{-1} (the third rate was applied during flowering). During growing season were used pesticides recommended for particular species. The seed yield per 1 ha was calculated for legumes and cereals for 15% moisture and WR for 8%.

Table 1. Crop rotation in 2012-2015

Rotation (harvest year)			
2012	2013	2014	2015
legumes + SB	WT	WR	-
-	legumes + SB	WT	WR
-	-	legumes + SB	WT

The present study showed that legumes as forecrops increased yield of all after-harvest crops in rotation. The lowest increase of yielding was observed after pea cultivation (WT 27.8%, WR 2.7%) and the highest after yellow lupine cv. Perkoz cultivation (WT 49.7%, WR 26.7%). Yielding of these crops also depend on nitrogen fertilization. In compared to the control, each dose of nitrogen fertilization caused significantly increase of WT and WR yielding and the highest increase of yield caused the dose 180 kg N·ha^{-1} (WT 27.8%, WR 42.3%).

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Hairy vetch biomass production and effect on the yield of spring barley

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Keywords: hairy vetch, cover crop, barley

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Legumes are often used as cover crops because of their ability to fix atmospheric nitrogen and improve the nutrition of subsequent cash crop [3]. Hairy vetch (*Vicia villosa* Roth) is considered to be an effective winter cover crop in northern climate conditions because of its cold tolerance, fast growth and high N fixation capacity [4].

A field experiment at Estonian Crop Research Institute (58° 44′ 59.41″ N, 26°24′ 54.02″ E) was conducted during the period of 2016–2018 to determine the hairy vetch cover crop biomass production, N accumulation and the effect on subsequent barley cash crop yield. Hairy vetch (cv. 'Villana', 50 kg ha^{-1}) was sown on the 3rd of August in both years, after harvest of winter wheat. Cover crop above- and below-ground biomass samples were collected from four randomly placed squares of 0.25 m^2 in each plot at the end of October and in the following spring before incorporating the cover crops into the soil, prior to the establishment of spring barley (on the 4th and 7th of May in 2017 and 2018, respectively). Dry matter (DM) yield was determined after drying the material at 65 °C to a constant weight. Plant total N in milled samples was analyzed by the Dumas Combustion method on a VarioMAX CNS elemental analyser ("Elementar", Germany). Barley was harvested at physiological maturity on the 30th and 6th of August in 2017 and 2018, respectively. The grain yield of dried and cleaned seeds was adjusted to 14% moisture content and expressed in kg ha^{-1}. Statistical analyses were carried out by statistical package Agrobase 20^{TM}. One-way analysis of variance (ANOVA) was used to test the differences of cover crop biomass, N and barley yield.

The provision of agro-ecosystem services depends on cover crop biomass production [2]. In the autumns of 2016 and 2017, hairy vetch produced equal amount of biomass (1420 kg ha^{-1} and 1360 kg ha^{-1}, respectively). Whilst in the springs, before cover crop incorporation, the biomass was different. Due to earlier beginning of vegetation period and higher temperatures in 2018, the cover crop biomass yields were 960 kg ha^{-1} higher compared to 2017. Depending of the year, N accumulation varied from 62 kg ha^{-1} to 84 kg ha^{-1} N.

Cover crop impacts on subsequent crop yields is reported to be very variable. Cover crops increase, reduce or have no effects on subsequent crop yields. The negative influence of cover crops on successive cereals is associated principally with reduction of soil water storage, especially in water-limited regions [1]. In present study, barley yield was positively impacted by hairy vetch in both years. In 2017, it was 220 kg ha^{-1} higher in the cover crop variant compared to control, but it was not statistically significant. In 2018, barley yield (3100 kg ha^{-1}) was significantly higher compared to control (2760 kg ha^{-1}). Therefore, it can be concluded that hairy vetch is suitable cover crop species for cereal production under Estonian conditions.

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Field pea productivity depending on pre-crop fertilisation

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Keywords: field pea, nue, pre-crop impact

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In advanced cropping system it is valuable to assess not only each crop productivity separately but also the impact of preceding crops (pre-crops) to subsequent crops. The aim of this experiment is to determine the impact of winter wheat with different nitrogen (N) fertilization rates to subsequent field pea (*Pisum Sativum*) dry matter yield (DMY) and nitrogen use efficiency (NUE). NUE indicates how much N has taken up by harvested grain per one kg N available resources, i.e. input N amount divided to harvested grain N amount. It is a valuable tool to access N utilization in crop production and to make first calculations of N surplus and the environment pressure due to N loss.

The field experiment was conducted at the experimental station of Estonian University of Life Sciences at Eerika, Tartu, Estonia (58° 22´ N, 26°40´E). The soil was sandy loam Stagnic Albic Luvisol according to the World Reference Base for soil resources 2014 (FAO, 2015). The soil texture was sandy loam (56.5% sand, 34% silt and 9.5% clay). The data was collected and analyzed during the period of 2012- 2018. The five-field crop rotation was designed in following order: barley undersown with red clover – red clover – winter wheat – field pea – potato. There were four different fertilization treatements in conventional plots and three different treatments in organic plots:

	Pre-crop (winter wheat)	Field pea
N1	mineral N 0 kg ha^{-1}	no fertilizers
N2	mineral N 50 kg ha^{-1}	N20P25K95
N3	mineral N 100 kg ha^{-1}	N20P25K95
N4	mineral N 150 kg ha^{-1}	N20P25K96
Org1	no fertilizers	no fertilizers
Org2	catch crops	
Org3	catch crops + cattle manure 10 Mg ha^{-1}	

The input for NUE calculation contains N amounts of mineral fertilizers, biological fixation, catch crops, atmospheric deposition, seed material, soil stock (plant available NO_3 and NH_4 before pea sowing). The output contains N amount in harvested pea grain.

The DMY of field pea was 40-48% higher in mineral fertilized N2, N3 and N4 treatments compared to non-fertilized treatment N1. The higher nitrogen rates (N3, N4) in precrop slightly suppressed the DMY of peas. However, the higher pre-crop fertilization produced more residues like roots and straw, which were incorporated in soil and increased N source for pea. Interestingly, no DMY rise was seen due to N uptake, despite the the pre-crop residues being available during sowing, which means that N surplus had to happen in soil. Higher NUE in N2, N3 and N4 indicates higher input N utilization of pea than non-fertilized N1.

There was no yield difference in organic system treatments Org1, Org 2 and Org3. The additional N amount from catch crops and manure did not increase N uptake, caused NUE reduction and possibly surplus N in the soil.

Figure 1. Field pea DMY and NUE

The amount of residue N and the C / N ratio affect the N release amount and rate of pre-crop residues through mineralization during the pea growth period. If the pre-crop has above mentioned criteria similar to winter wheat, and does not have any specific allelopathic effect on the pea, it can be assumed that the effect of a pre-crop is similar to this study.

The results of the study can help to design crop rotation sequence where crops are in the most beneficial order. The crops with higher N demand not should be placed before field pea if possible.

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The influence of seed coating on germination and field emergence in selected grass and legume species

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Keywords: arid areas, field emergence, laboratory germination

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The main aim of our research was testing the influence of seed coating on laboratory germination and field emergence in selected grass and legume species. The field experiment is located in dry area with continental climate near Hodonín (in the Southeast of the country) on sandy acid soils. Within the project different legume and grass species were evaluated. After two years of evaluation it can be concluded, that there is no positive effect of seed coating both on the laboratory germination and the field emergence and subsequent vegetation development. Seed coating prolongs germination / emergence period. The results, especially in the field emergence, significantly differ among tested species.

Table 1: Emergence rate displayed by the number of days from sowing to emergence. Coated and non-coated seeds were compared. Results of statistical testing of differences based on two years data (2017-2018).

Species / variant	Number of days to emergence	Significance
Melilotus albus coated	7.3	a
Anthyllis vulneraria non-coated	7.6	a
Medicago lupulina coated	8.0	a
Melilotus albus non-coated	9.1	ab
Trifolium repens non-coated	9.3	ab
Onobrychis viciifolia non-coated	9.4	abc
Anthyllis vulneraria coated	9.6	abc
Trifolium repens coated	9.8	abc
Medicago lupulina non-coated	10.7	abcd
Onobrychis viciifolia coated	11.0	abcd
Festuca rubra coated	12.3	bcde
Festuca pratensis non-coated	12.4	bcde
Lolium perenne non-coated	12.7	bcde
Lolium perenne coated	13.3	cde
Festuca pratensis coated	13.7	de
Phleum pratense non-coated	13.9	de
Festuca rubra non-coated	14.3	de
Phleum pratense coated	15.3	e
Poa pratensis non-coated	23.7	f
Poa pratensis coated	25.8	f

Table 2: The results of germination testing in the laboratory – coated and non-coated seeds. Results of statistical testing of differences based on two years data (2017-2018).

Species / variant	Germination (%)	Significance
Onobrychis viciifolia coated	77.8	a
Festuca rubra coated	81.0	a
Anthyllis vulneraria coated	85.0	ab
Festuca rubra non-coated	87.2	bcd
Melilotus albus non-coated	88.5	bcd
Onobrychis viciifolia non-coated	90.8	bcdef
Anthyllis vulneraria non-coated	91.3	bcdef
Phleum pratense coated	92.4	bcdef
Poa pratensis coated	93.6	cdefg
Melilotus albus coated	94.5	cdefg
Medicago lupulina non-coated	94.8	cdefg
Poa pratensis non-coated	95.4	cdefg
Medicago lupulina coated	95.5	cdefg
Trifolium repens non-coated	96.8	cdefg
Phleum pratense non-coated	97.2	efg

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Healthy novel extruded gluten-free snacks based on legumes and rice: bioactivity evaluation

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Keywords: Bean/rice/carob fruit; extrusion; bioactivity; antitumoral and antimicrobial activity

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Extrusion-cooking technology is a high-temperature and short-time process, necessary to cause structural, physico-chemical and nutritional changes of raw materials, forcing the material to flow under different conditions (temperature, moisture, screw speed, and feed). Rice and legumes have a great potential in the development of healthier gluten-free products than traditional snacks [1].

Rice flour (*Oryza Sativa* L.) has been reported as a good raw material to obtain expanded food products [1]. Dry beans (*Phaseolus vulgaris* L.) are rich in proteins, dietary fibre, complex carbohydrates (leading to low glycaemic index), minerals and numerous phytochemicals endowed with useful biological activities [2]. Carob fruit (*Ceratonia siliqua* L.*)* is a tree leguminous native to the Mediterranean region. The fortification with carob fruit would provide adequate fibre content and a good amount of bioactive compounds. In previous studies, carob evidenced therapeutical properties against several diseases, such us, regulatory effect in blood glucose level, reduction in low-density-lipoprotein cholesterol in hypercholesteraemic patients, body weight benefits, and improved digestion, and lipid utilization [3]which were associated with endothelial dysfunction, inflammation, and fibrosis. Furthermore, sirtuin-1 (SIRT1.

In this study, novel gluten-free extruded foods (composed by rice: 50-80%, bean: 20-40%, and carob: 0-10%) were performed using a twin-screw extruded at CARTIF (Valladolid, Spain) and the effects of extrusion were evaluated regarding bioactive properties. Commercial extruded rice was used as external control. Raw materials, non-extruded, and extruded samples were extracted with ethanol/water (80:20, v:v) and purified using C18 SepPak**®** ***Vac 3 cc cartridge*** [4,5], in order to obtain an extract rich in bioactive compounds. The cytotoxicity, anti-inflammatory, and antimicrobial activity were evaluated following previously reported procedures [6, 7].

Carob, commercial extruded rice and most of the extruded samples, showed cytotoxicity in the majority of the tumour cell lines tested (HeLa - cervical carcinoma, HepG2 - hepatocellular carcinoma, MCF-7 - breast adenocarcinoma, and NCI-H460 - non-small cell lung cancer). While, bean, rice, and most of the non-extruded samples presented no toxicity (GI_{50} value >400 *µg/mL*) using a non-tumour porcine liver cell culture (PLP2). In general, it was observed that extrusion process improves the cytotoxic potential in the rice-legumes sample mixtures, revealing lower GI_{50} concentrations (ranging between 115 and 362 *µg/mL*). The anti-inflammatory activity revealed a high heterogeneity, presenting bean and carob samples, the highest activity in comparison to the extruded samples. Concerning the antimicrobial activity, which was tested using a panel of multi-resistant isolated clinical strains, a low potential was observed, with non-extruded and extruded samples revealing higher values of minimum inhibitory concentration (MIC) and minimal bactericidal concentrations (MBC).

In conclusion, rice-legumes flours, rice blends, beans, and carob fruits are a great alternative for the development of new gluten-free snacks products, in a market dominated mainly by cereals, due to the presence of different bioactive compounds, such as phenolic compounds, which can give healthier benefits to the consumers.

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Determination of bioactive compounds in rice-based pasta fortified with bean and carob fruit

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Pasta was largely consumed all over the world and these products were classified as the ideal vehicle for the fortification by the World Health Organization (WHO). Additionally, nowadays the population with celiac disease is growing as well as the population who exclude gluten traditional products for other health reasons. In general, gluten-free (GF) products were not nutritionally and quality better than the gluten containing products. It is well known that legumes can improve the nutritional characteristic of the pasta products, since they are gluten-free seeds, good source of fibre, minerals, vitamins and proteins. Gluten is considered the most significant factor related to pasta cooking quality, because it is responsible for the formation of the structure; however, in the GF samples, starch has to assume the structuring role [1]. Carob fruit, (*Ceratonia siliqua*) a leguminous tree native to the Mediterranean region, contain caroubin, a protein similar to wheat gluten, that is associated to great cooking quality and firmness in GF pasta [2].

The aim of this study was to analyse the presence and the content of some bioactive compounds in experimental fettuccine elaborated with mixtures of rice (*Oriza sativa*) (0-100%), bean (*Phaseolus vulgaris*) (0-100%) and carob fruit (0-10%). Results were compared with a commercial rice pasta, as external control.

Fettuccine were elaborated following the methodology (with minor modifications) of [3,4] and the bioactive compounds: individual inolsitol phosphates (IP), soluble sugars and α-galactosides, protesase inhibitors and lectins, were determined following [5–7] methods, respectively.

Inositol phosphates, mainly phytic acid (IP6) has been related with a low incidence of some cancer, atherosclerosis or type II diabetes [8]. As expected, the higher bean percentage caused the higher total IP content in the fettuccine. In comparison to the commercial rice pasta the experimental fettuccine showed from 4 to 15 times more content of IP6. The cooking process did not revealed significant differences between cooked and uncooked counterparts. Thus, pasta elaboration steps were not effective on phytate destruction. In consequence, pasta could retain the health benefits associated to this bioactive compounds. The α-galactosides are considered as bioactive component with some prebiotic activity [8]. In general, the pasta elaboration and cooking process reduced the content of each individual sugar determined in the fettuccine samples; the total α-galactosides content were also significantly reduced, around 40-70%, by pasta processing and cooking. The main α-galactoside detected in all the samples was stachyose. Trypsin and chymotrypsin inhibitors are considered nutritionally active factors associated with a reduction of the risk of some chronic diseases [8]. As expected, the higher bean content showed the higher inhibitor activities in the raw samples. In general, the cooking process did not reduce significantly the protease inhibitor activities. PHA lectin in the fortified pasta was reduced completely after the cooking process, avoiding the toxic effects, such as vomiting, bloating, pancreatic enlargement, etc., associated to the consume of raw or uncooked samples rich in this glycoprotein [8].

We can conclude that the pasta elaboration and the cooking process did not affect in the same extent to the different bioactive compounds studied. In comparison to the commercial rice pasta studied, the amount of the phytochemical compounds remaining in the cooked pasta revealed that they could exert healthier functions.

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Profile of crude protein, mineral elements, polyphenols and dpph scavenging activity of lima bean (*Phaseolus lunatus* L.) seeds

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Lima bean is one of the lesser used crops in Ghana despite its importance as food for man and fodder for animals. The experiment was conducted to evaluate lima bean for seed quality traits. Twenty accessions of lima beans were planted in single row plots, their seeds were harvested and pulverized and phytochemical analysis was conducted to collect data on percent crude protein content, percent mineral element content, polyphenol content and DPPH scavenging activity expressed in mg/l. The mineral elements were phosphorus, copper, iron, manganese, zinc and magnesium. The polyphenols were 2,5-dihydroxylbenzoic acid, caffeic acid, chlorogenic acid, gallic acid, p-coumaric acid, syringic acid, vanillic acid, rutin and quercetin. Antioxidant properties were based on DPPH scavenging activity. Percent crude protein ranged between 19.40±0.09% and 8.85±0.19% with a grandmean of 13.18±0.29%. Mean phosphorus content ranged from 21.08±0.008% to 13.39±0.21% about a grandmean of 6.16±0.24%. Mean copper content ranged between 0.10±0.00% and 0.0002±0.00002% with a grandmean 0.03±0.004%. Mean iron content ranged from 0.001±0.00000% to 0.01±0.000001% with a grandmean of 0.002±0.00003%. Mean magnesium ranged from 0.39±0.0001% to 0.13±0.0001% with a grandmean of 0.25±0.0008%. Mean zinc ranged between 0.0005±0.001% and 0.02±0.002% with a grandmean of 0.01±0.0001%. Percentage mean content of manganese ranged from 0.03±0.0001% to 0.003±0.00001% with a grandmean of 0.001±0.0001%. Mean caffeic acid content ranged from 145.69±0.25 mg/l to 67.57±0.63 mg/l with a grandmean of 89.25±2.90 mg/l. Mean chlorogenic acid content ranged from 148.40±0.30 mg/l to 53.85±0.76 mg/l with a grandmean of 80.09±3.51 mg/l. Mean content of syringic acid ranged from 146.42±0.30 mg/l to 57.85±0.76 mg/l with a grandmean of 78.09±3.51 mg/l. Mean content of gallic acid ranged between 177.08±0.48 mg/l and 27.36±1.21 mg/l, grandmean value was 68.90±5.56 mg/l. Mean 2,5-dihydroxybenzoic acid content ranged from 116.667±0.213 mg/l to 50.123±0.538 mg/l and the grandmean recorded was 68.58±2.47 mg/l. Mean content for vanillic acid ranged from 109.789±0.303 mg/l to 15.228±0.764 mg/l with a grandmean of 41.46±3.51 mg/l. Means of p-coumaric acid content ranged from 123.285±0.412 mg/l to 0 mg/l with a grandmean of 30.55±4.77 mg/l. Mean content of quercetin ranged from 439.291±1.102mg/l to 175.958±1.816 mg/l with a grandmean of 273.68±8.26 mg/l. Mean content of rutin ranged between 48.857±0.082 mg/l and 15.333±0.171 mg/l with a grandmean of 29.12±1.00 mg/l. DPPH scavenging activity mean values ranged from 45.403±0.094 mg/l to 3.146±0.216 mg/l with a grandmean of 20.34±1.55 mg/l. Multiple regression analysis suggests that 48.52% of variation of scavenging activity of the lima bean extracts results from the contribution of the six mineral elements and the nine polyphenol compounds. The study also showed that the lima bean accessions used for the study can be good natural sources of phenolic compounds and antioxidants.

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Thiamine (vitamin B$_1$) and riboflavin (vitamin B$_2$) content of field peas (*Pisum sativum* L.), faba beans (*Vicia faba* L.) and lupins (*Lupinus* spp. L.)

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A deficient supply of B vitamins leads to health problems and decreased performance in poultry and swine. Since B vitamins are water-soluble and continually excreted by mammals, they must be provided frequently with the diet to guarantee a sufficient supply.

Thiamine (vitamin B$_1$) plays a major role in the function of the nervous system and in the energy- and amino acid metabolism. Deficiencies lead to severe health problems. Cereal-based diets are supposed to contain sufficient amounts of thiamine for poultry and swine. However, recent information on the actual content of thiamine in grain legumes is scarce.

Riboflavin (vitamin B$_2$) also takes part in the energy and amino acid metabolism. Deficiencies often lead to a loss of mobility and other health problems. Riboflavin is reported to be contained in grain legumes in large amounts. However, recent information on the riboflavin content of grain legumes is also scarce.

To improve the knowledge on the thiamine and riboflavin contents of grain legumes, 87 field peas (*Pisum sativum* L.), 73 faba beans (*Vicia faba* L.), 110 blue lupins (*Lupinus angustifolius* L.), and 4 yellow lupins (*Lupinus luteus* L.), were analysed via high-performance liquid chromatography methods (HPLC) with fluorescence detection (FLD) according to EN 14122 (2014) and EN 14152 (2014). The samples were taken in the years 2011, 2012, and 2013 from organic variety field trials throughout Germany. The effects of variety and environmental conditions on the thiamine and riboflavin content of field peas, faba beans, and blue lupins were tested statistically with generalized linear models. Due to an unbalanced sample-set a data reduction was necessary. Six field pea varieties from three years and four harvest sites, six faba bean varieties from two years and three areas [1], as well as five blue lupin varieties from three years and three sites were tested. In total, 41 field pea samples, 57 field bean samples, and 31 blue lupin samples were used for statistical analysis. Furthermore, exemplary diets with high amounts of the three grain legume species field peas, faba beans and/or blue lupins were calculated for piglets (30%), lactating sows (35%), gestating sows (25%), pre-fattening pigs (25%), and fattening pigs (52%) as well as chicks (28%), laying hens (25%), broiler chickens (33%), geese (25%), and turkeys (40%). Those calculations were used to determine the value of these grain legumes in terms of thiamine and riboflavin supply to monogastric animals in organic farming.

The thiamine contents of all grain legume species varied widely (Figure 1). The variation of the riboflavin content of grain legumes was less distinct.

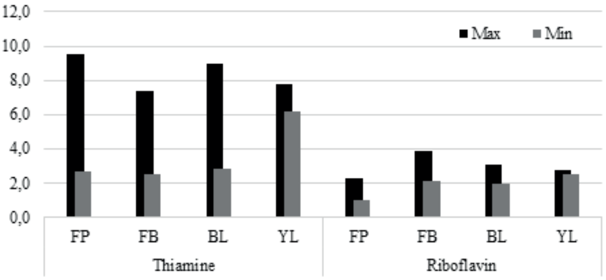

Figure 1. Thiamine- and riboflavin contents in field peas (FP, n=87), faba beans (FB, n=73), blue lupins (BL, n=110), and yellow lupins (YL, n=4) in mg/kg DM

Variety affected the thiamine content of faba beans as well as the riboflavin content of field peas and faba beans. There was also an effect of harvest site or area on the thiamine and riboflavin content of all tested grain legumes. Cultivation year affected the thiamine content of field peas and faba beans as well as the riboflavin content of field peas and blue lupins.

The thiamine recommendations of the GfE (Society of Nutrition Physiology, 1999, 2006) were met in all exemplary diets. Depending on their individual thiamine content and their proportion in the diet, the thiamine amount from grain legumes accounted for 32 to more than 100% of the recommended amount of thiamine. However, the riboflavin recommendations of the GfE were only met for laying hens, piglets, lactating sows, and pre-fattening pigs, because their exemplary diets contained milk powder, whey powder, green meal, and/or brewer`s yeast. The riboflavin amount from grain legumes accounted for 11 – 30% of the recommended amount. Grain legumes can contribute to the thiamine and riboflavin supply of organic diets, but wide variations of the contents must be considered.

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Genetic and environmental influence on *Lathyrus sativus* mature seed metabolome

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Worldwide, consumers are increasingly concerned with food quality and environmental sustainability. Grain legumes answer to these concerns. On one hand, grain legumes have a rich nutritional composition, health-promoting components and a functional food status. On the other hand through their unique nitrogen-fixing ability and ease of incorporation into crop rotations, grain legumes contribute to mitigate the adverse effects of agricultural production on the environment, increasing the sustainability of ecosystems.

At food quality level, grain legumes are known for their high seed content in protein, vitamins, fibres, resistant-starch, and polyphenols and for their antioxidant activity. However not all grain legumes have been characterized to the same extent and limited data on seed quality is available as the primary focus has been hitherto on other phenotypic and agronomic trait improvement [1]. This is the case for *Lathyrus sativus*, also known as grass pea, an underused grain legume but one of the most promising sources of calories and protein for populations in drought-prone and marginal areas [2].

Seed quality traits may be defined in terms of the synthesis of a number of key metabolites [3]. In order to characterize the metabolite profiles of large numbers of plant genotypes, metabolomics tools have been developed to analyse a wide range of metabolite classes in a single run and in a high-throughput manner [4].

Nuclear magnetic resonance (NMR) spectroscopy metabolite profiling is one of such metabolomics approaches that provide a broad and unbiased assessment of the changes in the plant metabolome in response to genetic and/or environmental factors [5].

To understand the genetic and environmental influence on grass pea metabolome as key steps for devising quality breeding approaches, a collection of 200 grass pea accessions representative of the cultivated world diversity was studied. Mature seeds were harvested during three years from a repeated field trial and milled into flour before metabolite extraction. After extraction of 1 g of flour in 20 mL of 50:50 ethanol:water, samples were evaporated, resuspended in NMR buffer and evaluated by untargeted NMR using a Bruker AdvanceII+ 500 MHz spectrometer. The data were processed and normalized in NMRprocflow and compared through multivariate analysis using MetaboAnalyst. Metabolite concentrations were annotated in the NMR spectra using the Chenomx NMR Suite Professional Ed.

The NMR spectra were binned into approximately 650 uniform bins/metabolome features. Analysis of variance detected differences among the accessions metabolite profiles in each of the harvested years, with no clear accession clustering. Nevertheless, it was possible to clearly discriminate the three different years based on the seed composition variation.

Several metabolites were contributing to this genetic and environmental differentiation, and their identification is still ongoing by matching with NMR databases. As an example, one of the compounds that contributed the most to the year discrimination was betaine. Betaine is a derivative of choline, produced as a response to environmental stress. Betaine has a major role in the protection of the cells adjusting the osmotic balance, consequently helping the plants to overcome the stress caused by water, cold and salinity. The climate variation felt through the cultivation years have had an effect on this metabolite and probably also on other, influencing the final quality of the seed [6,7].

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Genetic diversity of protein, iron and zinc contents in lentil (*Lens culinaris* Medic.) seeds

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Micronutrient deficiency, popularly known as hidden hunger, is among the most serious global health problems affecting more than two billion people worldwide [1]. This problem is mainly caused by low dietary intake of micronutrients, especially Zn, Fe and Se, leading to significant health disorders.

Micronutrient deficiency can be address through different approaches such as mineral supplementation, food fortification and dietary diversification. However, these measures are beyond the reach of the poor households. To alleviate this problem, biofortification, through plant breeding techniques, holds great promise for improving the nutritional value of staple food crops.

Among grain legumes, lentil (*Lens culinaris* Medik.) contains a relatively high concentration of protein (~30%) and is an excellent source of essential micronutrients in forms that are highly bioavailable to humans [2]. In addition, a wide range of genetic variability has been reported among lentil germplasm, indicating that it is possible to breed lentil cultivars with enhanced ability to accumulate iron and zinc.

The main objective of this study was to determine the protein content and the concentrations of iron and zinc in lentil seeds. For this purpose, a set of 150 lines originating from 47 countries were planted at ICARDA research farm, Rabat, Morocco. Seeds were harvested at maturity stage during 2016-2017 cropping season. Seeds mineral concentrations were measured using a previously described modified HNO_3-H_2O_2 method [3], followed by inductively coupled plasma-optical emission spectroscopy (ICP-OES). While, the crude protein content was determined by micro-Kjeldahl Method [4].

Analysis of variance showed significant variation ($p<0.01$) for iron, zinc and protein content among the tested lentil genotypes. Mineral Concentration varied from 44.72-108.58 mg kg^{-1} for Iron and from 24.74-64.92 mg kg^{-1} for Zinc. The maximum concentration was obtained for iron in ILL 6281 (108.58 mg kg^{-1}) and zinc concentration in ILL 6350 (64.92 mg kg^{-1}). Crude protein content ranged from 22.42 % for ILL 7223 to 32% for ILL 8018.

Overall, Lentil has the potential to provide an excellent natural source of Fe, Zn and protein. Thus, lentil could be used as a staple food crop to eradicate hidden hunger especially in developing countries.

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Glycation affects differently the main soybean Bowman-Birk isoinhibitors, IBB1 and IBBD2, altering their anti-proliferative properties against HT29 colon cancer cells

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Thermal processing of soybean meal, a common procedure used in food industry, might affect the bioactivity of certain compounds, including protease inhibitors of the Bowman-Birk family. In previous studies, Bowman-Birk inhibitors (BBI) from legumes have demonstrated to exert their potential chemopreventive and/or therapeutic properties *via* protease inhibition [1,2]. It has been suggested that, under thermal treatment, Lys and Arg residues within the BBI protease inhibitory domains might react with reducing sugars and could affect the ability of these proteins to inhibit serine proteases [3]. Therefore, the aim of this work was to investigate the molecular pattern of the major soybean BBI isoinhibitors, IBB1 and IBBD2, under glycation conditions and how this affected their well-reported anti-proliferative properties against HT29 colon cancer cells. Both soybean isoinhibitors showed remarkable stability against heat treatment (95ºC, 90 min). In the presence of glucose, IBBD2 loses most of its trypsin inhibitory activity after 90 min of heat treatment while IBB1 is not affected. In order to investigate the glycation pattern of both soybean BBI isoinhibitors, MS spectra data was obtained and further *in silico* analysis to identify glycated peptides was carried out. Although glycation of both isoinhibitors took place, Arg residues within the inhibitory domains of IBBD2 were more prone to be glycated than those of Lys in IBB1. As a result of the differential glycation process, the anti-proliferative properties of IBBD2 against HT29 colon cancer cells were significantly diminished whereas IBB1 was unaffected.

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Figure. Inhibitory activity of IBB. (**A**) Percentage of trypsin and chymotrypsin inhibitory activity of IBB1 during heat treatment in the presence or absence of glucose, being 100% of inhibitory activity that of the purified protein without heating. (**B**) Percentage of trypsin inhibitory activity of IBBD2 during heat treatment in the presence or absence of glucose, being 100% of inhibitory activity that of the purified protein without heating.

Selection of pea genetic resources based on phytic acid content screening of different varieties and lines for subsequent biofortification

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Improving feed quality is an important goal for breeders. Non-ruminants cannot fully utilize phosphate bound in the form of phytic acid. Our goal is to develop biofortified pea lines with a lower phytic acid content and / or a higher content of native phytase and an increased content of digestible forms of mineral elements (P, Ca, Mg, Fe, Zn, Se), which are an essential part of a minerally nutritious diet for creation of varieties with improved nutritional utilization. We deal with a complex solution of the issue of mineral nutrition in peas for feed and food use. To improve the quality of feed, today‘s society puts a great deal of pressure on farmers and secondarily on the part of consumers. Improving the whole feed process is a desirable trend.

Peas are one of the most widely grown and cultivated legumes in the EU. For its balanced content of protein, fat, glycids, fiber, minerals and vitamins, peas are considered good quality food and feed. Recently, the importance of using peas in feed doses for monogastric animals as sources of energy and protein has begun to grow in the EU, and its cultivation has been adequately subsidized. Seeds of peas are an important source of protein for human and animal nutrition. They contain mostly 21-25% of crude protein. An objective evaluation of feed quality is a prerequisite for their effective use in the feed.

Biofortification is a process of improving the quality of feed and food, which evaluates the amount of minerals in the product and also their quantity in the available form. All seed plants synthesize a large amount of phytic acid - myo-inositol-1,2,3,4,5,6-hexacisphosphate (PA), a cyclic alcohol with ester-bound phosphate groups. This molecule is predominantly used in seeds, serving as a phosphorus stock (75% of total P in seeds) and minerals bound to it. By its chemical properties, phytic acid acts as antinutrition factor. Phytic acid salts are called phytates, forming about 1.5% dry weight of seed [1]. Monogastrics are not able to hydrolyze the phosphodiester bound between phosphate and myo-inositol. The result is inefficient use of feed and increased phosphate content in excrements that significantly pollute groundwater and soil. Low-phytate crops are rich in inorganic phosphorus in seeds and have a low phytic acid content.

The goal of screening in the first year of the project was to search for genetic resources of low phytic acid pea (PA). A set of varieties and pea lines were selected for screening and determination of phytic acid content and basic qualitative compounds.These varied from growth type, flower color, seed and contend of nitrogen, starch and resistant starch. From these materials a set of seed samples (100g per sample) was created to determine the basic characteristics suitable especially for human nutrition and livestock nutrition. In 2018, phytic acid (PA) analyzes were performed, first on samples from genetic sources (including breeding lines) and further obtained low-phytate lines from Canada [2]. In our analyzes and trials, Canada‘s low-phytic lines 1-2347-144 and 1-150-81 were used for comparison, which originated from the CDC Bronco variety. In these lines, the phosphate phosphorus concentration was reduced by approximately 60%, compensating for an increase in inorganic phosphorus. Values of phytic acid for the canadian lines are (0.22-0.57%) and for the CDC Bronco variety 0.74% [2]. These lines were mutagenized by sodium azide and ethylmethanesulfonate (EMS)**.**

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Effect of processing on the content of total dietary fiber, choline and folate in flours from Swedish grown legumes

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Keywords: legumes, total dietary fiber, methyl donors

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The trend towards sustainable food consumption and healthier lifestyle in Sweden have driven a demand on plant-based foods thus increasing the opportunity to incorporate more legume-based ingredients in food formulation. Such ingredients could be whole legume flours; which are thermally processed to reduce or eliminate the content of anti-nutrients in the raw legume seeds [1]. Legumes provide a high amount of dietary fiber and methyl donors, e.g., folate, for both the average intake is below the recommendation in Sweden [2]. Increased use of locally sourced whole legume flours in food products will be beneficial in terms of sustainable production. Increased consumption of legume-based foods might increase the dietary intake of fiber and folate of the Swedish population.

There has been no study on the characterization of nutrients in flour from Swedish grown legumes. Therefore this work aimed to study the effect of three different processing techniques (boiling, roasting and germination) on the nutrient content of whole flours from four types of Swedish grown legumes. The data generated in this work will be valuable for food industries in deciding which type of legume flour that is best suited for their products.

Four types of legumes (yellow and grey pea (*Pisum sativum*), faba bean (*Vicia faba*) and white bean (*Phaseolus vulgaris*)) were selected according to their suitability to be cultivated in Sweden. Three types of processing technique (boiling, roasting and germination) prior to drying and milling were used to produce the legume flours. The effects of each processing technique on the content of total dietary fiber, total choline and folate in legume flours were investigated.

Dried legume seeds were soaked in tap water (1:3 w/v) overnight at room temperature. Thereafter, the seeds underwent different processing: boiling, roasting (at 180°C 15-25 min) and germination (24 h and 48 h). Boiled and germinated seeds were dried at 50°C for 16 and 8 h, respectively. Raw and dried processed seeds were milled into flours. Total dietary fiber was determined according to AOAC 991.43 [3], and total choline and folate was quantified using established HPLC methods [4,5].

In raw legume flours the content of total dietary fiber was between 16 g to 18 g/100 g DM, of total choline between 136 mg to 209 mg/100 g DM and of folate between 73 µg and 246 µg/100 g DM. Boiling led in all legumes to an up to 1.2 to 1.3-fold increase of total dietary fiber, whereas it induced depending on the type of legumes a reduction of total choline and folate between 7-27% and 15-32%, respectively, as compared to the raw flours. Roasting resulted in an increase of total dietary fiber by 1.1 to 1.2-fold and total folate by 9-26%. Roasting did not affect the total choline content in flours of yellow pea, grey pea and white bean but resulted in a 12% decrease in faba bean flour. Germination induced a rise in the content of total dietary fiber 1.1 to 1.3-fold and folate content 1.6 to 3.5-fold in pea and up to 1.2-fold in bean (Figure 1). However, germination did not affect the total choline content of yellow pea, grey pea and faba bean but led to a 5-15% reduction in white bean compared to the raw flours. Overall, treated bean flours had a higher nutrient content than treated pea flours: total dietary fiber by 1.2-fold, total choline up to 1.5-fold and folate by 1.1 to 2.6-fold compared to.

In conclusion, roasting and germination enhanced the nutrient content of legume flours, particularly folate, compared to boiling. Hence roasted and germinated legume flours could be potentially used for biofortification to enhance folate and total dietary fiber content in food.

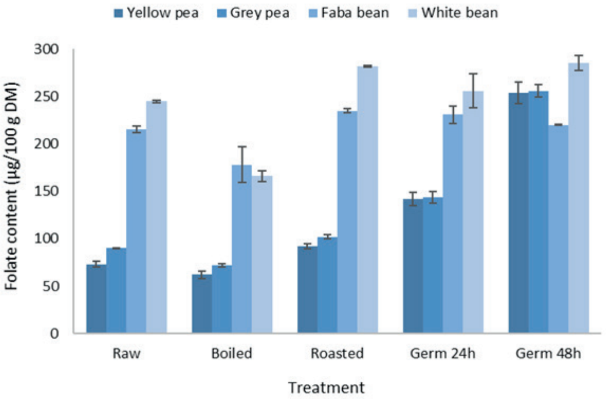

Figure 1. Folate content in raw and treated legume flours. Bars represent the mean (m± sd) from duplicate analysis of two trials (n=4). Folate content is the sum of folate, expressed as µg folic acid/100 g from H_4folate, 5-CH_3-H_4folate, 10-HCO-PteGlu, 5-HCO-H_4folate

Acknowledgments: This work was supported by the Swedish Research Council FORMAS [942-2016-38].

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Narrow-leafed lupin (*Lupinus angustifolius* L.) seed β-conglutin proteins induce G0/G1 arrest and apoptosis in human colorectal cancer cells

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Keywords: autophagy, colorectal cancer, vicilin, molecular nutraceutics, P53 gene, apoptosis

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Lupins seed proteins seem to be particularly promising as innovative source of functional food ingredients with different positive health aspects, preventing cardiovascular disease, and reduction of glucose and cholesterol blood levels [1].

Interestingly, *Lupinus angustifolius* (narrow-leafed lupin, NLL) seeds are a valuable source of proteins for human consumption with multiple nutritional and nutraceutical properties [1]. Among these proteins, particular research attention has been focused to those from the vicilin or beta-conglutin family, which are the most abundant proteins in NLL seeds [2].

Recently, we have demonstrated the potential anti-diabetic [3,4], antioxidant and anti-inflammatory activities of particular beta-conglutin proteins (β1, β3 and β6) [5], due to their capacity to induce the transcriptional decrease of iNOS expression and the reduction of nitric oxide production; as well as the decrease in the production of pro-inflammatory cytokines; the inhibition of chemotaxis and cell adhesion capacity [5]. These properties could be attributed to the ability of these conglutins to interact with insulin [3] and their particular structural characteristics [6].

In the present study, we purified NLL recombinant beta-conglutin proteins (rβ1, rβ3, and rβ6) using affinity–chromatography, and evaluated their effects on colon cancer cell lines HCT-116 (p53 wild-type), and HCT-116 p53 null (p53 inactive).

The results of the current study showed that β1, β3 and β6 conglutins are capable of inhibiting the growth of colorectal cancer cells. In all cases, the effect is observed at a very low concentration. In HCT-116 cell line, the IC50 values are 0.8, 5.8 and 30.1µg ml for β1, β3 and β6 conglutin, respectively. Moreover, when the HCT-116 p53 null line is treated, the IC50 values are 3.0, 3.4 and 51.8µg/ml for β1, β3 and β6 conglutin, respectively. Thereby, in both cell lines β-conglutin proteins effect is higher in the case of β1, followed by β3 and β6.

Additionally, these three conglutins decrease cell proliferation by inducing cell cycle arrest, independently of p53. Specifically, the β1 and β3 conglutins induce accumulation in G0/G1 phase, while β6 induces, in addition, the accumulation in G2/M phase. In all cases, very few cells were found in S phase.

On the other hand, the treatment with the three conglutins considerably increases the apoptosis, independently of p53 (Figure 1).

In summary, β1, β3, and β6 conglutin proteins from blue lupine seeds, affect the viability in colorectal cancer cells at very low concentration, inducing apoptosis and decreasing cell proliferation by cell cycle arrest, either in G0/G1 phase or in G2/M phase.

Therefore, our results suggest that NLL β-conglutin proteins may be potential chemotherapeutic agents with potential uses for treatment of human colon cancer.

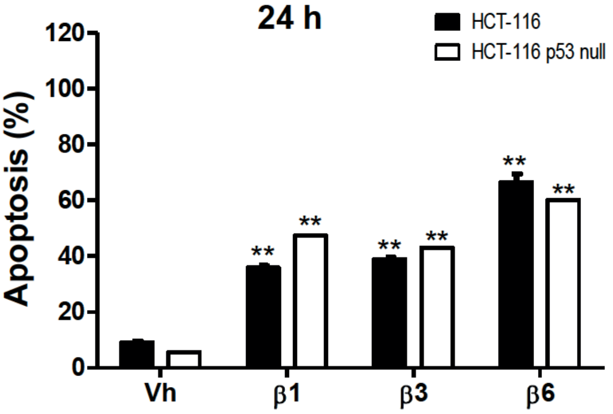

Figure 1. β1, β3 and β6 conglutins induce cell death by apoptosis after 24 h of treatment. The results represent the mean ± standard deviation of three separate experiments. Vh represents the control, where the same process was performed, except for the conglutin addition. ** $P < 0.001$ *vs* Vh

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POSTER SESSION

5.11

Pasta elaborated with lupine and wheat by cold extrusion. Effect of process on colour and cooking quality

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Keywords: lupine, pasta, extrusion

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Pasta, made from durum wheat semolina, has become one of the most consumed and popular food all over the world. Therefore, the World Health Organization (WHO) and Food and Drug Administration (FDA) consider pasta as a good vehicle for addition of nutrient and bioactive compounds to prevent diseases and promote population health. Due to their nutritional and functional value, (protein, polysaccharides, phenolic compound, etc.) legumes are considered interesting foods to be included in balanced diets because of their effects in treatment and prevention of some chronic diseases (cancer, type II diabetes, hypercholesterolemia, cardiovascular diseases) [1]. Particularly, sweet lupine seeds represent an excellent opportunity of using natural materials to fortify pasta, in order to enhance human health. Apart from that, lupine seeds are interesting for food industry because of its nutritional, functional and nutraceutical properties. However, a growing number of allergic reactions to this legume have recently been reported as a consequence of a more widespread consumption of lupine-based foods. Our previous studies have demonstrated that processing lupine seeds in an autoclave at 2.6 bar for 20 min drastically reduces IgE binding, allowing to obtain hypoallergenic flours [2].

In this study, nutritional properties of pasta elaborated by cold extrusion with 0-100% raw or autoclaved lupine (*Lupinus albus* var. Rumbo) at 138°C, 20 min and 0-100% durum wheat semolina (*Triticum durum* L.) were evaluated. The colour attributes and the pasta cooking quality were also determined.

The inclusion of raw and autoclaved lupine flour in pasta increased the protein, ash, lipid and insoluble fibre content compared to traditional pasta (100% durum wheat semolina).

In general, the optimal cooking time was increased by addition of raw lupine flour to pasta (3.74 min to 6.64 min) in comparison to wheat pasta. All elaborated pastas presented a cooking loos up to 12%. There was a significant increase in cooking loss when raw lupine flour was added (6.4% to 17.61%). The use of ingredients different to wheat produces an increase in cooking loss, due to the presence of fibers, weakening the gluten network responsible for the textural properties of pasta, while water uptake was similar in all studied pasta. The The fortification up to 25% autoclaved lupine flour produced negative changes in the pasta cooking quality.

Colour is an important quality factor to the acceptability of pasta, because consumers prefer pasta with bright yellow colour [3]. Pasta elaborated with lupine flour decreased brightness (L*), particularly with autoclaved lupine, while, redness (a*) and yellowness (b*) of pasta were increased by addition of lupine flour, in comparison to wheat pasta

Taking into account the nutritional composition and the dietary fibre content, pasta elaborated with lupine flour could be considered as a healthier and higher quality alternative to the traditional pasta.

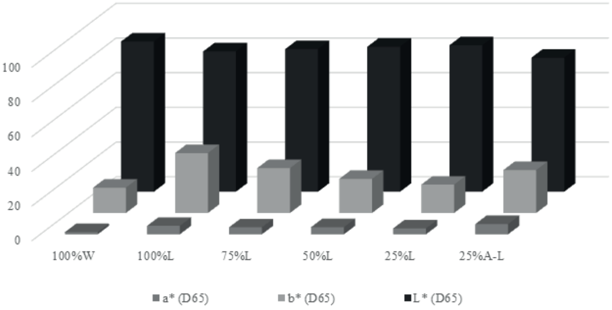

Figure 1. Colour of pasta elaborated with wheat (W) and 25-100% lupine (L) and 25% autoclaved lupine (A-L)

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POSTER SESSION

5.12

Ethanol and SFE legume seeds extracts as potential carbon source for lactic acid bacteria

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Keywords: alpha-galactosides, prebiotics, *Lactobacillus*

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Raffinose family oligosaccharides (RFOs) called also α-galactosides belong to saccharides widespread in the plant kingdom. RFOs are non-reducing, low molecular weight soluble saccharides in water. Generative parts of plants belonging to *Leguminoseae* family are rich source of these saccharides.

They perform many protective physiological functions in plants. From the nutritional point of view α-galactosides are considered as an arduous factors, because they are not hydrolysed in the small intestine of monogastric animals. Undigested saccharides go to the large intestine where they are fermented with releasing of gas. This feature means that in the light of new reports on the importance of probiotic bacteria for animal and human health, it is important to provide nutrients that they consume. Compounds which can modify the composition of colon bacterial microflora are called prebiotics. On the other hand, seeds free of onerous oligosaccharides can be an attractive raw material for the production of food and feed.

The main aim of these studies was performing extraction of soluble in water fraction containing high concentration of α-galactosides from of legumes growing in Poland, determination of their chemical composition and influence on the growth of selected *Lactobacillus* bacteria.

The seeds were subjected to ethanol extraction and then concentrated and dried. The time temperature and ethanol concentration were variable. Seeds were also extracted using supercritical carbon dioxide in various pressure, flow temperatures and the presence of co-solvent variants.

Analysis of saccharide composition in these extracts was determined by using GC-MS.

Extracts with the highest oligosaccharides content were selected for further testing as their usefulness as a carbon source for lactic acid bacteria. Two control media were prepared. One contained glucose - the best source of carbon for bacteria and the other one without saccharides. In the test variants, glucose was replaced by the same amount of obtained extracts. The media was inoculated and the lactic bacteria were grown for 72 hours with optical density (OD) measurement.

It was found that the extraction method has no significant effect on the saccharides composition in the extracts. Good dynamics of growth of *Lactobacillus* bacteria was found on media containing extracts from leguminous plant seeds. This dynamics was lower than on media with glucose, but significant higher than on control media without saccharides. Described results indicate, that obtained extracts can be potential prebiotics.

Utilization of legume-cereal mixtures for feeding in the silage form and its influence on milk quality

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Keywords: intercropping, dairy cow, milk quality

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Feeding of silages from legume-cereal mixtures (LCM) spreads considerably in the diet of cattle and also dairy cows in the Czech Republic. In 2015 the area of LCM cultivation increased by 31 %. By a qualified estimation, LCM silages can now be included up to 48 % (max. 70 %) of cases of dairy cow cattle feeding rations. Earlier, in 2010, it was up to 25 %.

Current climate change (increasing droughts) and the rise in protein feed prices are therefore reasons for increasing interest in growing and producing and increasing of portions from LCM silages in dairy cow feeding rations. Soil contamination of plant roughage feeding can contribute to bacterial contamination of raw milk [1] correlation between silage and milk for sporulated microorganisms 0.69) in stables and milking parlors in particular by spore-forming and psychrotrophic microflora, which can consequently caused the endangering of quality and safety of dairy products.

In 2015-2018, controlled plot trials were conducted with field pea (leafy and semi-leafless types), spring wheat and spring barley in monocultures (M) and legume-cereal mixtures (LCM) to verify the use of LCM to support fodder self-sufficiency. Green and dry matter (GM, DM) yields of M and LCM in two seed ratios 70:30 and 50:50 were compared. The highest yield of DM at a four-year mean was achieved by a mixture of semi-leafless pea with barley in a ratio of 70:30. In the monitored period 2015-2018, the influence of the year was the dominant factor (especially drought), which had a predominant influence on the monitored yield parameters (see Figure 1).

For samples of LCM and M, the nutrient composition was determined. An increase in the concentration of nitrogen compounds in LCM was found compared to M. It can be evaluated as a positive LCM effect.

A case study to assess the possible impact of LCM feeding on milk composition and properties and reproduction of dairy cows was conducted. There was carried out a three-year comparison of the milk quality and composition (bulk and individual samples) of two similar (from zootechnical point of view) cow herds with and without LCM silage (LCM and NOLCM feeding). Differences between LCM and NOLCM were significant for all milk indicators except for free fatty acids. Inhibition in milk has not been captured. There were not clear reproduction differences. A pilot and case field study indicated that application of LCM silage does not lead to deterioration in a milk quality and cow reproduction.

The LCM silage application in feeding rations can be considered as a risk-free, neutral, practically adequate replacing way of part of the roughage component of dairy cow feeding rations with regard to milk quality, according to the actual constellation and needs of the relevant agrotechnical conditions. The values of the reproduction indicators were better in the group in which the LCM silage was fed and this feeding influenced positively the dairy cow pregnancy rate. However, this phenomenon may be partly associated with a slightly lower milk yield in the LCM herds.

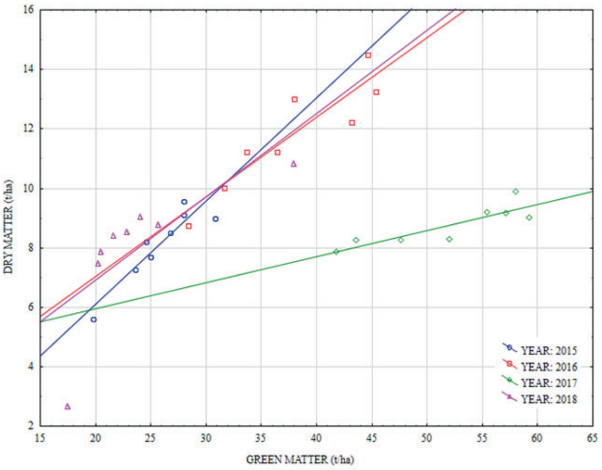

Figure 1. Green and dry matter yields of legume-cereal mixtures in 2015-2018

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Effect of biologically active products to soybean content of fatty acids

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Soybean is globally important crop providing protein for feed and food. The quality of protein - its fatty acid composition - is as important as high yield of soybean. Soybean proteins contain almost all essential amino acids are most similar to proteins from animal sources [3]. Several factors influence the concentration of amino acids present in soybean seeds, such as climate changes, genetics, topography, and soil fertility.

To find out the effect of different biologically active products to seed quality of soybean a field trial was carried out at the Estonian Crop Research Institute (58° 44′ 59.41″ N, 26°24′ 54.02″ E) in 2017 and 2018. Eight products were used: 1. 'EM+' - *Rhodopseudomonas palustris, Rhodobacter sphaeroides, Lactobacillus plantarum, Lactobacillus cesei, Streptococcus lactis, Saccharomyces* spp., *Streptomyces* spp. 2. 'AlgeaFert Meal' - seaweed meal obtained from pure *Ascophyllum nodosum*, a brown alga that grows in the pure habitat of the Arctic regions; 3. 'SEA-90' - natural mineral crystals produced by solar dehydration of sea water; 4. 'Albit' - natural biopolymer poly-β-hydroxybutyrate (PHB) synthesized by soil beneficial bacteria *Bacillus megaterium* and *Pseudomonas aureofaciens*; 5. 'MAX Black Pearl' - humic acids: 25%, amino acids: 8%, NPK: 17%, organic matter: 25%; 6. 'Baikal EM-1-U' - a large number of effective microorganisms (EM): lactic acid bacteria, photosynthetic bacteria, nitrogen-fixing bacteria, yeast fungi, enzymes, amino acids, etc; 7. 'Raskila' - dry matter 81.33%, NPK: not less than 6–1–14 g l^{-1}, humus: not less than 3 g l^{-1}, microelements: Fe, Zn, Mg, Mn, Mo, B, Cu; 8. 'Delfan plus' - total of organic matter 444 g l^{-1}, organic N 108 g l^{-1}, organic C 276 g l^{-1}, amino acids 288 g l^{-1} from the acid hydrolysis of animal protein.

The Estonian soybean variety 'Laulema' was tested. Before sowing the seeds were soaked for four hours in dilutions of preparates or in water (if the product was used during growing period (8) or the product was a granulated fertilizer (5)).

The total content of the following amino acids were quantified in the soybean samples using LC-UV method: Histidine (His), Serine (Ser), Arginine (Arg), Glycine (Gly), Aspartate (Asp), Glutamate (Gly), Threonine (Thr), Alanine (Ala), Proline (Pro), Cystine (Cys), Lysine (Lys), Tyrosine (Tyr), Methionine (Met), Valine (Val), Isoleucine (Ile), Leucine (Leu), Phenylalanine (Phe) and Tryptophan (Trp).

It has been widely documented that soybean seed composition varies with environmental factors, especially during the seed filling period when accumulation of the seed chemical components occurs [1, 2, 4]. The same was in our trials - the weather conditions of the tested years were very different: in 2017, there was a large amount of precipitations and in 2018, there was a drought and high air temperature. Therefore, the results of the trials are various. The biggest differnces occurred in the content of the free amino acids: in 2017, the average was 8.0 mg g DM^{-1}, and in 2018, only 1.4 mg g DM^{-1}. All products and their mixtures, used in trial, increased the content of free amino acids on soybean seeds in 2017, the best result was obtained, using 'Baikal EM-1-U' (total amount 8.84 mg g^{-1} DM, control 6.79 mg). Only the treatment with 'EM+' increased the content of free fatty acids, all other products did not give positve results in 2018. The content of total amino acids increased 5.76 mg by using 'Baikal EM-1-U', but impact of other products was negative - by using 'Delfan plus' total amino acids content increased 27,93 mg in 2017. In 2018, none of the products had clear positive effect for the amount of total fatty acids. The largest amount of total fatty acids (44.35 mg) occurred with 'MAX Black Pearl'. Content of Met increased by using 'Albit' 0.87 mg, content of Trp increased by using ''Baikal EM-1-U' 0.19 mg, content of Gly increased by using ‚Delfan plus' 0.84 mg in 2018.

As a result, it can be stated that biologically effective products influence the content of amino acids in soybean seeds.

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Effect of the addition of Apulian black chickpea flour on the physico-chemical and sensory properties of bakery products

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Keywords: composite flour, chickpea, bread, pizza, focaccia

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Chickpeas (*Cicer arietinum* L.) are commonly classified in two main groups, *kabuli* type, characterized by large seeds with beige coat, and *desi* type, characterized by small and rough seeds with a dark colored coat [1]. A previous study, focused on assessing the genetic diversity of a wide collection of chickpea accessions of *kabuli* and *desi* types, reported about the existence of an uncommon type of black chickpea, historically cultivated in Apulia (South of Italy), apparently similar to *desi* but genetically different [2]. This *Apulian black* type, which is being replaced by modern cultivars and is therefore at risk of genetic erosion, has an interesting potential for further commercial development [2]. The aim of this work was, therefore, to assess the feasibility of using *Apulian black* chickpea flour in the production of bakery products and to assess the effect of this enrichment on the physico-chemical and sensory properties of the end-product.

Bread, "focaccia", and pizza crust were made with composite flours of durum wheat and black chickpea. "Focaccia" is a traditional Italian fatty flat bread widely consumed as street food in Southern Italy [3]. Pizza crust (eventually frozen or packed in MAP), instead, is the object of an increasing interest by both producers and consumers as a convenient food that can be seasoned at home.

In order to choose the best durum wheat/black chickpea ratio, doughs at different ratios (90/10, 80/20, 70/30, and 60/40 w/w wheat/chickpea flours) have been subjected to the evaluation of farinographic and alveographic parameters in order to assess the suitability to the production of fermented bakery products. The addition of black chickpea flour progressively and significantly ($p<0.05$) increased farinograph water absorption and dough development time, whereas shortened the stability time. At the same time, the alveograh P/L ratio increased and the strength decreased. These results were all imputable to the fiber, contributed by whole meal chickpea flour.

Considering the data obtained, the 60/40 w/w durum wheat/chickpea flour ratio was selected, in order to fulfil the legal requirements of the nutritional claim "high fiber content" in the end-products.

The black chickpea-enriched bakery products showed significantly higher levels of phenolic compounds and anthocyanins and higher antioxidant activity than control.

Regarding the sensory profile, although having a similar inner structure (Fig. 1), all the enriched products were significantly harder and more consistent than the corresponding control and were characterized by a moderately intense and pleasant odor note of chickpea.

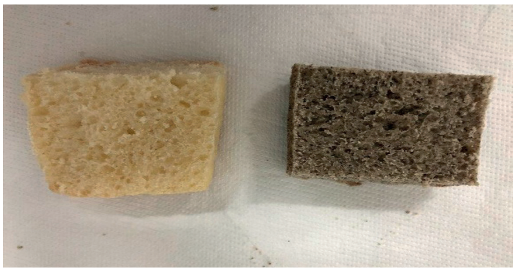

Figure 1. Inner structure of bread with and without black chickpea flour

The obtained results show that the addition of an innovative ingredient as the flour of *Apulian black* chickpeas, in mixture with durum wheat flour, allowed producing bread, "focaccia", and pizza crust with enhanced nutritional features and antioxidant properties, without eccessively affecting the sensory properties.

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Proximate composition and bioactive compounds content on gels based on two commercial pea protein isolates. A comparison

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Keywords: Pea protein isolate, gelation, phytochemicals

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There is increasing consumer demand for convenient and healthy foodstuffs of vegetable origin. Vegetable proteins have high potential for use in the food industry in the formulation of new meat and seafood analogue products as a more accessible and sustainable alternative to animal proteins.

Dry peas, as well as other pulses, are good sources of proteins, fiber, starch, vitamins and minerals. Moreover, they are also rich in phytochemicals, which have generally been associated with several health benefits, such as hypoglucaemic, antihypertensive, anticarcinogenic, hypocholesterolaemic and antioxidant properties, and they are viewed as bioactive compounds [1].

Dry peas can be processed into different products including pea protein isolate (PPI). The commercial PPIs are mainly focused to athletes as a food supplement due to their high protein content, although it can also be used as a functional ingredient to improve the nutritional value to fortified foods. Most of the scientific literature is focused on the protein and carbohydrate content of these PPIs and on their functional properties, such as gelation, water and oil absorption capacity or emulsifying properties. However, there is little information about the bioactive compounds presents in these PPIs able to exert healthy roles.

In the Spanish market, it could be found different commercial PPIs, obtained following different extraction procedures, which can include or not isoelectric precipitation. Thus, the main aim of this study was to determine the differences in the nutritive and bioactive compounds content of two different commercial PPI (PPI-A and PPI-B) as well as in the gels elaborated a different concentration of PPI plus microbial transglutaminase (MTGase).

Proximate composition was evaluated using the corresponding AOAC methods [2]. Bioactive compounds (α-galactosides, inositol phosphates and protease inhibitors) were analyzed according to Pedrosa et al., [3].

Gel samples of both commercial PPIs were prepared at 20% and 23% of PPI concentration with 2% NaCl, 4.5% sodium caseinate (as MTGase substrate). Blends were homogenized at 80ºC/5 min, then cooled to 40ºC and added 0 and 5 U/g of enzyme.

Total and soluble protein contents of both PPIs did not differ significantly ($p>0.05$). In the present study, PPI-A had lower ($p<0.05$) fat, and ash, but higher ($p<0.05$) carbohydrate content than PPI-B. In general, these differences could be due to the different extraction procedures of both PPIs. Although PPI-B had lower amounts of carbohydrates, it contained a higher ($p<0.05$) amount of starch than PPI-A. Both PPIs showed a similar total dietary fiber content.

In relation to the bioactive compounds, phytic acid (IP6) was the main inositol phosphate in all the samples. Although PPI-B contained higher (24%) amount of total inositol phosphates than the PPI-A. This fact was also observed in the gels. For example, 20% gel of PPI-B contained a 38% more total inositol phosphate than the same gel elaborated with PPI-A. There were no significant differences between gel with or without MTGase.

Both PPIs showed a similar amount of total α-galactosides, being stachyose the main sugar detected. The gels elaborated with PPI-B retained a 40% more amount of these compounds. There were no significant differences between gels with or without MTGase.

Regarding the protease inhibitors content, the PPI-B contained 5 times more trypsin inhibitor activity than the PPI-A. As the pea variety used by both industries to obtain these PPIs is unknown, these differences could be due both to the pea variety, the environment where grew the seeds. Moreover, taking into account the thermolabile nature of these compounds, the temperature used in the drying process could inactive the protease inhibitors. Gels elaborated with 5U/g of MTGase showed lower trypsin inhibitor activity.

In conclusion, there are small differences in the proximate composition of both PPIs, however, there are significant ($p<0.05$) differences in their bioactive compounds content. From a healthy point of view, it is more interesting elaborate gels products with the PPI-B

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5.17

Bioactive compounds of gluten-free formulations based on rice and chickpea enriched with passion fruit: analysis of extrusion effect

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Keywords: legumes, functional snacks, extrusion

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Chickpea is currently considered gluten-free functional foods since legumes are a rich source of nutritional and healthy compounds such as protein, dietary fiber, starch, vitamins and minerals. They also contain phytochemicals such as α-galactosides, inositol phosphates and trypsin inhibitors, whose effect as bioactive compounds or as antinutrients factors depend on their concentration in the final food products, time of exposure, and their interaction with other dietary components [1]. Extrusion cooking is a high-temperature, short time, versatile and modern food process that may have beneficial effects on the nutritional value of legumes improving protein digestibility and decreasing the negative effects of some antinutritional factors. In recent years, the food industry has focused their attention on the development of gluten-free food products [2]. In this sense, the use of extruded flours based on legumes and gluten-free cereals is a good alternative for the development of new snacks suitable for the celiac population. Moreover, the incorporation of legumes in these ready-to-eat products may contribute to increase its consumption, mainly in children and teenagers [1, 2].

The aim of the present study was to evaluate the extrusion process effect on α-galactosides, inositol phosphates and trypsin inhibitors content in 10 different formulations based on rice and chickpea flours (70:30) and enriched with different percentages (0-20%) of passion fruit (a total of 20 samples).

The concentration of α-galactosides (raffinose, stachyose and verbascose) was determined using HPLC equipped with a refractive index detector [1]. Individual inositol phosphates (IP4, IP5 and IP6) were identified and quantified by ion exchange HPLC [1]. Trypsin inhibitor activity (TIA) was measured using a small-scale quantitative assay [3]. Results of TIA were expressed as units of trypsin inhibitor (TIU) per mg sample.

In raw formulations, total α-galactosides content ranged from 5.55 to 14.71 mg/g. These values are lower than those reported by other authors in lentil flour formulations [1]. Generally, raffinose was the main α-galactoside, followed by stachyose, while verbascose was no detected in the analyzed samples. Regarding extrusion effect, the total content of α-galactosides was higher in extruded samples comparing with the corresponding raw samples. Extrusion process may cause modifications in cell membranes increasing the extractability of oligosaccharides [1]. This positive extrusion effect increase α-galactosides that are considered as prebiotic compounds, with several health benefits, such as cholesterol and glucose blood levels control, as well as a protective effect against colon cancer [1].

Total inositol phosphates content ranged from 2.03 to 2.97 and from 1.68 to 3.52 mg/g, in raw and extruded samples, respectively. IP6 was the prevalent isoform in all analyzed formulations, followed by IP5 and IP4. This tendency was previously reported by other authors [1, 4]. Inositol phosphates are considered as antinutrients compounds, reducing the bioavailability of some minerals [4]. After extrusion process, a reduction in total inositol phosphates content was observed, improving the nutritional value of analyzed formulations.

Trypsin inhibitors are protease inhibitors found in raw legume seeds. These compounds have been indicated to hinder the protein digestion considering antinutrient factors. Raw analyzed formulations contain between 2.47 and 6.21 TIU/mg, while in extruded samples this content ranged from 0.22 to 0.33 TIU/mg. The decrease of these compounds improves the nutritional quality of the analyzed formulations and is in accordance with other authors [1, 4].

The results of the present study showed that extrusion processing is a valuable technological treatment for enhancing the nutritional value of cereal-legume based formulations. Extrusion cooking increases the content of oligosaccharides, which have health benefits due to their prebiotic effect, and reduce the amounts of inositol phosphates and trypsin inhibitors, which are considered as antinutritional factors. Therefore, the analyzed extruded snack-type products formulated with rice and chickpea could be a healthy gluten-free alternative to potentially increase legumes consumption.

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Tempe-type fermentation as a useful tool in decreasing potentially toxic compounds of faba beans

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Keywords: *Faba bean*, vicine, L-dopa

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The faba bean belongs to *Fabaceae* family and similarly to other representatives from this group is a good source of protein but also carbohydrates, vitamins from the group B and minerals. Considering its great potential as an ingredient for food products or for feed one may ask why this plant is still underutilized. In 2017 the production of faba beans accounted only for 1.4% compared to total production of the soybean [1].

Legumes despite their great nutritional value may contain significant amounts of antinutritional factors such as condensed tannins, protease inhibitors, lectins which limit their use. Faba beans apart from aforementioned compounds contain also pyrimidine glycosides *e. i.* vicine and convicine. These alkaloids are the causative agents of favism, a genetic disease which manifests in haemolytic anaemia. Moreover, these compounds were recognized as factors reducing productivity and viability in broiler chickens and laying hens [2].

The other compound contained in faba beans is L-3,4-dihydroxyphenyloalanine (L-DOPA). This compound is used in the therapy of Parkinson's disease. However, its use can cause many side effects such as serious hallucinations and dyskinesias in addition to gastrointestinal disturbances like nausea and vomiting. Thus it can be seen as antinutritional factor when ingested by healthy individuals.

Different processing methods like boiling and roasting were examined in the context of reducing the pyrimidine glycosides but the decrease was not satisfactory. Only L-DOPA was completely degraded [3]. An effective method is fermentation by *Lactobacillus plantarum* which reduced markedly the vicine and convicine content (by 91%) [4].

The aim of the study was to evaluate the influence of tempe-type fermentation on vicine, convicine and L-DOPA content in faba beans. Tempe fermentation is conducted by food grade fungi *Rhizopus oligosporus* and is widely used to process different beans in the south-eastern Asia. It was demonstrated that this kind of bioprocessing method improves the nutritional value of the tempe products.

In this study fermentation using three different strains of *Rhizopus oligosporus* namely NNRL 2549, NNRL 2710 and NNRL 6495 were used to ferment powdered dry faba beans *var.* Bonus. The biomass was mixed with water in the ratio 1:2.5 in the Erlenmeyer flasks and autoclaved. The fermentation in each case lasted 6 days and the samples were taken every day. Subsequently, the samples were frozen, lyophilized and stored in 4 °C. The qualitative and quantitative analyses of the compounds of interest were carried out using high performance chromatography equipped in diode array detector.

The results show that all strains of *R. oligosporus* caused the decrease of the vicine, convicine and L-DOPA. The highest vicine decrease was achieved by strain NNRL 6495 (by 86%) while in the case of convicine it was NNRL 2549 and the compound was not detected at all. Also, each strain led to L-DOPA reduction to the level below detection. Strain NNRL 2549 eliminated L-DOPA from the biomass in the 2nd day of fermentation while strain NNRL 2710 in the 5th.

This research describes for the first time the possibility of decreasing the content of vicine, convicine and L-DOPA by using the bioconversion of faba beans by different strains of *R. oligosporus*. The eliminating effect of pyrimidine glycosides can be explained by β-glucosidase activity of the fungi. While the results concerning L-DOPA are in the opposite than those reported so far. Randhir *et al.* (2004) noted the increase of L-DOPA level by 46% in the 6th day of fermentation in the faba bean by an unspecified strain of *R. oligosporus* [5]. The mechanism of reducing the L-DOPA by *R. oligosporus* require further investigation.

The fermentation by *R. oligosporus* was proved to be a useful tool in decreasing the content of potentially toxic compounds in the faba bean. The selection of the appropriate strain and faba bean variety having the lowest content of vicine, convicine and L-DOPA can shorten the fermentation time to assure effectiveness of the process and allow obtaining the product with the desired quality.

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Nutritional characteristics of marketable grain legume seeds (Fabaceae)

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Keywords: grain legume seeds, multi-mineral fingerprint, phenolic profiles

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Grain legumes belonging to the Fabaceae (Leguminosae) family are of prime importance for human and animal nutrition. Members of legume family play a distinct role in agricultural ecosystems with their capability of fixing nitrogen from the air, through association with specialized soil bacteria [1]. Food legumes are typically consumed as dry seeds, which have the high protein content, and in some cases as immature seeds or pods [2]. Besides the nutritional value, legumes also contain non-nutrients or bioactive compounds, such as inhibitors of proteases and amylases, lectins, saponins, phytic acid and phenolic compounds [3]. Within this study nutritional characteristics of marketable grain legume seeds of different species were studied. A total of 20 legume samples belong to grain legumes of common bean (*Phaseolus vulgaris* L.) as dry bean and snap bean; scarlet runner bean (*Phaseolus coccineus* L.); white lupin (*Lupinus albus* L.); narrow-leafed or blue lupin (*Lupinus angustifolius* L.), yellow lupin (*Lupinus luteus* L.); faba bean (*Vicia faba* L.); lentil (*Lens culinaris* L.); chickpea (*Cirer arietinum* L.); field pea (*Pisum sativum* L.); and soybean (*Glycine max* (L.) Merrill). Legumes were grown at the experimental fields of the Infrastructure Centre Jablje, Agricultural Institute of Slovenia, Slovenia (304 m above sea level; 46.151°N 14.562°E). The dried samples were homogenised using a ball mill (Retsch MM 400). Moisture was determined by heating at 103°C for 4 h (EC 152/2009 App. III A); proteins were analysed by method ISO 5983:2, using factor 6,25; and fats with petroleum ether extraction (152/2009 App. III H). Phytic acid was determined by indirect spectrophotometric determination of phytic phosphorus. For multi-mineral analysis, ICP-MS (Agilent 7900) was used and for determination of phenolic profiles HPLC-MS/MS (Agilent 6400 Series Triple Quad LC/MS). The data of the following nutritional characteristics were obtained in different grain legume samples: moisture (7.8 – 20.2 % dry weight, DW), proteins (14.05 – 43.05 % DW), fats (0.63 – 18.52 % DW), phytic acid content (113 – 2566 mg/100 g DW), phenolic profiles (a total of 27 phenolic compounds belonging to phenolic acids and flavonoids; see *Figure 1*) and multi-mineral fingerprint (a total of 24 minerals belonging to macro-, micro- and trace minerals). The results showed significant differences between analysed grain legume species and/or among different cultivars for investigated nutritional characteristics.

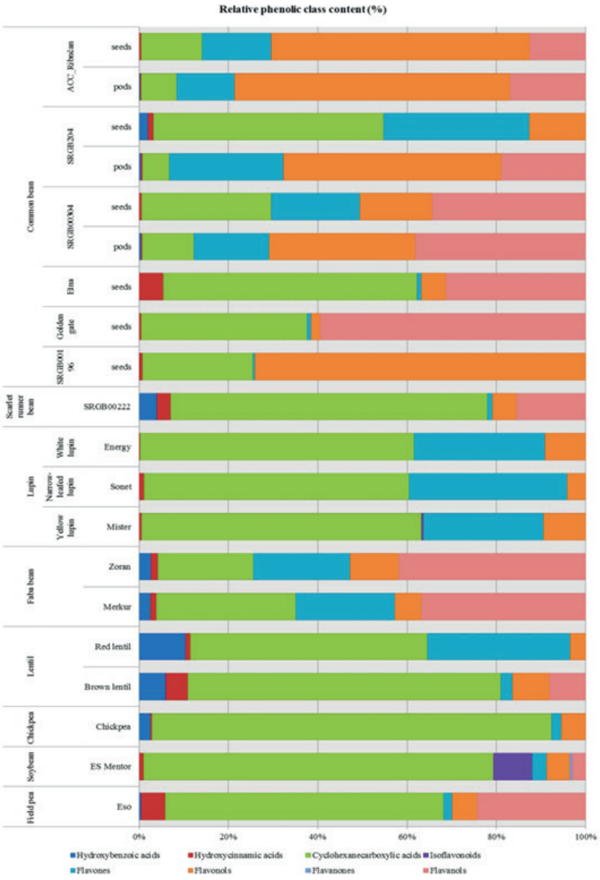

Figure 1. Principal qualitative profiles of the phenolic classes in grain legume seeds/pods

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The polyamine spermidine as a health component in soy-foods: from soybean breeding to food production

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Soybean is a major protein crop with a seed protein content of over 40%, which makes it a highly desired source of protein in human nutrition and livestock feeding. Although more than 95% of the world soybean production is utilized in feeding, the production of various soy-foods and soybean-based food-ingredients is gaining in importance. Moreover, the consumption of soy-foods has been recognized to be associated with a number of positive health effects mediated by particular proteins, lipids and minor constituents such as saponins and isoflavones (phytoestrogens).

A recent accumulation of evidence in cell biology and metabolomics is revealing significant anti-aging and other health effects associated with the polyamine spermidine [1]. Spermidine is a strong inducer of cellular autophagy, which mediates cardioprotective, neuroprotective and anti-inflammatory effects. As spermidine is a natural phytochemical, it is considered a high-value candidate for anti-aging interventions.

Soybean is one of the richest sources of spermidine among all food raw materials [2]. Thus, spermidine concentration of soybean could partly explain the long-term health benefits and longevity associated with soy-food consumption in human populations.

As spermidine content might become increasingly relevant in food-grade soybeans in the future, a set of early maturity genotypes was grown in replicated field experiments near Vienna, Austria for three seasons, and spermidine and other polyamine concentrations were determined from seed samples using ultra-high performance liquid chromatography (UHPLC) [3].

The results confirm earlier reports on soybean polyamine levels: Spermidine concentration was in the range from 167-291 mg/kg dry seed (Figure 1), whereas putrescine and cadaverine were between 3 and 29, and spermine between 31 and 179 mg/kg, respectively. Statistically significant genetic as well as environmental variation was found for all polyamines analyzed. Concentrations of putrescine, spermidine and spermine were highly correlated to each other, which is due to their common biosynthesis pathways. In contrast, seed protein content was not related to spermidine. Estimates of heritability for spermidine concentration were medium to high and comparable to other seed quality traits suggesting that spermidine can be selected for in food-grade soybean breeding.

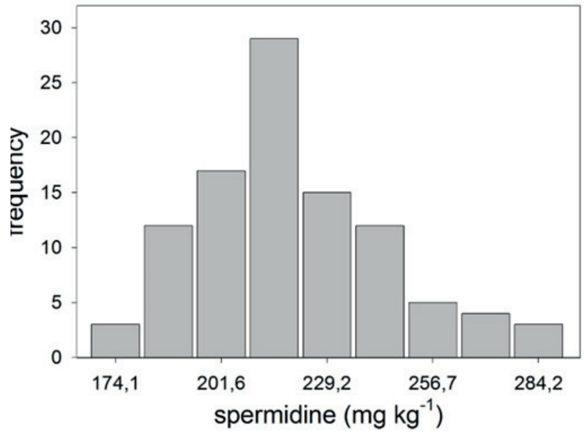

Figure 1. Spermidine concentration in soybean samples (n=100, mean=220.5, dry matter basis)

Natto, a fermented soy-food is the richest known source of spermidine, whereas tofu, protein isolates or soy-drinks are considerably lower in spermidine concentration [2]. Apart from genetic differences between small-seeded natto-style soybeans and other genotypes, differences in spermidine might also be due to food processing procedures. Preliminary results from seed sectioning and fractioning experiments suggest a higher concentration of spermidine in the germ than in the cotyledon section of soybean seeds. Thus, separation of germs and cotyledons during food processing might explain the variation in spermidine content between different soy-food products.

The present results suggest that both plant breeding and food processing are bearing potentials to regulate spermidine content of soy-foods. Thus, an additional health benefit from soy-foods or soybean-based functional food supplements can be generated in the context of their spermidine content.

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POSTER
SESSION

5.21

Effect of variety and crude protein content on the air-classification of field peas

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Keywords: peas, air-classification, fractions

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Pulses are rich sources of complex carbohydrates, protein, vitamins and minerals and are generally consumed after cooking both in whole seeds and decorticated splits. Recently, interest has grown in separating pulses into protein and starch fractions using wet (isoelectric precipitation) or dry (air-classification) fractionation process. A study was undertaken to investigate the effect of variety and crude protein content on characteristics of air-classified fractions from field peas. A laboratory air classifier was used to separate pea flour into protein-rich and starch-rich fractions.

Four pea varieties, each with two levels of protein content, were selected for this study. Crude protein content overall ranged from 18.3 to 27.9 g/100 g dry matter (DM). Composition of air-classified pea fractions were analyzed according to published methods [1], [2].

Analysis of variance showed that variety and crude protein content in pea flour had a significant effect on protein and starch content in starch-rich fraction, and starch content in protein-rich fraction. Yields for both starch-rich and protein-rich fraction were significantly affected by variety. Protein content in pea flour was positively correlated with protein contents in both starch-rich ($r=0.98$, $P<0.001$) and protein-rich fraction ($r=0.98$, $P<0.001$), but negatively correlated with starch contents in both starch-rich fraction ($r=-0.88$, $p<0.001$) and protein-rich fraction ($r=-0.84$, $P<0.001$). Protein content in pea flour was also positively related with ash content ($r=0.81$, $P<0.001$) and phytic acid content ($r=0.77$, $P<0.001$) in the starch-rich fraction, but negatively related with total dietary fiber ($r=-0.91$, $P<0.001$) in the protein-rich fraction. The fractions will have potential for use in food manufacturing as ingredients.

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The effect of different methods of processing lentil seeds on their nutritional value

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Keywords: lentils, processing, nutritional value

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The intensification of controversial decisions regarding the use of GMO soybean products in animal nutrition caused a rapid increase in the cultivation of leguminous plants, including lentils. *Lens culinaris* L. has the ability to bind atmospheric nitrogen due to the presence of papillary bacteria in the growths on the roots of the plant so do not require intensive nitrogen fertilization, which provide great economic importance. It also has a well-developed root mass, which promotes the growth of organic substances and improves physical structure of the soil. Lentil is a valuable food and feeds material because of high protein content. The factor limiting usage of seeds in the feed industry is the low content of sulfur amino acids and the presence of numerous anti-nutritive substances. Lentils also play an increasingly important role in human nutrition as a results of greater health care and consumer awareness, especially in developed countries; as well as culinary preferences and promotion of a new lifestyle (vegetarianism and veganism) [1, 2].

The aim of this study was to investigate the chemical composition of 4 different species of lentil seeds (*Lens culinaris*) and the effect of various processing methods as microwaving, boiling, soaking and autoclaving on the chemical composition of processed seeds.

Black, brown, red and yellow lentil seeds (Bioplanet) were subjected to the process. Tree portions (100g) of each seed were prepared separately for boiling (1), autoclaving (2), microwaving (3) and soaking (4). First group of seeds were soaked in warm water for 4 hours. After this time, each seed sample was drained and boiled in water for 40 minutes; (2) seeds were covered with 250 ml of distilled water and placed in 500 ml bottles. The bottles were placed in an autoclave at 121°C for 20 minutes; (3) seeds were placed in glass dishes and 500 ml of warm distilled water was added. Then the seeds were boiled in the microwave for 15 minutes; (4) seeds were placed in glass dishes and 500 ml of warm distilled water was added. The seeds were left in water for 24 hours. All the final products and raw seeds were dried and ground. Chemical composition: dry matter, crude ash, total and digestible protein as well as ADF, NDF and phytic phosphorus, were analyzed. Analysis results were statistically analyzed using univariate analysis of variance, and significance of differences at $P < 0.05$ was tested by Duncan`s test.

It was found that black and red lentil seeds contain more protein than green and brown varieties. However, the highest starch content was found in the seeds of red and brown lentils compared to green and black. In the seeds of green and red, a higher proportion of phytic phosphorus was found compared to other varieties. The levels of ADF and NDF varied among varieties.

The various methods of processing had different effects on the chemical composition of the tested lentil seeds. In the treated seeds generally an increase ($P < 0.05$) of the protein and starch content and reduction of the phytic phosphorus ($P < 0.05$) content were observed. Soaking of seeds improved the digestibility of protein and contributed to a reduction in the ADF and NDF content in the seeds of red lentils. All treatments caused the increase of the NDF content in the black lentil seeds. In the brown lentil seeds, the level of NDF increased after boiling and autoclaving.

From the nutritional point of view, the most beneficial effect on the seeds composition of studied cultivars had soaking and microwaving [3].

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SuBFer – plant product rich in well available iron

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Keywords: lupine, sprouts, ferritin

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Iron deficiency (ID) is a common malnutrition and induces 50% of anaemia cases, affecting more than 2 billion people worldwide [1]. ID strongly influences human organism, especially children. Properly balanced human diet is a good source of iron. However, ID usually is noted as a consequence of malabsorptive disorders, application of restriction diets or blood loses.

Interesting source of iron in human diet are legume seeds, containing an increased amount of ferritin. Ferritin is a protein responsible for detoxication and storing of iron. The protein is unique because it accumulates a huge amount of iron (up to 4500 atoms of iron) and is absorbed in human intestine by another mechanism than heme or ionic iron [2].

Ferritin is intensively expressed by lupine seeds in time of sprouting in stress conditions (i.e. excess of iron in the growing medium) [2].

The aim of the studies was to prepare supplement rich in iron absorbed by lupine sprouts and analyse the nutritional value of the obtained product.

Lupine sprouts were obtained from the *L. angiustifolius* seeds, Jowisz variety. On the 3rd day of sprouting, watering the plants with 25 mM $FeSO_4$ started. After 7 days the sprouts (Fig. 1A) were homogenized with water. The obtained homogenate was subjected to the tyndallization process to ensure adequate microbiological quality. After drying, resulting powder was directly encapsulated in hard capsules (Fig. 1B) or subjected to a tabletting process (Fig. 1C). Composition of main nutrients were analyzed.

The total iron content in the powder was ~9012 mg/100 g of the powder. As a consequence produced capsules and tablets contained 4.7 mg of iron per 1 dose; thus, consumption of 3 tablets or capsules was enough to provide the daily requirement for iron. The content of total protein in the powder was high - 29.4%. The powder was a valuable source of indispensable amino acid except of sulfur amino acid, which were reduced compare to sulfur amino acid in lupine seeds. Amino acid score for these amino acid was only 22%. The content of lipids was on the level of 4.7% and the composition of the lipids was quite good. 66% of unsaturated amino acids were present in those lipids; however, a lot of linoleic (precursor omega 6) was noted and as a consequence the ratio of omega 3 : omega 6 was strongly not desired. In the 100 g preparation, 62.5 mg of sterols were determined. Interestingly, a high amount of cycloartenol and a small amount of squalene alsowere noted. The applied process of sprouting strongly reduced the vitamin content, both vitamins soluble in water as well as lipids. Moreover, the sprouting under oxidative stress conditions influenced the accumulation of antioxidants. Total antioxidant content increased 5-fold compare to dry seeds.

It may be concluded that the obtained iron supplement is an interesting, natural source of iron. It does not contain such a huge amount of iron as usually found in supplements containing synthetic iron salts but the content of iron is higher than in natural supplements, much higher than in spirulina. It is also a good source if antioxidants and some amino acids.

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Figure 1. Lupine sprouts (A) enriched in ferritin iron and produced from them (B) capsules (C) tablets

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Early nodule detection by staining with 2,3,5-triphenyltetrazolium chloride

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Keywords: pea, nodule primordium, coloration method

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In a context of increasing protein demand and limitation of nitrogen fertilizers and pesticides use, legumes utlization either in sole crop or in intercrops is seen as a means to move towards productive systems more sustainable and resilient to climatic and economic hazards [1]. Ecosystems services provided by the legumes in such cropping systems highly depend on biological nitrogen fixation (BNF), which can be difficult to assess with isotopic methods [2, 3], especially in the early growth or in the farm fields. The objective of the present work was to develop a simple method to identify, count and measure nodule structures at different stages among a pea root system. We used 2,3,5-triphenyltetrazolium chloride (TTC), which is reduced by the activity of the mitochondrial respiratory chain and leads to the red-colored insoluble triphenylformazan. TTC is commonly used to measure tissue viality [4].

Pea plants (*Pisum sativum* L. 'Frisson') were grown in a culture chamber. The roots were harvested at different stages up to 14 days after germination. After sampling and washing with distilled water, the roots were stained in a medium containing a phosphate buffer solution (pH 7.5), a TWEEN 20 solution and a TTC solution (concentration 0.005% to 0.6%) [5]. They were protected from light and incubated at 30°C during either 20h or 25h. Image acquisition was performed with a high-definition scanner (EPSON Perfection V700, Regent Instruments Inc.) on a blue background to increase the contrast with the TTC stained roots. The scanner was calibrated using directly TTC stained fragments of fresh roots and dehydrated roots (white) as control.

A slight pink coloration of the roots was observed for low TTC concentrations (from 0.005% to 0.01%). From 0.1% TTC concentration, the roots remained white (or very slight pink) except a dark red color located at the root apex. The nodules were also stained dark red for TTC concentrations greater than 0.1% (Figure 1B), and could be detected at the early stages of their formation (Figure 1A).

Among the limitations of the TTC staining method, reported in literature, microorganisms can alter final staining by their ability to reduce TTC to triphenylformazan [5]. Thus, the coloring of pea roots with TTC concentrations greater than 0.1% has given us an unexpected result by strongly colouring nodules contents, where *Rhizobium* is present. Phytotoxic effect of TTC at high concentrations were also reported [5] and may explain the absence of coloration on the rest of the pea roots, whose color did not change after staining with TTC.

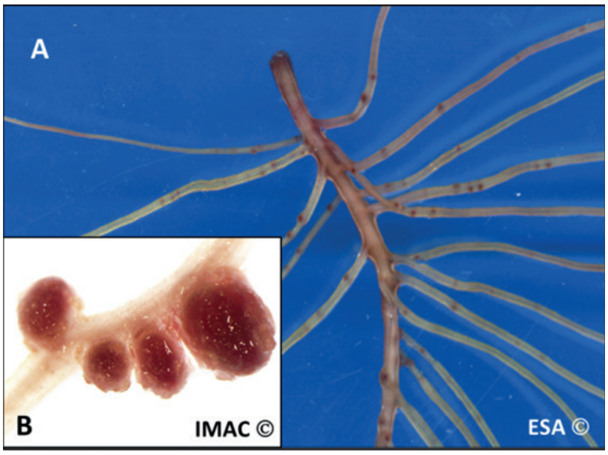

Figure 1. A. Scanned image sections of a two weeks old pea showing primary root (white/slight pink) and nodules primordia (red spots). B. TTC coloured nodules (× 1.6)

The technique combining TTC staining and image analysis can be successfully used to early identify nodule primordia and/or quickly quantify the number of nodules on a root system. Thanks to the specific coloration of the nodules, using WinRHIZO™ can help counting them and assess their volume.

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Uncovering seed yield traits in *Trifolium pratense*

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Keywords: seed yield, flower morphology, meiotic abnormalities, *Trifolium pratense*

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Introduction: Red clover (*Trifolium pratense* L.) is a valuable forage crop, but its seed yield is often unsatisfactory, especially in tetraploid varieties. Aiming to unravel the factors underlying seed yield in red clover, we investigated the relation between seed yield per plant, plant architecture, flower morphology, pollinator preference and abberations in the reproductive organs.

Materials and Methods: In this study, 600 genotypes from 15 diploid and 15 tetraploid cultivars, representative for European breeding efforts, were used. Multiple linear regression was used to reveal relations between seed number per plant, number of ripe and unripe flower heads per plant, seed number per ripe flower head, flower number per head, corolla tube length and diameter, and flower anthocyanin content (flower colour) (Vleugels *et al.* 2016). The relation between seed yield vs. nectar production and composition, determined through HPLC, was studied among a subset of 54 genotypes from our first study with known differences in seed yield. In addition, pollinator preference was studied on 6 diploid and 6 tetraploid genotypes with contrasting seed yields, by evaluating pairs of genotypes for attractiveness to *Bombus pascuorum* bumblebees: the main pollinator of red clover in our region (Vanommeslaeghe *et al.* 2018). Ovule development, the frequency of aberrations in male meiosis, and pollen quality were investigated among 19 diploid and 17 tetraploid genotypes with extreme seed yields, or subsets thereof (Vleugels *et al.* 2018).

Results: Seed number per plant was well predicted by the number of flower heads per plant and the seed number per flower head. Tetraploid genotypes produced fewer flower heads and fewer seeds per flower head than diploids. Seed yield was not correlated to floral characteristics such as flower number per head, flower colour, corolla tube dimensions, nectar volume or nectar composition. Furthermore, no preference for diploids or tetraploids could be demonstrated in choice experiments with *B. pascuorum*. High degrees of ovule abortion occurred in genotypes from either ploidy level, but no clear link with seed yield appeared. However, tetraploid genotypes displayed more meiotic aberrations than diploids, and lower pollen germination.

Conclusion: Seed yield in tetraploid red clover is lower, predominantly because fewer seeds are produced per flower head, which is not explained by differences in flower morphology or pollinator attractiveness. Instead, although further research is needed to fully unravel the mechanisms underlying the lower fertility in tetraploids, meiotic aberrations, ovule abortion and pollen quality likely are part of the complex process of seed yield in red clover.

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Transcriptome analysis reveals the diversity and importance of hormonal signaling network in developing pods and seeds of *Lupinus luteus*

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Keywords: phytohormones, seed development, transcriptome

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Biological processes that are the most important form agricultural point of view, as well as survival of a plant species are flowering and seed development. As a result, these stages of plant life cycle are complex and strictly controlled by different signaling pathways, often associated with hormonal homeostasis. Yellow lupine (*Lupinus luteus* L.) among other widely cultivated legume plants such as soybean (*Glycine max*) or chickpea (*Cicer arietinum*) holds a great practical and agricultural importance [1]. However, unlike soybean its regulatory pathways still remain relatively undiscovered. Unfavourable growth conditions may result in excessive generative organ shedding or decline in seed quality, which is why the study of lupine's regulatory pathways and differentially expressed genes (DEG) over the course of pod development is an important task.

Abscisic acid (ABA), among other phytohormones such as ethylene, indole-3-acetic acid (IAA), cytokinins, brassinosteroids and many others is responsible for regulation of almost every aspect of plant growth and development, including pod and seed formation. Abscisic acid is recognized as a major regulator of plant growth and development [2], implicated in processes of growth and senescence, as well as drought stress response. Studies show that level of this hormone is highly varied in both spatial and temporal manner, with soybean seeds exhibiting much higher level of ABA than pod walls. The ABA level in seeds also correlates with the changes of growth rate across whole developmental period [3]. The transcription factors as well as key proteins responsible for hormone signaling for both ABA and ethylene, such as ABI4 and ABI5 have been identified in both pods and seeds, along with other transcription factors such as WRI1 and ABR1, which could suggest a cross-talk between ABA and ethylene in seed development [4].

For the purpose of this study, yellow lupine's fruit development was separated into 8 stages based on post-anhensis time and pod size. The pods have been collected from field-grown plants, separated into seeds and pod walls (containing pedicels) for each stage of development, and then pooled into 3 arbitrary groups for RNA sequencing purposes. The obtained transcriptome data allowed us to assess the expression levels of various genes that may be potentially involved in phytohormone signaling and homeostasis in developing pods of yellow lupine.

The results of our preliminary DEG analyses revealed that genes such as Ethylene-responsive transcription factors (ERF), proteins regulated by ABA and GA (Gibberelic acid), and enzymes participating in synthesis of Ethylene and Jasmonic acid (JA) and many other were highly upregulated in certain developmental stages. This strongly suggests that ABA, ethylene, and jasmonic acid, as well Gibberelins play a major role in yellow lupine's pod development and precise control over their metabolism and perception is required.

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Impact of bacterial vaccine on the yield and quality of soybean seeds (*Glycine max* (L.) Merrill)

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Keywords: inoculation, *Bradyrhizobium japonicum*, soybean

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Soybean (*Glycine max* (L.) Merrill) represents the legume family (*Fabaceae* Lindl.,). As a result of symbiosis with nodule bacteria (*Bradyrhizobium japonicum*) soy plants can fix atmospheric nitrogen. Notable advantages of soy as a crop include the versatile usage of the seeds. It should however be remembered that soybeans contain antinutrients.

The importance of soy in Poland has increased and new high-yield varieties have been introduced in agricultural practice. Many authors [1], [2], [3], [4] point out that vaccination of seeds with nodule bacteria, performed before planting, is of particular importance in soy cultivation. The treatment is highly consequential as it potentially results in better yield and reduced need for mineral fertilisation with nitrogen. The purpose of the study was to determine response of soy plants to selected bacterial vaccines, compared to controls.

A structured field experiment was carried out in 2017 and 2018. It was held in an individual farm located in the village of Makowisko (Podkarpackie Region, Poland). Firstly, the experiment involved a use of bacterial vaccines designed for soybean seeds: HiStick**® Soy,** Nitragina from Puławy, Nitrazon and a control. Secondly, it applied the following cultivars: Annushka, Lajma, Madlen, Violetta, Mavka, Atlanta and Smuglyanka.

The use of bacterial vaccines was justified, by reference to the controls. Root nodulation was most beneficially affected by HiStick® Soy. In 2017 the lowest effect in terms of the number and dry mass of nodules was produced by Nitragina from Puławy and in 2018 by Nitrazon. On average during the years of the experiment, the yield of the seeds following the use of HiStick® Soy, Nitragina from Puławy, and Nitrazon was higher by 0.53 $t \cdot ha^{-1}$; 0.60 $t \cdot ha^{-1}$ and 0.66 $t \cdot ha^{-1}$ respectively, compared to the control. Vaccination of the seeds produced the most positive response in the plants of Annushka cultivar. The use of bacterial vaccines resulted in higher contents of total protein in the seeds. Inoculation of the seeds with HiStick**® Soy** increased the degree of lodging in the plants and prolonged the crop vegetation period, compared to the controls. Out of the cultivars examined, the highest yield was produced by Madlen and Violetta (on average during the years of the study, 3.70 $t \cdot ha^{-1}$). In the case of Atlanta and Smuglanka there was a problem with harvesting the seeds, due to the longest vegetation period.

In summary, the yield and quality of soybean seeds were affected by weather conditions and the two experimental factors investigated.

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The influence of soil management on the amount of biologically reduced (fixed) nitrogen estimated by isotope dilution method (^{15}N) by yellow and blue lupins and its uptake by winter wheat

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Keywords: soil management, biological nitrogen reduction, lupines, winter wheat

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In natural field conditions during 2012-2015 years three different type soil management: conventional tillage, reduced tillage and no-tillage[1] on the yield and amount of biologically reduced nitrogen by yellow and blue lupins and its uptake by winter wheat was determinated. The experiment with lupins were carried out in 2012, 2013 and 2014 but with winter wheat 2013, 2014 and 2015. These crops were cultivated in two rotations: yellow lupin – winter wheat and blue lupin – winter wheat. The amount of biologically reduced nitrogen was estimated by isotope dilution method with ^{15}N.

In rotaion yellow lupin – winter wheat , the mean yield of yellow lupin seeds reached 2.84 t·ha^{-1}. The impact of soil management was not significant however the highest yield of seeds (2.94 t·ha^{-1}) was obtained on the reduced tillage system, similar to the residue 10.54 t·ha^{-1}. This state is very important because using this system of soil management it is possible to reduce the cost soil management. The mean value of total nitrogen in biomass of lupin was 245 kg·ha^{-1}. Seeds contained 160 kg·ha^{-1} of nitrogen, it is 1000 kg·ha^{-1} of crude protein and in lupin residue was 85.4 kg·ha^{-1} of total nitrogen which was ploughed. In biomass of yellow lupin %Ndfa was 58.8%, what equal to 144.5 kg·ha^{-1} from air, including 105.1 kg·ha^{-1} in seeds and 39.4 kg·ha^{-1} in residue. Yield winter wheat cultivated after yellow lupin as average from 2013-2015 reached 5.01 t·ha^{-1} of grain (the highest 5.43 t·ha^{-1}) on the reduced tillage system of soil management similar to yellow lupin and the lowest 4.32 t·ha^{-1} on no-tillage system. Yield of wheat residue reached 7.51 t·ha^{-1} and was very similar in all cases of soil management system. The value of nitrogen utilization coefficient from yellow lupin residue was differentiated by impact of soil management and reached 71,0% on the conventional and reduced tillage system and 59,3% on the no-tillage system of soil management (mean value 67,1%). In the rotation of blue lupin – winter wheat, the yield of lupin seeds (as a mean for three series) reached 2.90 t·ha^{-1} and 6.15 t·ha^{-1} of residue. It was no significant differences in seeds yield caused by the system of soil management, however the highest yield was got on the conventional system of soil management. The amount of nitrogen derived from air was 45.0%, what equal of 70.6 kg·ha^{-1} N per hectare including 59.2 kg·ha^{-1} in seeds and 11.4 kg·ha^{-1} in residue. The total nitrogen amount in blue lupin residue ploughed contained 43.0 kg·ha^{-1} including 11.4 kg·ha^{-1} Ndfa. It was no significant impact of soil management on the yield of winter wheat cultivated after blue lupin (mean value 1.57 t·ha^{-1} of grain and 6.97 t·ha^{-1} of residue). However the highest yield of grain was reached on the conventional system and the lowest on the no-tillage. The value of nitrogen utilization coefficient from blue lupin residue was not differentiated by the system of soil management and the mean value was 66,8%.

Generally, the kind of lupin did not significantly differentiated the yield of winter wheat.

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POSTER SESSION

7.6

Using phytohormone application to optimise nodule characteristics and increase nitrogen fixation

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An agricultural system so reliant on industrially fixed nitrogen (N) is fundamentally unsustainable due to the huge quantities of fossil fuels necessary for its production. There are both global environmental impacts from greenhouse gas emissions caused at each stage of N fertiliser processing, as well as local environmental consequences through nitrate leaching into watercourses. The unique ability of legumes, in a symbiosis with soil bacteria (rhizobia), to achieve biological nitrogen fixation (BNF) offers an opportunity to enhance agricultural sustainability by reducing reliance on industrially fixed N. However, BNF rates are highly variable and stress sensitive, especially to drought.

Legume nodulation is tightly regulated by plant-microbe phytohormone signalling. Exogenous phytohormone applications can increase nodulation and/or BNF, however studies do not always examine how the two are related. Although it is commonly assumed that increased nodule number and/or size will increase BNF and subsequently yield, optimum nodulation phenotypes are not known. However, stunted hyper-nodulating mutants indicate that plants can only support a certain number of nodules. Furthermore, the relationship between nodule characteristics and stress tolerance has received little attention.

This work hopes to 1) establish whether exogenous phytohormone treatments are effective in altering nodulation and 2) if these changes represent an improved nodule phenotype for both BNF and stress tolerance. The agronomic benefit of altered nodulation, in terms of yield, will also be determined.

Effect of bacterial inoculation and boron fertilization on the soybean Augusta cultivar's root parameters

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Keywords: soybean, root system parameters, bacterial inoculation

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In the world there is a continuous increase in interest in sustainable agriculture, which assumes an increase in the diversification of crop rotation, biodiversity and promotion of legumes [1]. As part of the policy of sustainable development, the importance of legumes is growing, as a result of which, in recent years, soy is becoming more and more important for the economy. In Europe, non-GMO soy is a valuable source of fodder protein, nitrogen and the source of organic matter in soil [2]. The popularity of soybean cultivation in Europe is still small and results mainly from the low plant yield potential. This results from inadequate knowledge about growing soy, compared to other legumes, including peas. An emerging trend in global agriculture is the adaptation of new soy cultivars to unfavourable habitat conditions. This is achieved through the development of the root system and the augmentation of the nodulation process, the effect of which would be to reduce the level of mineral fertilization with nitrogen. One of the agrotechnical factors enabling the growth of plant root system activity, especially in suboptimal conditions, is the use of bacterial vaccines associated with foliar fertilization, which has been successfully confirmed in the cultivation of pea [3].

The aim of the work was to assess the impact of integrated soybean inoculation, with commercially available bacterial or fungal inoculants, combined with boron foliar fertilization on the formation of root system parameters and soy root nodules.

The experiment was carried out in the Experimental Station of the University of Agriculture in Prusy, near to Krakow. The experiment was carried out on 1st Class Soil, a wheat soil complex. Three inoculants were compared in the experiment: two bacterial (Nitagine, Nitroflora) and a one fungal (Mykoflorin) depending on the boron fertilization. During seeding, seeds inoculation was carried out. The borate application was conducted twice: in the seedling and budding phases. At the flowering stage, the root system and roots' nodules were taken to determine their mass and selected biometric parameters.

On the basis of the statistical analysis we have shown that the combined application of bacterial inoculation of Nitagina or Nitroflora along with foliar fertilization with boron significantly increased some parameters of the soybean root system. Significantly higher root length density (RLD) 8.00 cm cm^{-3} was obtained in objects with the combination of boron and Nitagina and 7.67 cm cm^{-3} after application of boron and Nitroflora (Fig.1). In addition, significantly higher parameters for the root surface area density (RSAD) were found for the combination of Mykoflorin and boron, at the level of 1.13 cm^2 cm^{-3} and slightly lower for the combination of Nitroflor and boron, at the level of 0.96 cm^2 cm^{-3}. The combinations used had a negative effect on the mean root diameter (MRD). In the case of root nodule analysis, we showed that the associated application of bacterial vaccines with boron significantly increased the mass of nodules. We obtained the best results after the application of Nitragina and boron, which resulted in an increase in the mass of nodules to 0.480 g. In the case of the applied mycorrhizal inoculant, we obtained a lower mass, equal to 0.337 g. The least effective inoculant was Nitroflora.

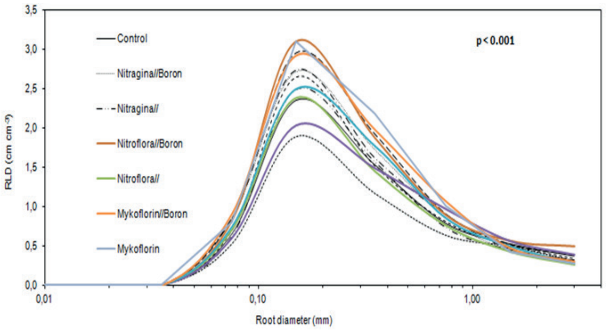

Figure 1. Characteristic on root length density classes dependin on bacterial inoculation and boron fertilization

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Flowers under construction. Transcriptome-wide exploration of genes involved in late flower development in yellow lupine (*Lupinus luteus* L.)

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Keywords: RNA-Seq, yellow lupine, flower

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Yellow lupine (*Lupinus luteus* L.) is a legume plant originating from Mediterranean and has a great potential to become the main source of plant protein in Europe [1]. It is especially important in the face of increasing demand for plant-derived protein and frequent occurrence of soy allergy. The high seed yield can be achieved only after successful flowering, anthesis and fertilization [2]. One of the first steps towards improving crop performance is understanding complex net of regulatory mechanism that orchestrates floral development.

For this purpose we have constructed RNA-Seq libraries using RNA extracted from yellow lupine flowers at four different stages of development. Flowers at the first stage are green buds with closed anthers and small, intensively growing gynoecium. Second stage comprises flowers where most anthers started to open, third one is a stage of full anthesis and flowers at the fourth stage are fertilized with slightly enlarged pistil.

Gene Ontology (GO) analysis of differentially expressed genes (DEGs) showed, that when flowers enter the 2nd stage (2vs1), expression of quite a large number of genes essential to cell walls and development significantly changes. When they reach the 3rd stage (3vs2) the number of DEGs associated with cell walls and hormones increases and DEGs involved in developmental processes become less numerous. After entering the 4th stage (4vs3), the number of genes engaged in development increases and of these associated with cell walls decreases (Figure 1).

Among phytohormones, auxin is best known integrator of plant development. Our results indicate, that it plays the biggest roles in flowers at 3rd stage of development, where genes encoding biosynthesis enzyme (YUCCA3) and auxin-responsive proteins (IAA11, AIR12) increase their transcriptional activity. When flower enters 4th stage, expression level of genes encoding auxin transport protein BIG decreases while transcripts of genes encoding GH3.5 and GH3.6 proteins inactivating IAA by conjugation are more accumulated. Regarding other hormones, in all library comparisons studied here there are DEGs associated with gibberellins and jasmonic acid.

These results indicate that for their proper development and functioning lupine flowers require dynamic changes in expression level of genes among others associated with hormones, cell walls, and development.

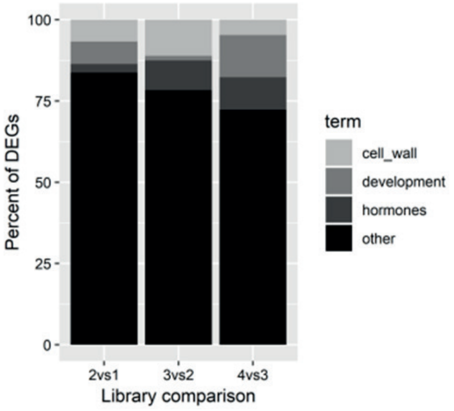

Figure 1. Percentage of DEGs categorized to GO terms concerning: cell walls, development, and hormones in comparisons of RNA-Seq libraries generated from yellow lupine flowers at different stages of development

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Characterization of plant growth promoting traits of rhizobia isolated from chickpea (*Cicer arietinum* L.) plants

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Keywords: legume, PGPB, phylogeny

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Chickpea (*Cicer arietinum* L.) currently ranks second in the world's production of food legume and it is considered a valued crop for a growing world population and its high nutricional demand.

On smart strategies that favors soil health sustainability, plant growth promoting bacteria (PGPB) are multifunctional agents that can be used as tools to maximize the crop productivity and alleviate biotic and abiotic stress. Rhizobacteria are able to form beneficial relationships with legumes through direct and indirect mechanisms that enhance the availability of essential nutrients, mineralize organic compounds and production of phytohormones.

One of the most abundant group is the bacterial order Rhizobiales that induce nodule organogenesis in legumes roots and are recognized for its ability to fix atmospheric nitrogen. However, rhizobia typically exhibit multiple functional traits such as solubilize inorganic phosphorous, produce siderophores and indoleacetic acid (IAA) that have beneficial interactions on the rhizobia-legume symbiosis that can enhance soil health management and plant growth.

This work featured a total of 29 isolates collected from chickpea root nodules, grown in Portugal under different edapho climatic conditions. All isolates were evaluated for their ability to induce root nodules.

Furthermore, the occurrence of four plant growth promoting mechanisms were evaluated. Phosphate solubilization activity was performed by plate assay using National Botanical Research Institute's phosphate growth medium (NBRIP) [1]. Siderophore production was estimated qualitatively by the Chrome Azurol S assay [2]. Indole acetic acid production was assessed by a colorimetric method described by Brígido and Glick [3] and bacterial hydrogen cyanide production was detected by a qualitative method [4].

Additionaly, the genetic identification of the isolates was performed through the amplification of the full-length 16S rDNA, *nodC* and SMc 00019 gene analysis. The aligned sequences were used in the phylogenetic analysis.

Considering the isolates ability to solubilize inorganic phosphorus, only four presented negative results, whilst the remain were able to solubilize tricalcium phosphate or/and aluminum phosphate.

From all the isolates and type strains tested, 16 isolates produced siderophores. Regarding IAA production, 20 isolates were considered positive, presenting values lower than 5 µg ml^{-1} of IAA.

All the isolates were negative for the hydrogen cyanide production.

The present study indicates that the majority of the isolates possess two or more plant growth promoting traits, being phosphate solubilization and IAA production the most common.

The phylogenetic analyis revealed that all strains belong to *Mesorhizobium* and *Burkholderia* genera.

This study revealed multiple biological mechanisms of chickpea microsymbionts with potencial as plant growth promoting bacteria, indicating that inoculants of the orders Rhizobiales and Burkholderiales can benefit sustainable agriculture production of chickpea.

Further research of the isolates is required under greenhouse and field conditions since its ultimate utility depends upon how effective they are in competing under diverse soil livig microbial communities.

Acknowledgments: This work was supported by portuguese national funds through Programa Operacional Competitividade e Internacionalização (POCI), Project 3599 – Promover a Produção Científica e Desenvolvimento Tecnológico e a Constituição de Redes Temáticas (3599-PPCDT) and Fundo Europeu de Desenvolvimento Regional (FEDER) under Project POCI-01-0145-FEDER-016801 and by FCT under Project PTDC/AGR-TEC/1140/2014". This work is supported by National Funds by FCT – Portuguese Foundation for Science and Technology, under the project UID/AGR/04033/2019. The first author also acknowledges the financial support provided by the FCT-Portuguese Foundation for Science and Technology PD/BD/128271/2017, under the Doctoral Programme "Agricultural Production Chains – from fork to farm" (PD/00122/2012).

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POSTER SESSION

7.10

Root growth and development do not directly relate to shoot morphogenetic strategies in temperate forage legumes

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Keywords: legumes, root growth, root branching, architecture, taproot, shoot:root ratio, allometry

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Little is yet known about the component traits that control the deployment of root architecture in most grassland species. The aim of this study was to compare patterns of root growth in contrasting forage legumes and to analyse their dependency to shoot development.

The parameters of a dynamic simulation model (Archisimple, [1]) were identified for six contrasting legume species (i.e. alfalfa, white clover, red clover, sainfoin, birdsfoot trefoil, kura clover) in a series of greenhouse experiments in order to characterize root elongation, root branching and taproot growth. The model allowed us to explore the interactions between root development and whole plant growth.

Most of the root morphogenetic parameters studied displayed significant inter-specific variability. Several traits appeared to be correlated, indicating a close association between extreme root tip diameters, the branching ability of roots and their maximal elongation rate. The species differed along two PCA axes which accounted respectively for the branching ability of fine roots and taproot development. Quite remarkably, the group of species identified for root development did not coincide with those previously identified for shoots [2], suggesting a degree of independence between the morphogenetic traits that control shoot and root architecture. However, both plant parts remained tightly linked through the trophic relationship represented by root biomass allocation.

Our results suggest that the concomitant analysis of root and shoot development is necessary to properly decipher rooting strategies in forage legumes [3].

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Tissue and cellular localization of jasmonic acid during late stamen development of yellow lupine

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Keywords: jasmonic acid, stamen, yellow lupine

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Yellow lupine (*Lupinus luteus* L.), as one of the Fabaceae family members, is economically important plant species. Among the many desirable traits, the most important is (1) the symbiosis with bacteria that allows the usage of atmospheric nitrogen for growth and development, and additionally enrichment the soil with this macroelement; (2) a specific type of storage substances, which are proteins accumulated in seeds. Yield and productivity of many crop species depend on successful reproductive development to produce fully valuable seeds for animal and human nutrition. Nevertheless, in yellow lupine the development of generative organs is associated with the undesirable phenomenon of excessive and premature flower abortion. It is assumed, that in our latitude, on the first whorl of the inflorescence falls up to 60%, on the second whorl almost 90%, and on the higher whorls all flowers. The consequences of this phenomenon are significant from an economic perspective, and the causes are not fully understood. Until now, several non-exclusive hypotheses describing the reasons of flower abortion have been proposed, among which the most significant are insufficient quantitative and qualitative pollination of flowers and varied activity of plant hormones, including jasmonates (JAs). Studies conducted mainly on *Arabidopsis thaliana* have shown that plants with deficiency of JAs displayed retarded filament elongation, delayed anther dehiscence, and reduced pollen viability that leads to male sterility. This proves that the JAs play a key role during late stamen development. Moreover, the level of JAs varies in each tissues and cells in different stages of stamen development [1]. Therefore, the main purpose of this study was to determine the immunolocalization of jasmonic acid (JA) at several late stages of anther and filament development in yellow lupine variety Taper.

The plant material were harvested and fixed in buffer containing paraformaldehyde, glutaraldehyde and EDAC. Then, the tissues were dehydrated in ethanol with dithiothreitol, supersaturated and embedded in BMM resin under UV light for polymerization. The sections were cut on an ultracut microtome and placed on glass slides covered with Biobond. The sections were incubated with the primary antibody (anti-JA) overnight, washed, and then a secondary antibody was applied. Negative control reaction required for validation of the immunohistochemical findings was performed by omitting the incubation with the primary antibody and showed no labelling. All the samples were observed under a fluorescence microscope.

The first selected stage was characterized by presence of four separate locules with pollen grains. In the subsequent phases, the successive processes of differentiation or degeneration of several tissues that led to dehiscence and finally senescence of stamen were observed. Results of our study shown that JA was located in both the anther and the filament during late stages of stamen development. The strong fluorescence signal indicating a large amount of JA was noted when anther contained tricellular pollen grains and became bilocular. Then, the increased signal was mainly observed in cells of gradually degrading and breaking septum. The signal was also noticeable in initially degenerating middle layer/tapetum cells. Furthermore, vascular bundle and adjacent cells were filled with hormone molecules. In other cells, the signal was definitely weaker or not recorded at all. The subsequent stamen developmental stages, such as breakage along stomium and pollen release (dehiscence) and shrinkage of cells and anther structure (senescence) showed relatively similar pattern or lack of JA signal.

Based on the results of these preliminary studies, it can be concluded that JA occur more frequently in cells undergoing degeneration. Additionally, the presence of JA in vascular bundle indicates intensive transport between the filament and the anther. There is no doubt that JA plays an important role in the late stamen development in yellow lupine. Further research, at the cytological and molecular levels, will allow us to fully understand the mechanism of stamen development in the studied plant, which may contribute to the improvement of the pollination and fertilization process, thus increasing the number of flowers on the plant and improving yield.

Acknowledgments: This work was supported by the Polish Ministry of Agriculture and Rural Development grant no 222/2015.

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POSTER SESSION

7.12

Contribution of traits related to plant architecture and pod type in yield improvement of field pea in Australia

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Keywords: semi-leafless, semi-dwarf, yield advantage, non-shattering pod

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Field pea is an important pulse crop for human consumption and stock feed. Field pea is a crucial component of crop rotation in cropping belt of southern Australia.

Field pea breeding in Australia shifted from a focus on conventional trailing types in the 1970's and 1980's to semi-leafless and semi-dwarf (SLSD) types through the 1990's and 2000's leading to the first widely grown SLSD variety, Kaspa, released in 2002. Traditionally, the conventional varieties were tall, fully-leaved and had shattering pods whereas more recent varieties are relatively short, semi-leafless and mostly have non-shattering pods.

A set of historical and current field pea varieties were grown in field experiments in the Victorian Mallee and the Wimmera regions to assess the contribution of the traits related to plant architecture and pod type to yield improvement. The traits related to plant architecture were leaf type (semi-leafless and fully-leaved) and internode length (short and long); and the traits related to the pod type were shattering and non-shattering. The experiments included 41 varieties released in 1970's to 2017. Trials were grown in 8 sites from 2012 to 2017 resulting in 11 site × year combinations. Normalized Difference Vegetative Index (NDVI) were recorded at selected sites to quantify early vigour. Seed yield was analysed using factor analytic model and predicted means were used to compare genotypes.

The average yields of the experimental sites ranged from 0.32 to 3.65 t/ha. Conventional varieties, on average, had significantly higher NDVI than SLSD varieties at Horsham in 2016. Yield advantage of SLSD over conventional varieties ranged from 10.6% yield penalty in low yielding environments to 15.7% yield advantage in higher yielding environments. Varieties with non-shattering pods yielded higher than those with shattering pods in all the environments. Varieties with non-shattering pods had yield advantage of 2.0 to 21.7% over those with shattering pods in all the environments. Poor performance of SLSD varieties in low rainfall environments might be associated with their inferior vigour as indicated by NDVI when compared to the conventional varieties.

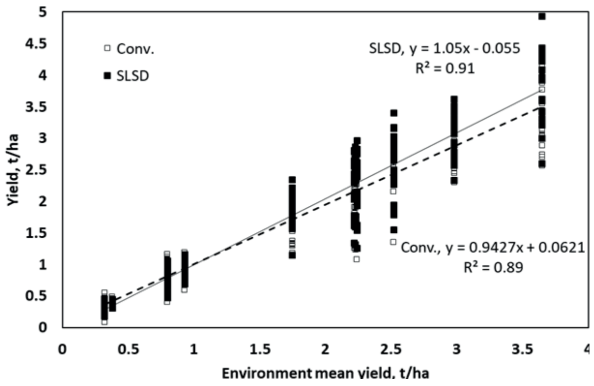

Figure 1. Performance of conventional (Conv.) and semi-leafless semi-dwarf (SLSD) varieties in a range of environments in field

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Late stamen development of *Lupinus luteus* involves both cell differentiation and degeneration processes

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Keywords: stamen, structure, *Lupinus luteus*

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Proper development of male reproductive organs is essential for plants to achieve their life cycles. Stamen, which is composed of a filament and an anther, is subject to a specific developmental process, and any abnormalities lead to male sterility. In model plant *Arabidopsis thaliana* 14 stages of stamen development were distinguished [1]. During early stages 1 to 4, cell division events occur within the primordia to establish a bilateral structure with locule, connective, and vascular regions characteristic of the mature anther. At stage 5, archesporial cells within the anther primordia divide periclinally to give rise to distinct parietal and sporogenous cell lineages that differentiate into the endothecium, middle layer, tapetum and microspore mother cells of the locules. Microspore mother cells undergo meiosis between stages 5 and 7 within each of the four locules and generate tetrads of haploid microspores. Microspores are released from the tetrads at stage 8 and differentiate into three-celled pollen grains between stages 9 and 12 [1]. At stage 13, filaments elongate rapidly, and anthers dehisce to release viable pollens onto the stigma for pollination and subsequent fertilization [2]. It is assumed that late stamen development comprises the phases that occur after the opening of the flower; however, in many plant species including members of Fabaceae family, there is phenomenon called cleistogamy, in which the dehiscence occurs before the flower opening, leading to self-pollination. Due to the fact that the stamen development may differ in various species and the structure of yellow lupine (*Lupinus luteus* L.) stamens has not been investigated until now, this has become the main purpose of this study. In order to describe the changes that occurred at the tissue and cellular level, the anthers and the filaments of yellow lupine var. Taper in several late developmental stages were collected.

The plant material was fixed in a mixture of paraformaldehyde and glutaraldehyde in phosphate-buffered saline buffer solution with the addition of EDAC, and then dehydrated using increasing concentration of ethanol with ditiotreitol, supersaturated and embedded in BMM resin. Thus prepared stamens were cut into sections using ultramicrotome and placed on slides. Then they were stained with methylene blue and observed in the light microscope.

The first collected phase of late stamen development in yellow lupine corresponded to the 11th phase described in *A. thaliana*. Anther had four separate locules and each of them contained microspores differentiated into three-celled pollen grains. Coordinated with pollen development was a general increase in anther size and differentiation or degeneration of particular cell layers. We observed expansion of the endothecium layer and secondary wall thickenings in endothecial and connective cells. The beginning of middle layer/tapetum degradation was noticed, but significantly less than in *A. thaliana*. Differentiation of the stomium was in progress. The breakage of the septum, taking place between this and the next selected phase, generated a bilocular anther. This corresponded to the 12th phase determined in *A. thaliana*. At this point in yellow lupine, degradation processes were dominated. The progressive degradation of the third layer of cells surrounding the locules, as well as rapid and intense degradation of septum cells has been observed. All these visible changes in specific anther cell types preceded the release of pollen grains during dehiscence. In this phase, which corresponded to the 13th phase of *A. thaliana*'s stamen development, breakage along stomium and pollen release occurred. Cells that formed septum further degraded. In the last selected phase, the shrinkage of the cells and anther structure took place. The stamen senescence occurred, the cells degraded, the remaining non-degraded cells were deformed, and size of connective was decreased. Finally, the stamens fell off the plant.

Detailed knowledge about the stamen structure of yellow lupine and precise description of the key events that occurred at each stage of development can facilitate further research at the cytological and molecular levels. In yellow lupine, understanding the mechanisms of stamen development may result in improved pollination and fertilization. This, in turn, can prevent excessive and premature abortion of flowers, and finally increase yielding.

Acknowledgments: This work was supported by the Polish Ministry of Agriculture and Rural Development grant no 222/2015.

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POSTER SESSION

7.14

Characterization of plant growth promoting traits of bacteria isolated from cowpea (*Vigna unguiculata* (L.)) Walp nodules

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Drought-tolerant crops, such as cowpea, are of great interest for sustainable agriculture, due to its tolerance to low water availability, high temperatures and soils with low fertility, wich make cowpea extensively cultivated in the semi-arid tropical regions.

Some bacteria promote plant growth through different biological mechanisms and they can do so endophytically, in symbiosis or as free-living cells. These plant growth-promoting bacteria (PGPB) can establish symbiosis with some legumes such as cowpea, and can act directly by facilitating plant nutrients acquisition or influencing plant hormone levels, or indirectly by reducing or preventing the deleterious effects of phytopathogens.

Furthermore, PGPB can exhibit several functional traits such as solubilization of inorganic phosphorous, production of siderophores and indoleacetic acid (IAA), that can enhance soil health management and plant growth.

The objective of this work was to identify strains of PGPB and evaluate the occurrence of four plant growth promoting mechanisms. Bacteria were isolated from cowpea root nodules, grown in different Portuguese soils. All isolates were evaluated for their ability to induce root nodules. The identification of the isolates was performed through the amplification of the full-length 16S rDNA, *nodC* and SMc 00019 gene analysis.

Phosphate solubilization activity was performed by plate assay using National Botanical Research Institue's phosphate growth medium (NBRIP) [1]. Siderophore production was estimated qualitatively by the Chrome Azurol S assay [2]. Indole acetic acid production was assessed by a colorimetric method described by Brígido and Glick [3] and bacterial hydrogen cyanide production was detected by a qualitative method [4].

Most of the PGPB strains characterized belonged to *Rhizobium* sp., *Burkholderia* sp., *Bradyrhizobium* sp., *Pseudomonas* sp. and *Bacillus* sp. genera. The majority of the isolates presented two or more plant growth promoting traits, being phosphate solubilization and siderophore production the most frequent.

Rhizobium sp. were the strains that presented more positive tests for all the growth promoting traits evaluated.

These PGPB strains are potential inoculants for sustainable production of cowpea. Additional research with the strains tested is essential under greenhouse and field conditions, once their effectiveness depends of the behavior in different soils and climate conditions.

Acknowledgments: This work was supported by portuguese national funds through Programa Operacional Competitividade e Internacionalização (POCI), Project 3599 – Promover a Produção Científica e Desenvolvimento Tecnológico e a Constituição de Redes Temáticas (3599-PPCDT) and Fundo Europeu de Desenvolvimento Regional (FEDER) under Project POCI-01-0145-FEDER-016801 and by FCT under Project PTDC/AGR-TEC/1140/2014". This work is supported by National Funds by FCT – Portuguese Foundation for Science and Technology, under the project UID/AGR/04033/2019. The first author also acknowledges the financial support provided by the FCT-Portuguese Foundation for Science and Technology PD/BD/128271/2017, under the Doctoral Programme "Agricultural Production Chains – from fork to farm" (PD/00122/2012).

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Soybean development and productivity in response to organic management under a cool temperate climate in Europe

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Keywords: *B. japonicum*, row spacing, sowing date

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Soybean (*Glycine max* (L.) Merr.) is a common crop in southern part of Europe and has never widely spread in Scandinavian or Baltic states as they lie above the northern boundary of its typical distribution region. Therefore, the demand for locally organically grown soybeans has prompted new research on soybean cultivation feasibility in cool-climate European countries.

Field trials were set up in Lithuania, Institute of Agriculture (LAMMC) in the organically managed sites in 2015, 2016, 2017 and 2018. This research examined the effect of the following organic management practices: two sowing dates, 25-cm and 50-cm row spacing, inoculation with *Bradyrhizobium japonicum* and their interaction on soybean development, nitrogen fixation, productivity and yield quality. The study involved seven soybean varieties (*Merlin, Violetta, Bohemians, Silesia, Brunensis, De-013-130660, H-15-007-0072*) and two *B. japonicum* strains (‘*AGF78*’ and ‘*2490*’).

The findings suggest that soybean can develop relatively well in the locations above the present northern soybean distribution region but its development, productivity and yield quality significantly depend on the management practices. The yield of soybean varied from 673 to 3154 kg ha^{-1} in response to the management practices. The plants cultivated at 50 cm row spacing tended to accumulate a higher aboveground biomass, more pods and higher TKW and seed yield. Yield components reached the highest values of 16.8 g DW biomass, 19.9 pods plant^{-1} and 7.9 g seeds plant^{-1} when inoculated soybean had been sown on 20 May with a 50-cm row spacing. Protein content significantly varied from 27.4 to 35.3 %, fat content from 17.4 to 21.5 %.

The most productive soybean cultivars in the organic agroecosystem were found to be *Merlin*, *Silesia* and *Bohemians*. The cultivars *Silesia* and *Bohemians* exhibited the highest TKW of 229 g and 215 g respectively. The shortest vegetation period was recorded for *Merlin* and *Bohemians*, The length of soybean vegetation season for all varieties varied from 142 to 161 days at latitude 55°N. The varieties *Brunensis*, *De-013-130660, H-15-007-0072* were the last to reach full maturity and harvesting was technically hard due to the weather conditions.

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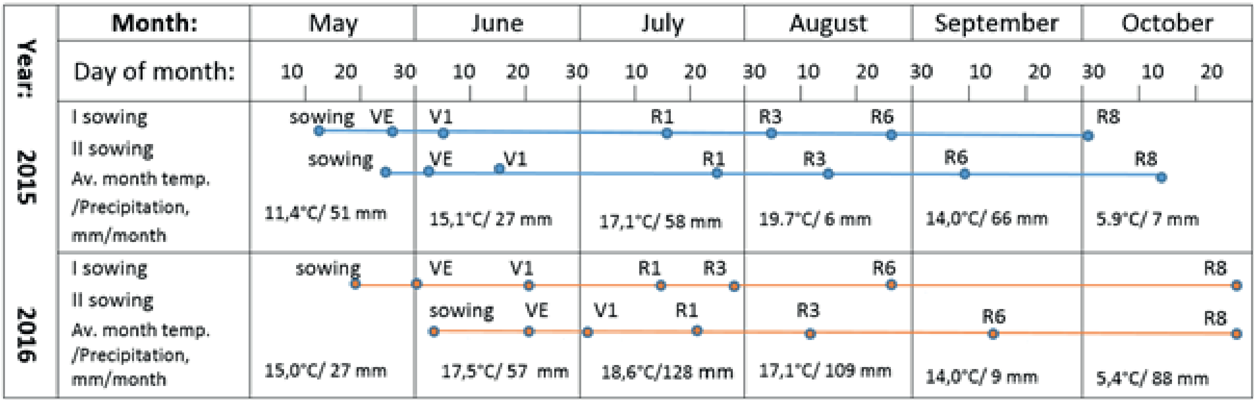

Figure 1. Development stages of soybean Merlin sowed in different dates in 2015 and 2016
Indices: VE – emergence; V1 – first trifoliate, R1 – beginning flowering, R3-beginning of pod formation, R6-full seed, R8-full maturity

Rhizode position as a functional trait in legumes. Study of trade-off for plant productivity and resilience

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Keywords: legumes, rhizodeposition, plant and microbiome interactions,ecophysiology, phenotyping.

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Rhizodeposition is the release of organic carbon (C) to the soil that connects the biotic and abiotic components of the C cycle. It can promote C storage to soil but also mediates plant-microbe interactions (Jones *et al.*, 2009). These interactions are complexes as rhizodeposition will influence the composition and functioning of microbial populations which in return are able to increase the availability of nutrients in soil and provide protection against pathogens (Sasse *et al.*, 2018). Despite their importance for current agriculture challenges, plant-soil microbes interactions remain poorly understood due to the methodological challenge they represent and the complexity of actors and processes involved (Oburger & Jones, 2018). Indeed in the context of climate change, our current agriculture needs to reconcile with ecology and mitigate these interactions to benefit mutually environment and food production while selecting more resilient ideotypes. As plants and soil health are highly dependent on trophic and signaling interactions in the rhizosphere, it is worth noting that plants from natural ecosystems tend to exhibit more rhizodeposition than in agrosystems (Pausch & Kuzyakov, 2018). Thus, it is crucial to evaluate why crop species exhibit less rhizodeposition and what are the plant physiological processes governing rhizodeposition in terms of quantity and type (exudation, mucilage, root cap and border cells loss) at the temporal and spatial scale. Identifying potential trade-off for carbon allocation would assist the selection of resilient ideotypes and development of sustainable farming practices.

Because of their potential for agroecology, we study the relationship and potential trade-off of carbon allocation in legumes with focus on the specie *Pisum sativum*. Carbon budget for biomass, growth, storage, nodulation and rhizodeposition at the whole plant level is performed with state of the art phenotyping and isotope labelling plateforms.

We show the different rhizodeposition processes and methods to assess them in order to evaluate their respective contribution to carbon budget and microbial population management. Root mucilage is a hydrogel composed mainly of polysaccharides actively secreted by exocytosis. It provides multiple benefits such as hydraulic continuity with soil, metal complexation and rhizosphere stabilization (Kroener *et al.*, 2016). Border cells are cells that detach from root apex. They lower frictional stress during growth but also have a major role in mediating microbial populations in legumes through the release of secondary metabolites (Watson *et al.*, 2015). Exudates are soluble plant derived primary and secondary metabolites that can be released through active and passive mechanisms (Jones *et al.*, 2009). Second, we present our ecophysiological structure-function approaches at the root level focusing on sugars, taking into consideration carbon and water transport. The developmental gradient of roots is studied using anatomy in order to link it to functions such as growth, storage, respiration and exudation. Further the use of hydraulic methods informs on radial and axial resistance for water flow but also passive diffusion of sugars to decipher to which extent loss of organic carbon is an unfortunate necessity for water absorption through passive loss (Zwieniecki *et al.*, 2002).

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Evaluation of leaf water potential and chlorophyll stability index variation for chickpea genotypes in abiotic stress conditions of Turkey

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Keywords: leaf water potential and chlorophyll stability index, chickpea, water use efficiency

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Chickpea (*Cicer arietinum* L.) is one of the important legumes widely grown for dietary proteins in semi-arid Mediterranean climatic conditions. To evaluate the genetic diversity with improved heat and drought tolerance capacity in chickpea, thirty-four selected chickpea genotypes were tested under different field-growing conditions (rain fed winter sowing, irrigated-late sowing and rain fed-late sowing) in 2015 growing season. A factorial experiment in randomized complete block design with 3 reps was conducted at the Eastern Mediterranean Research Institute Adana, Turkey. Leaf water potential and chlorophyll stability index were measured throughout the growing seasons. Leaf water potential variation throughout both growing seasons, indicated greater adaptability for Seçkin, Aksu, FLIP 03-126 C, FLIP 03-108 C, FLIP 01-24 C, FLIP 01-54 C, F4 09 (X 05 TH 69-16124-8), EN 808, and EN 1750. Besides, FLIP 05-150 C and FLIP 05-170 C chickpea genotypes showed higher chlorophyll stability index values under Mediterranean climatic conditions.

Identification of faba bean sources for herbicide resistance

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Keywords: herbicide tolerance, post-emergent herbicide, Metribuzin, Imazethapyr.

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Faba bean (*Vicia faba* L.) is a grain legume grown in many countries for its importance as a source of protein for food and feed and as a source of Nitrogen (N) for the succeeding crop. Even though it is proved that the inclusion of faba bean in the cropping systems may contribute to their sustainability. The cultivation of this crop has declined in the past years due to its susceptibility to various biotic and abiotic stresses. Among them, weeds are a major constraint due to being a poor weed competitor during the early stages of crop cycle. A few herbicides are available but all present limitations of selectivity, critical time of application. Furthermore, options for post-emergence broadleaf weed control are limited. Therefore, the deployment of herbicide resistance in faba bean is most desirable for weed management. The purpose of this research work was to identify sources of resistance to two post-emergence herbicides in faba bean germplasm for further use in the breeding programs.

A set of 210 faba bean accessions were evaluated for three seasons at two locations (Terbol and Marchouch ICARDA stations) during 2014/2015 to 2015/2016 in alpha lattice design with two replications except the trial conducted in Marchouch during 2014/2015 in augmented design. The experiments consisted of three treatments comprising two post-emergent herbicides applied at the pre-flowering stages and an untreated treatment (control). Herbicides used were Metribuzin and Imazethapyr at the recommended rate (250 g a.i./ha and 75 g a.i./ha, respectively). The 25 most resistant accessions were selected and further evaluated in a third season in alpha lattice design with three replications, where additional treatments were added to evaluate their tolerance to higher dosages (1x, 1.5x and 2x of recommended rate).

The herbicide tolerance score (HTS) [1,2] was recorded at 2 and 4 weeks after herbicide treatment from 1 (no damage observed) to 5 (Susceptible) scale. In addition crop performance was assessed by recording days to flowering (DFLR), days to maturity (DMAT), number of branches per plant (NBPLT), plant height (PLHT), number of pods per plant (NPPLT), number of seeds per pod (NSPLT), hundred seed weight (HSW) and grain yield per plant (GYPLT).

Significant differences among genotypes were observed for all the recorded traits. Significant differences among the treatments were observed for all the recorded traits except for the HSW and NPPLT.

There was a significant reduction of plant height, yield and yield attributes in some accessions treated with 1.5x and 2x of the Metribuzin during the third year and therefore were discarded.

Among the 25 accessions reevaluated during 2017/2018, the sixteen described in the Table 1 were validated as resistant accessions as there were no significant difference among treatments for GY, DFLR, DMAT, PLHT and other yield components even at the higher dosages.

Table 1. Name, Pedigree and Source of the selected lines

Line Name	Pedigree	Source
SelTer2017/7663	[1]F7/8983/05 X [2]sel2004lat393-1	ICARDA
SelTer2017/7633	[1]F7/8983/05 X [2]sel2004lat393-1	ICARDA
FLIP03-69FB	S 98 023, Fam 797-2/1804/03	ICARDA
Hama-2	[3]HBP/S0-EM-Lat/2000	ICARDA
Najeh	[4]Sel.88Lat.18025 x SP49C	ICARDA/ TUNISIA
SelTer2017/7630	Selection fromVF335	RUSSIA
SelTer2017/7668	Selection from IG126172	ECUADOR
SelTer2017/7449	Selection from INRA1482	NETHERLAND
SelTer2017/7617	Selection from INRA2583	FRANCE
SelTer2017/7621	Selection from INRA2574	FRANCE
Saraziri	Selection from Iran Landraces	IRAN
Basabeer	Bulk1/3-1993 landrace	SUDAN
SelTer2017/7616	Selection from INRA1512	FRANCE
SelTer2017/7625	Selection from INRA2568	FRANCE
Syrian Local Large	ILB1814	SYRIA
SelILB1814/2013	Selection from ILB1814	SYRIA

[1] Lines selected from populations developed with wide range of genotypes including the main source F402.
[2] IG12923 originated from Ukraine
[3] Population developed at ICARDA
[4] Selected at ICARDA from the cross between F216 x INIA06.

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Development of faba bean (*Vicia faba*) host differentials for assessing variation on *Uromyces viciae-fabae* populations and pathotype nomenclature system in Australia

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Keywords: faba bean rust, host-pathogen interaction, virulence

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Faba bean rust caused by *Uromyces viciae-fabae* (Pers.) J. Schröt.) is an important disease limiting faba bean production across countries in Australia, Africa, Asia and Europe. The development of rust resistant cultivars provides economical and viable solution to overcome this disease. Although at least two genes for resistance have recently been characterized in Australia [1] there is a lack of understanding on the pathotype variation. Faba bean rust is an obligate parasite exhibiting all five spore forms in its life cycle on a single host. This allows sexual recombination and the chances of variation in the pathogen are quite high. Based on isozyme variation, differences in virulence were found among rust isolates collected in Australia [2]; however, since then neither continuous surveillance for the pathogen variation has been conducted nor a host differential set to asses the variation has been developed.

This study examined differences in the virulence of Australian *U. viciae-fabae* isolates by developing a differential set of faba bean genotypes. Ten rust isolates were collected from the major faba bean growing states in Australia; South Australia (SA), New South Wales (NSW) and Queensland (Qld). A single spore culture was generated and multiplied by inoculating each isolate in isolation on susceptible cultivar Fiord, under greenhouse conditions. In the absence of a differential set, these pathogen isolates were used for assessing virulence on 40 diverse faba bean genotypes.

Based on the host-pathogen interactions, 12 putative host genotypes (Doza#14916, Doza#12034, Ac1257#14904, IX552Rb-2-4, Ac1655, IX114#15033, IX474/4-3, IX585c/1-11, Ac1227#14908, IX524Rb-2-1, PBA Nanu and PBA Warda) were identified that displayed variation in the rust isolates. These genotypes have been proposed as a differential set for assessing pathotype variability in the future.

We found a variation in the pathogen, but there was no system of naming the pathogen isolates. For the first time, a nomenclature system was developed using the binary pathotype naming system similar to developed for common bean rust [3]. According to the binary nomenclature, each isolate was given a two-digit code separated by a hyphen. The differential set genotypes were ranked (1 to 12) on the basis of resistance; genotypes showing resistance against most isolates were arranged first followed by the genotypes with resistance on fewer isolates. Each susceptible reaction of a differential genotype was attributed a binary value. In this arrangement, the genotypes were separated in two groups (genotype 1 to 6 and 7 to 12) and the name of each pathotype was assigned by the concatenation of the corresponding binary values attributed to the susceptible reaction of the differential lines within each group. Nine virulence patterns on the differential set were detected and the isolates were named using the new nomenclature system. The SA isolates were named as pathotypes 0-10, 0-46, 40-31 and 40-55; NSW isolates as 24-40, 63-53 and 63-49; and Qld isolates as 55-63 and 63-53.

The virulence among *U. viciae-fabae* populations can be predicted; SA being the least virulent, NSW moderately virulent and Qld highly virulent. This fits well with the development of rust in these regions; rust appaears only at the later stage of growing season in SA and southern NSW and damage is negligible, whereas it occurs from seedling satge in the warmer and sub-tropical region of northern NSW and southern Qld causing considerable damage. The pathotype 63-63 from the southern Qld was highly virulent and overcame all host differentials posing a risk of rust outbreak in the region. This is the first report of the well characterised *U. viciae-fabae* pathotypes based on differential set in Australia. The differential set will allow to detect any new variation in pathotypes before it becomes epidemic in the region. However, a routine surveillance will be required to detect these variations. This information will facilitate breeding for rust resistance in faba bean according to the prevalence of pathotypes in the target region and monitor the breakdown of resistant genes.

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Medicago ABCG protein acts as selective modulator in the phenylpropanoid pathway

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Keywords: ABCG transporter, phenylpropanoids, *Medicago*

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The phenylpropanoid pathway is one of the most investigated metabolic pathways leading to the biosynthesis of secondary metabolites. Phenylpropanoids are engaged in many physiological processes, as well as they are involved in plant-environment interactions. In *Medicago truncatula* pterocarpan medicarpin, a product of phenylpropanoid pathway, is a main phytoalexin. It has been shown that several genes from mediacrpin biosynthesis route are strongly upregulated upon biotic stress/pathogen infection. It is also the case of *MtABC10*, a gene encoding a plasma membrane transporter belonging to the ABC (ATP Binding Cassette) protein family.

It was revealed that upon biotic stress the MtABCG10 protein is required for efficient *de novo* production of medicarpin. The *MtABCG10* silencing resulted *inter alia,* in a lower accumulation of medicarpin and its precursors, leading to an increased susceptibility of plants to fungal infections [1]. Metabolomic phenotype observed in *MtABCG10*-silenced roots was averted by exogenous application of early medicarpin precursors, namely: 4-coumarate (the core phenylpropanoid pathway) and formononetin (isoflavonoid branch). Transport experiments of various Medicago compounds conducted in tobbaco BY2 suspension cells overexpressing *MtABCG10* and membrane vessicles isolated from them revealed that MtABCG10 is responsible for the selective membrane translocation of 4-coumarate and liquiritigenin [2]. The recognized MtABCG10 substrates constitute key intermediates and branching points in phenylpropanoid pathway. The proper distribution of such precursors and identification of transporters participating in it appears as an important step in understanding of the phenylpropanoid biosynthesis.

Based on obtained results, it could be proposed that the MtABCG10 acts as a selective modulator od carbon flow in the phenylpropanoid pathway upon biotic stress that is responsible for directing early medicarpin precursors, from the general phenylpropanoid and flavonoid pathways to the isoflavonoid phytoalexin routue.

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Screening of cowpea lines for drought tolerance

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Keywords: cowpea, drought, tolerance, susceptible, screen house.

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Thirty nine cowpea lines were screened for drought tolerance to provide information on cowpea lines that can be used for genetic improvement of the acceptable varieties. The experiment was carried out using wooden boxes filled with top soil. Cowpea lines were planted in three replications and the seedlings were watered daily using a small watering can of four litres for two weeks until partial emergence of the first trifoliate leaves of all the varieties was observed. Thereafter, watering was stopped for thirty five days and wilted plants in each variety were counted daily. Watering was resumed for fourteen days to ascertain regeneration potentials of each variety. Significant differences were observed among the cowpea lines for drought tolerance Stress effect was first noticed on the unifoliate leaves twelve days after watering was stopped, followed by the emerging trifoliates and finally the growing tip dried. Wilting percentage at different days after termination of watering indicated TVx 3236, NG/SA/07/132 and IT95K-193-12 to be the most susceptible to drought. The recovery percentage after watering ranged from 0% for NG/SA/01/09/004 to 100 % for IT81D-994 and Oloyin. All the cowpea lines were grouped into three based on the findings from this study. Group 1 comprises of three lines (TVx 3236, NG/SA/07/130 and NG/SA/01/09/004) which are highly drought susceptible while Group 2 and 3 comprise of 14 and 22 lines respectively that were tolerant at varying degrees. The screening provided information on cowpea lines that can be used in breeding for drought tolerant cowpea varieties.

Characterization of genotype by environment interactions in soybean in agro-climatic conditions of Poland

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Keywords: *Glycine max*; AMMI; genotype x environment interaction

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Interest in soybean in Poland has more than 150-year history and the attractiveness of soybean went through various fluctuations. In the late 1980's, soybean varieties with the appropriate earliness for the conditions of Polish, with good yield potential and adapted to mechanized harvesting were released. There were eight soybean varieties on National List, of which Progres, Polan, Aldana, Jutro and Mazowia developed in IPBA- Radzików and Nawiko, Gaj and Augusta developed in Agricultural University Poznań.

Changing climatic conditions, good results of soybeans crop for its producers, the development of organic farming and the national need for plant protein caused a renewed interest in this species both breeding and cultivation. In recent years, significantly increased the number of soybeans cultivars on National List (COBORU 2019). Also in cultivation are the varieties from CCA. An important aspect of the evaluation of the varieties in agricultural production is the stability of the crop, resulting from the genotype x environment interaction. The presence of GxE interaction can be attributed to predictable factors such as soil type, pest and disease management and unpredictable factors such as precipitation, temperature and humidity in each environment. However, information on the genotype by environmental interaction (GEI) in soybean in the country is limited. The objectives of this study was to characterize GEI in soybean grown in Poland for seed yield, protein and oil content.

Ten soybean (*Glycine max* (L.) Merr.) cultivars were evaluated at six locations in Poland for three years. Field experiment were set as randomized complete block design with four replication. Currently accepted of management and cultural practices for soybean were applied. Seed yield form each plot was converted to dt·ha^{-1} at 15% moisture. After harvest, average 1000-g sample from 4 replications of every genotypes was collected for protein and oil analysis using near-infrared reflectance whole grain analyzer (Foss Infratec 1241). Analysis of AMMI was performed using procedures of SAS (Institute, Cary, NC,USA).

The diversity of the environment requires the availability of soybean varieties with both a high yield and relatively small seed yield fluctuation location and growing seasons. The overall seeds yield of tested soybean cultivars varied from 26,2 dt·ha^{-1} to 38,0 2 dt·ha^{-1} with an average 30,6 dt·ha^{-1}. The descending order of soybean yield for locations was followed: Przecław > Tarnów > Strzelce > Kawęczyn > Radzików > Przebędowo.

Analysis of weather conditions for each of the location explains to some extent the diversity of soybean yields in the locations. The higher the value of the hydrothermal index and higher of the sum of the heat units had a determining influence on the soybean yield regardless of soil conditions. The average protein and oil content in seeds amounted to 39,1% and 21,9% with 2.5% CV and 3,7 % CV variation among cultivars.

The analysis of variance of AMMI model elucidate that seed yield were significantly affected by G, E, and GEI. The highest percentage of variation was explained by E 63,1%, while G and GEI explained 16,0% and 11,7% respectively. The AMMI analysis partitioned summ of squares of GEI into nine principal components (PC). The PC1 and PC2 captured 37,6% and 27,6% of GEI respectively. In the AMMI1 biplot, the genotypes and environment scores close to zero express general adaptation. Three groups of varieties were separated: stable Madlen and Mavka with seed yield slightly above grand mean; Abelina, Merlin and Lissabone with high seed yield in the relatively (more specifically adapted) favorable environments and with high contribution to the interaction (GEI), and the remaining LP-2, Progres, Augusta, Aldana and Annushka the early maturing varieties with yield below average. Six environments gave the highest and two the lowest mean seed yield. Based on AMMI2 biplot five early maturing cultivars create cluster of similar reaction to environment conditions. The remaining cultivars showed considerable dispersion.

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Genome-wide association study (GWAS) for resistance to *Sclerotinia sclerotiorum* in common bean

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Keywords: *Phaseolus vulgaris*, GWAS, white mold resistance

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White mold (WM), caused by the fungus *Sclerotinia sclerotium* (Lib.) de Bary, is a serious disease in common bean (*Phaseolus vulgaris* L.). This pathogen is responsible for significant yield losses, particularly in temperate and humid production regions. Resistance to WM is quantitative inherited and may be due to physiological in nature, or avoidance (morphological traits related to plant architecture or to phenological traits). The aim of this study was to conduct a genome-wide association study (GWAS) against WM resistance in order to i) better understand the genetic architecture of this complex trait ii) identify new QTLs associated with the resistance or iii) to verify previously reported QTLs [1].

The common bean SERIDA diversity panel was used [2]. This panel was constituted by 308 lines and genotyped using genotyping-by-sequencing [3] with 5394 SNP markers obtained after filtering missing values (<10%) and minor allele frequency (MAF > 0.05). Evaluation of the resistance was conducted in greenhouse tests using the straw method [4] and a local WM isolate. The panel was evaluated in two separate runs. Each run consisted of two replications arranged in a randomized complete block design. A replication consisted of four plants per line in a single pot. The severity of disease progression was assessed using a 1-to-9 scale where a value of 1 = no symptoms and 9 = total plant collapse [1, 4]. GWAS analysis was conducted with the total average value per line considering the mixed linear model (MLM) implemented in TASSEL V5 and a cutoff value of $-\log10(p)>2.0$ [5]. Manhattan plot was visualized using qqman package in R [6].

A total of 55 significant SNP-WM reaction associations were identified involving all bean chromosomes except Pv05 and Pv11 (Figure 1). These SNPs can be grouped into 20 regions considering a chromosome walk of 1 Mb up and down. Based on physical positions in the bean genome [7] of the underlying markers, some of these regions could correspond with QTLs previously reported [1]: 3 regions (Pv02: 3.63-5.63 Mb; Pv03: 38.95-41.54 Mb; Pv08: 36.14-38.24 Mb) could correspond to meta-QTLs [1], 6 regions (Pv01: 41.46-48.41 Mb; Pv06: 23.00-25.00 Mb; Pv07: 39.98-42.53 Mb; Pv08: 12.32-14.32 and 27.74-29.74 Mb; Pv09: 9.47-12.71 Mb) could correspond with QTLs identified in RIL populations [1], and 11 regions were newly identified in this study involving chromosomes Pv02 (33.64-37.40 Mb), Pv03 (0-1.95 and 4.85-6.85 Mb), Pv04 (1.34-3.37 Mb), Pv06 (16.95-18.95 Mb), Pv07 (0-1.14 Mb), Pv08 (6.31-8.31 and 50.78-53.40 Mb), Pv09 (15.42-17.78 and 34.94-37.68 Mb) and Pv10 (40.09-42.09 Mb). Results obtained in this work allow identifying new 11 chromosome regions candidate to be involved in the genetic control of such complex trait, as well as verify 9 regions previously described.

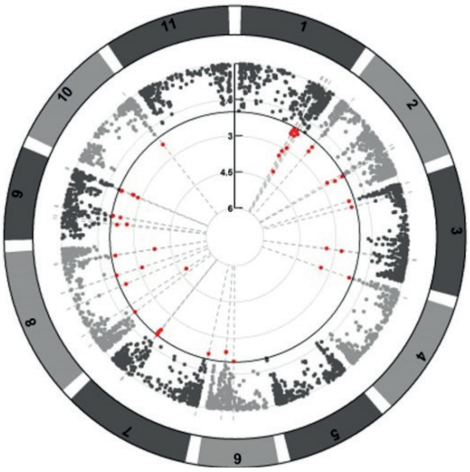

Figure1. Manhattan plot showing the SNPs significantly associated with WM response (red color) per chromosome

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Integrated weed management strategies for organic chickpea in the northern great plains of USA

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Weed management in organic chickpea (*Cicer arietinum* L.) production poses a substantial challenge. Without chemical herbicides, the weed control in organic production is dominated by cultural and mechanical methods. Field studies were carried out in 2016 and 2017 at the MSU-Western and MSU-Eastern Agricultural Research Centers to evaluate the integration of three types of weed control practices, which included crop variety (two chickpea varieties, Black and CDC Orion), seeding rate (standard seeding rate at 43 seeds m^{-2} or 1x and 50% increased rate or 1.5x), and pre-emergent weed control practices (flame weeding, shallow tillage, and untreated weedy control). Chickpea plant density, weed biomass, and chickpea yield were evaluated. The Black chickpea had consistently higher stand densities and yields and lower weed biomass relative to the CDC Orion, which is likely due to higher vigor and resistance to soil borne disease. Increasing seeding rates consistently increased yields by 15-26% and consistently increased crop densities in the Black variety. Flame weeding was not effective at reducing weed biomass neither at Corvallis and Sidney, but it increased chickpea yields at Corvallis in 2016. Shallow tillage consistently reduced weed biomass by 40-60% when combined with increased seeding rates. However, shallow tillage after chickpea emergence resulted damage to chickpea seedlings. Results suggest that competitive varieties, increasing seeding rates, and shallow tillage can be successfully integrated to improve yields and reduce weed pressure in organic chickpea, but efficacy of shallow tillage depends strongly on proper timing. Additional greenhouse and field trials were conducted to screen the seedling vigor indices of chickpea germplasms and their correlations to seed yields were analyzed.

Candidate genes involved in resistance to fusarium wilt in yellow lupin (*Lupinus luteus* L.)

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Keywords: yellow-lupin, fusarium wilt, resistance

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Yellow lupin has a potential to become highly important agronomic crop due to its high seed protein content and good adaptation to sandy soils [1]. One of the main threat in yellow lupin breeding is its susceptibility to fusarium wilt, a soilborne disease caused by *Fusarium oxysporum*. Owing to extensive breeding efforts, yellow lupin cultivars with improved level of resistance to this pathogen exists. Nevertheless, a molecular background of the resistance is still poorly understood [2].

The objective of this study was the analysis of transcriptome-derived candidate genes possibly involved in resistance to *Fusarium oxysporum*. Comparative transcriptome analysis of yellow lupin leaf samples, derived from susceptible and resistant accessions growing on a monoculture field naturally infested with *Fusarium* spp. was conducted earlier. Based on these results we selected three genes for which a functional annotation pointed a contribution in such processes as: response to the pathogenic invasion; wounding; abscisic acid and salicylic acid treatments; as well as a participation in cell-wall organization processes. Moreover, based on the hypothesis that lupin alkaloids may play a potential role in maintaining plant's resistance against fungal diseases [3] we have also examined three other candidates, which represented the nearest homologues of *Lupinus angustifolius* genes, already described as participating in quinolizidine alkaloids (QAs) biosynthesis. Gene expression changes were analysed in 3 susceptible and 3 resistant accessions of yellow lupin in response to fusarium infection. We found out that in the susceptible accessions all the investigated genes were significantly down-regulated. In case of the accessions characterised with tolerance/resistance to fusarium wilt, we observed the increase of the relative expression of genes involved in stress response, as well as slightly decreased (thus still notably higher than in susceptible lines), relative level of alkaloid genes expression.

Our results demonstrate the complex nature of yellow lupin response to fusarium wilt. We show that the response to pathogen infection triggers different regulatory pathways and underlying it diverse molecular components, which in consequence provide sufficient genetic background for a plant to manage the disease. Our study contributes to better understanding of molecular mechanism underlying resistance to *Fusarium* in yellow lupin.

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Germination and growth of soybean inoculated with *Rhizoctonia solani*

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Rhizoctonia solani is a common soil-borne fungal pathogen responsible for economic losses in soybean crops. It is dangerous already at the stage of germination causing pre-emergence and post-emergence damping-off [1]. Germinated seedlings can die out rapidly or they are weakened which leads to growth limitation. Typical symptoms of the disease are reddish-brown lesions on hypocotyls. The severity of symptoms depends on the virulence of an isolate and environmental conditions. Soybean cultivars studied so far differed with a degree of susceptibility but none of them showed a complete resistance.

The aim of the study was determining the effect of *R. solani* inoculation of seven soybean cultivars bred by AgeSoya: Annushka, Atlanta, Lajma, Madlen, Mavka, Smuglyanka and Violetta. Moreover, susceptible cultivar Amsoy [2] was included as a control. Amsoy is a cultivar not cultivated in Poland, its seeds were obtained from German GeneBank in Gatersleben. *R. solani* isolate used for the inoculation test was obtained from the Polish Bank of Plant Pathogens in Poznań and it was multiplied on PDA medium. Two-week old fungal cultures were the source of inoculum. The circles of agar (1 cm in diameter) with mycelium were put on sterile soil in pots. Next, disinfected soybean seeds were placed on the circles and then covered with thin layer of soil. In the similar way the control objects (non-inoculated seeds) were prepared except for adding inoculum. The experiment was carried out in a room where the temperature was 19°C, humidity 31% and photoperiod 16/8 h. Three weeks after inoculation (when plants reached BBCH phase 10-13) disease symptoms were recorded and fungal isolates were obtained from the tissues with visible lesions. Additionally, the dry mass of plants was determined.

In case of cultivars Mavka and Lajma, only few seeds germinated (also in non-inoculated treatment) which may be the result of their high sensitivity to disinfection. Due to the low number of seedlings these two cultivars were excluded from further observations.

None of the seedlings belonging to cv. Violetta showed disease symptoms. Only 1.9 % of plants within cv. Atlanta appeared to be susceptible. Over 90 % of plants within cv. Annushka and Madlen had no symptoms recorded. The lowest rate of asymptomatic seedlings was observed for cv. Smuglyanka (84.5 %) however it was high considering that 65.5 % of seedlings within control cv. Amsoy was infected by the *R. solani*.

Pure fungal cultures obtained from infected organs were observed macro- and microscopically. Mycelium obtained from inoculated plants was identified as *R. solani*. In contrast, among the fungal cultures originating from non-inoculated control plants *R. solani* was not detected.

Dry mass of seedlings grown from inoculated seeds was mostly lower comparing to non-inoculated ones but the difference was statistically significant only in case of some of tested cultivars. *R. solani* infection decreased the dry mass of plants belonging to cultivars Annushka, Madlen, Smuglyanka and Amsoy.

The presented results showed the differential level of susceptibility of tested cultivars to *R. solani.* However, it is worth emphasizing that generally this level is low for all tested cultivars.

R. solani is known for high diversity and different isolates may differ in their pathogenicity. Therefore, in the future, this research may be extended with the use of *R. solani* isolates obtained from soybean seedlings infected under field contitions.

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Poland-Portugal cooperation on cell wall remodelling under stress conditions in *Medicago truncatula*: achievements and prospects

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Legumes and crop legumes (pulses) have been shown to be important sources of proteins, edible oil and industrial fuel. Due to their ability to fix atmospheric nitrogen, they are important players for the sustainability of ecosystems, as well as for agricultural practices [1].

As other crops, legume productivity across different environments are limited by different biotic constraints caused by pathogens as well as distinct abiotic constraints such as availability of water (drought and flooding), extreme temperatures (chilling, freezing and heating), salinity, among others [1], [2].

Cell walls are essential cellular components in plants, responsible for a number of critical functions, including providing shape to many different cell types, forming the interface between adjacent cells, playing important roles in inter-cellular communication and in plant-microbe interactions as well as perception and plant adjustment to environment stress conditions. Despite the increased knowledge on woody species, such as pines (e.g. [3]), poplar and eucalyptus (eg. [4]), in legumes the understanding of the changes in cell wall characteristics in different environmental conditions is still limited.

Plant Cell Biotechnology laboratory (PCB) of the ITQB-NOVA (Oeiras, PORTUGAL) has a long experience with *M. truncatula*. Several resources for this model plant were developed including an efficient protocol for somatic embryogenesis and genetic transformation [5] and physiological methodologies for screening water stress resistance [6].

The Department of Integrative Plant Biology (DIPB) of Institute of Plant Genetics of Polish Academy of Sciences (IPG PAS) was created in the frame of the ERA-CHAIR Biotalent project. Within this project the research line "Cell Walls by design" main objective is focused on the regulation of secondary cell walls biosynthesis in plants at transcriptional and post-transcriptional levels. The research is being currently done in woody plants, using as example *Salix purpura* as a model plant (new Sonata Bis – PurpleWalls).

Having as common interest to understand how cell wall is affected by the imposition of abiotic stress in the model legume *Medicago truncatula* in this bilateral action we develop a strong collaborative network using multidisciplinary approaches, which included visits, workshops and on-line meetings in order to obtain preliminary data and to draft future international research projects. Here we highlight those activities and prospect future accomplishments.

Several thematic workshops were organized to provide state-of-the-art training for the PhD students and Post-docs involved in the action: 1- workshop on legume transformation (PORTUGAL); 2- workshop on cytogenetics and LCM applied to legumes (POLAND); 3- workshop on DNA methylation (measurement techniques) and attendance to INTEGRATIVE PLANT BIOLOGY CONFERENCE at IGR-PAS (POLAND). Dissemination of action accomplishments was achieved by launching a bilateral project website, using WordPress tools, publicly available at https://bilateralplptcellwall.wordpress.com/. The last action will be on plant physiology and the aim is to acquire basic concepts on stress physiology, and to analyse the plants under abiotic stresses.

Finally, the goals of this action have been achieved with the training of young researchers, which acquire new skills and knowledge, and the development of a strong collaborative network. We foresee joint publications and the establishment of a solid basis for future joint project submissions.

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Screening the FIGS set of lentil (*Lens culinaris* Medikus) germplasm for tolerance to terminal heat and drought stress

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Keywords: lentil, heat, drought

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Lentil (*Lens culinaris* Medikus) is one of the most important cool season food legume crops. It constitutes good source of seed protein, carbohydrates and minerals particularly to the people living in the developing countries [1]. Terminal drought and heat stress are important factors affecting lentil production especially in the dry and semi-arid regions such as Morocco [2]. We evaluated a FIGS set of 200 lentil accessions in alpha design with two replications at Marchouch experimental station in Morocco at three different temperature regimes: normal planting, late planting with irrigation; late planting without irrigation. Observations were recorded on plant height, days to 50 % flowering, days to 95 % maturity, number of primary branches, number of secondary branches, number of tertiary branches, total number of filled and unfilled pods, biomass, grain yield and hundred seed weight. Results indicated that heat and drought stress at reproductive stage adversely affected plant height, number of primary, secondary and tertiary branches, total number of pods and seed yield. It also shortened the duration of pod filling by accelerating the forced senescence and the maturity. Using HTI, we identified that ILL 7835, ILL7833, ILL6338, ILL6104 were the most heat tolerant lines. Heat tolerance index was possitively correleted to seed yield under heat stress (Fig.1). However, ILL7835 and ILL6075 performed relatively better than other genotypes under drought stress condition by using several indices (STI, GMP, TOL, HARM and MP).

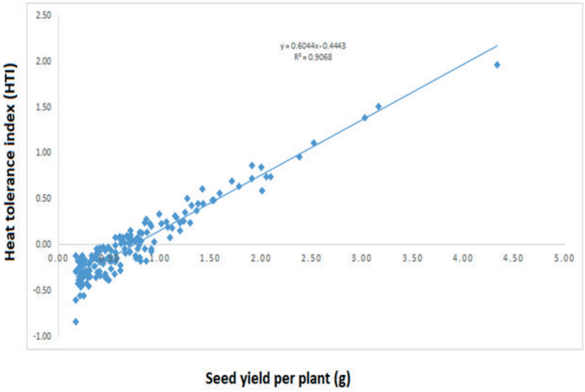

Figure 1. Correlation between Heat Tolerance Index (HTI) and seed yield under heat stress

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Monitoring relative prevalence of *Erysiphe pisi* and *E. trifolii* in pea in Spain and Tunisia

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Erysiphe pisi was thought to be the only causal agent of powdery mildew in pea. Currently, we know that *E. trifolii* and *E. baeumleri* can also cause this disease [1, 2, 3]. However, as the causal agents can not be discerned by the symptoms they cause, the actual incidence of other species different from *E. pisi*, and their relative prevalence, remains unknown. In an attempt to clarify this, we are developing markers able to identify and quantify *E. trifolii* and *E. pisi* and using them to monitor the prevalence of these pathogens along the pea crop cycle.

With this aim, we collected pea powdery mildew infected leaves from different pea accessions at several locations in Spain and Tunisia during two field seasons. In addition to susceptible accessions, we included accessions carrying *er1*, *er2* and *Er3* resistance genes in order to discern their influence on the prevalence of the different *Ersyiphe* species. Primers that specifically amplify *E. pisi* or *E. trifolii* were designed and are currently being used to quantify these pathogens by qRT-PCR. The analysis of the different field samples collected will allow us to figure out the relevance of *E. trifolii* and *E. pisi* as causal agents of pea powdery mildew in Spain and Tunisia.

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POSTER SESSION

8.13

Characterisation of *Medicago truncatula* root specific ABC transporter modulating lateral root density and nodule number

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Cytokinins are an important group of phytohormones in legumes. Their action influences root architecture and is essential for the inner root cell division at the onset of nodule formation. In response to environmental inputs, legumes use cytokinins as mobile signal to modulate nodule number and lateral root outgrowth.

Initiation of lateral root and nodule formation is influenced by various factors. Nitrogen starvation and drought play here a significant role. While nitrogen shortage increases number of nodules and decreases lateral roots density, drought suppresses nodulation and supports lateral roots emergence. In recent years, a lot of effort has been put into revealing the exact mechanism of such regulation, but this still remains unclear.

We used molecular approaches as well as phenotypic studies to investigate a role of ATP driven membrane transporters in distribution of cytokinins and shaping the root architecture in model legume *Medicago truncatula*.

Our attention has been paid especially to one of ABC transporters, present in root vascular bundles, its apical meristem and in developing nodules. Its expression is upregulated by (i) exogenously applied cytokinins, (ii) nitrogen limitation and (iii) Sinorhizobium meliloti. It is also down regulated upon drought stress and abscisic acid treatment. Upon nitrogen limitation plants without this transporter are characterized by lower lateral root density and nodule number.

We propose that this transporter takes part in the negative regulation of nodulation and lateral root formation.

Our results have implications both in legume crop research and determination of the fundamental molecular processes involved in drought response and nodulation.

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Seed coating as a method to improve resistance of legumes to biotic and abiotic stresses

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Keywords: film coating, polymer, germination, emergence

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Legumes are important source of food and animal feed which provide health benefits to humans and livestock. Moreover, due to their symbiosis with atmospheric nitrogen-fixing bacteria, cultivation of these plants improves soil fertility and facilitates sustainable farming. Despite all these advantages, legumes are greatly under-used crops; their production and yield gains over the past 50 years lag far behind that of cereals [1].

In Europe, yields of grain legumes are significantly more variable compared to other crops [2]. Part of this variability may be explained by the influence of biotic and abiotic factors. For example, species from phaseolid clade originating from a tropical climate (e.g. soybean) are considered to be more susceptible to cold stress [3]. Biotic factors include infections by a diversity of pathogens of which soil-borne fungi affect emergence and growth of many legume crops [4].

Seed coating is a technique of covering the seeds with a layer of substances which are usually expected to improve seed storability, sowing and plant emergence in the field. According to a nomenclature of Pedrini et al. [5], three types of coating treatments can distinguished depending on how much of the external material is applied to the seeds: film coating (up to 10% of seed weight), encrusting and pelleting. Chemical components of seed coats can be divided into binders (which are responsible for binding the coat to the seed surface; these are natural or synthetic polymers); and fillers which are inert, solid materials (e.g. lime, sand, talc etc.). In addition, seed coats may contain pesticides, nutrients and living organisms (e.g. rhizobia). We reviewed available published information (scientific articles and patents) on seed coating methods specifically designed for legume crops.

The most common coating treatment for legume seeds is film coating leading to forming only a thin coating layer. This can be explained by a large size and relatively smooth surface of these seeds; application of larger amounts of coating material is not necessary.

Legume seeds are usually subjected to rhizobial inoculation before sowing, therefore they cannot be coated with substances toxic or harmful to this inoculum. However, apart from this common characteristic, chemical composition of the described seed coating formulations for legumes is very diverse and depends on the coating objective. No single, universal seed coating can protect the seeds from the influence of all adverse environmental conditions.

Many published seed coating formulations are based on natural polymers derived from renewable sources (such as: chitosan, chitosan oligomers, starch, dextrins, cellulose derivatives) characterized by high bioactivity and biodegradability. Additionally, synthetic polymers may be added to the seed coatings as auxiliary and binding agents. This group of polymers includes: polyethylene glycol, polyethylene methacrylate, polyvinyl acetate, polyvinyl chloride, rosin, acacia and polyvinylpyrrolidone [6].

Fungal diseases are one of the major concerns in protecting legume seed material for sowing in many countries of the world, as fungicides are frequently used in the reported seed coatings. Interesting alternative approach to this problem involves binding on the seed surface organisms which can act as natural enemies of the fungal pathogens (e.g. *Bacillus* spp., *Burkholderia* sp., *Trichoderma* spp.).

Seed coating technique has a great potential for protecting leguminous seeds from adverse environmental conditions and soil-borne diseases. Many studies show that they improve germination capacity and germination rate. However, chemical composition of the coating formulations and their physical properties should be adjusted depending on specific field conditions.

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POSTER SESSION

8.15

Molecular profiling of narrow-leafed lupin (*Lupinus angustifolius* L.) resistance to anthracnose

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Keywords: antracnose, resistance, molecular markers, gene expression, PCR

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The narrow-leafed lupin (*Lupinus angustifolius* L.) is a grain legume crop appreciated as a source of protein for food and feed, as well as a plant enhancing mobilization of soil phosphorus and improving soil fertility through symbiotic nitrogen fixation. However, worldwide lupin cultivation has been hampered by high susceptibility to anthracnose, caused by the pathogenic fungus, *Colletotrichum lupini* (Bondar) Nirenberg, Feiler & Hagedorn [1]. Narrow-leafed lupin experienced high effectiveness of anthracnose resistance breeding, leading to the development of a large collection of resistant germplasm.

The resistance to anthracnose in narrow-leafed lupin is controlled by several single dominant genes that were discovered in different germplasm resources, namely, *Lanr1* in cv. Tanjil, *AnMan* in cv. Mandelup, and *LanrBo* in the breeding line Bo7212 [2-4]. To facilitate germplasm screening, several molecular markers linked to key resistance genes were developed. Markers for *Lanr1* include a pair of AntjM1 and AntjM2 markers based on the length difference of PCR-amplified products [2, 5], a pair of Anseq3 and Anseq4 markers developed by genotyping-by-sequencing approach and carrying the single nucleotide polymorphism (SNP) [6] and a DAFWA213 SNP marker identified by the whole-genome sequencing study [7]. Implementation of markers AntjM1 and AntjM2 into large-scale molecular selection revealed high ratio of false-positive scores [3]. Markers Anseq3, Anseq4 and DAFWA213 were evidenced to be applicable for breeding. The two other genetic anthracnose resistance resources, conferred by *AnMan* and *LanrBo* genes, have been exploited to a lesser extent than *Lanr1*. Just one co-dominant PCR marker, AnManM1, has been developed for *AnMan* resistance gene from Mandelup [3]. Attempts have been also undertaken to develop molecular markers tagging the last unexplored major narrow-leafed lupin anthracnose resistance gene, *LanrBo* [8]. A sequence-defined, HRM marker BoSeq196 has been developed, however, due to large distance from the gene (13.7 cM) its applicability in marker-assisted selection is limited [4].

In the present study, the narrow-leafed lupin collection (212 accessions including wild populations, landraces, cross derivatives, mutants and cultivars) was genotyped with Anseq3, Anseq4, DAFWA213 and AnManM1 markers. According to marker polymorphism 15 lines were revealed to carry resistant *Lanr1* allele and 11 lines resistant *AnMan* allele.

Based on the results of anthracnose marker genotyping 50 lines were selected for disease resistance surveys in controlled environment conditions. Three *C. lupini* isolates were tested. Lines 95842 "Population 22661", 95843 "Population 22660" and 96371 "Population 1" were the most susceptible, whereas lines 96233 "83A:476", 96230 "Mandelup" and 96214 "Tanjil" were the most resistant. These lines were selected for gene expression profiling of resistance response to *C. lupini*. Plant leaf tissue was collected 6, 12, 24, 36, 48, 60, 72 and 96 hours after inoculation. RNA sequencing (60 mln reads, 100 bp, paired ends) using NovaSeq 6000 System (Illumina) was done by Macrogen. Differential gene expression analysis is being performed using annotated *L. angustifolius* genome assembly as a reference [9].

Acknowledgments: This work was supported by the Polish National Science Centre SONATA 9 project no. 2015/17/D/NZ9/02112.

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Impact of different seed priming techniques on germination and seedling performance of common bean under water deficit conditions

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Keywords: root morphology, gas exchange, chlorophyll fluorescence

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Achieving rapid and uniform seedling emergence is a key point for crop performance especially under adverse environmental conditions [1].

Several authors [2], [3] have reported that seed priming treatments could accelerate germination and seed emergence and produce more vigorous plants particularly under abiotic stress. Most frequently used seed priming methods are hydropriming (seed soaking in pure water) and osmopriming (soaking seeds in osmotic solution with low water potential, such as polyethylene glycol (PEG), mannitol, sorbitol, KCl, KNO_3, K_3PO_4, $CaCl_2$ etc. [4].

The aim of this study was to investigate whether hydropriming and osmopriming increase tolerance to water deficit stress in seedlings of main Croatian common bean landraces.

Four most frequent Croatian common bean landraces; 'Biser'', ''Zelenčec'', ''Trešnjevac'' and ''Zlatni trešnjevac'' [5] were included in this research. Total number of 40 seeds per genotype was used in this research, of which 8 seeds per genotype were treated with different priming treatments:

- d H_2O, during 4 h
- 1.5% KNO_3, during 12 h
- 15 mM $CaCl_2$, during 3 h
- 2% H_2O_2, during 4 h
- -0.8 MPa PEG-6000, during 12 h

After application of priming treatments seeds were germinated in seed germination paper (Anchor paper company, USA), in drought treatment (-0.3 MPa) created using PEG 6000 concentration or in control treatment (0.0 MPa). Number of germinated seeds was counted at the 9th day [6], and on the 15th day of growth different morphological and physiological measurements were performed:

- root morphological traits (root length (RL), depth (RD), width (RW), surface area (RSA), volume (RV), average diameter (RAD), number of tips (RNT), number of forks (RNF))
- leaf chlorophyll content index (CCI),
- gas exchange parameters (net photosynthesis rate (A), stomatal conductance (g_s), transpiration rate (E), mesophyll CO_2 concentration (c_i), water use efficiency (WUE))
- chlorophyll fluorescence (F_v/F_m and F_v/F_o)
- root fresh weight (RFW), shoot fresh (SFW), root dry weight (RDW) and shoot dry weight (SDW).

Data were analysed using mixed model ANOVA. By combining drought treatments and priming treatments different groups were created and discriminant analysis (PROC DISCRIM) was performed to evaluate how valid the groups are, and which variables distinguish the best among them.

Reduced chlorophyll fluorescence (both F_v/F_m and F_v/F_o) indicate stress among plants grown in drought treatment. Drought treatment significantly decreased all measured root traits except average root diameter which increased. In addition, drought treatment increased average CCI, probably due to reduced SFW and SDW, but decreased average A, and g_s values. Irespectivly to drought treatments, priming treatments affected CCI (highest found in KNO_3 primed plants), and root traits (the smallest values, except RAD, were found in PEG primed plants and the highest in $CaCl_2$ primed plants).

The first axis of canonical correspondence analysisexplains more than 50% of the total variation and differentiates among drought treatment and control tretament groups. First axis is positively correlated to root traits (except the RAD which is negatively correlated) and negatively to CCI. Second canonical component explains more than 20% of the total variation and differentiate among: drought_H_2O, drought_$CaCl_2$ and control_H_2O from other groups and is more correlated to gas exchange parameters.

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The cropping system matters – contrasting responses of winter faba bean genotypes to drought stress

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Keywords: intercropping, *Vicia faba*, water deficit

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Intercropping systems including legumes have the potential to reduce inputs and to promote yield production in comparison to monoculture crops. Higher species richness also decreases the vulnerability to abiotic stresses such as water deficit [1]. Seasons of limited water resources are expected to increase in frequency and intensity [2] and can drastically affect the yield production of crops. This highlights the importance of drought tolerance and high water use efficiency (WUE) which are important breeding targets [3]. However, breeding efforts in legumes are traditionally performed in pure stands. We therefore evaluated contrasting genotypes of winter faba bean comparing pure and intercropped stands for their performance under water deficit.

In order to identify genotype-specific traits of drought tolerance, a pot experiment was conducted under controlled environmental conditions in the greenhouse. Two genotypes (S_004 and S_062) of winter faba bean (*Vicia faba* L.), differing in growth habitus and productivity, were cultivated in pure stands as well as in intercropping with winter wheat (*Triticum aestivum* L.; var. Genius) under control and water deficit conditions. Water deficit was induced by deficit irrigation. Plant biomass production, WUE (calculated as total aboveground biomass per water consumption during the growth period) and other drought stress related parameters were investigated.

Surprisingly, water deficit did not cause impaired productivity in all treatments. Instead, faba bean genotypes showed different drought stress responses in pure stands or in intercropping. With water deficit, biomass for genotype S_004 was reduced only in pure stands, whereas genotype S_062 showed a reduced biomass only in intercropping. Moreover, these physiological properties of maintained photosynthetic processes lead to increased WUE of intercropped stands with faba bean S_004 as well as pure stands of faba bean S_062 (Fig.1). These observations were verified by other parameters such as proline content and relative water content of leaves.

In summary, winter faba bean showed little interference of water deficit for genotype S_062 in pure stands while genotype S_004 exhibited little interference of water deficit when grown in intercropping. It can therefore be concluded that the drought tolerance of intercropping systems can be improved by the right choice of the winter faba bean genotype regarding complementary stand architecture and rooting patterns. These genotype-dependent interactions in intercropping further point to a demand for selection specific to intercropping in order to develop suitable cultivars.

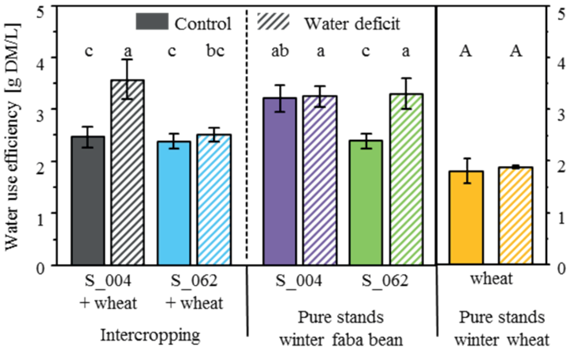

Figure 1. Water use efficiency of the winter faba bean genotypes S_004 and S_062 and winter wheat in intercropped and pure stands. Error bars: standard error. Different letters indicate significant differences. Duncan-test, $p < 0.05$, $n = 4$

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Can an orphan legume offer durable solutions for crop protection? – The continuum of *Lathyrus sativus* responses to powdery mildew

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Lathyrus sativus (grass pea) is a promising source of proteins and calories for drought-prone and marginal areas. Little is known on the availability of resistance in grass pea against powdery mildew, an important air-borne foliar disease affecting legumes worldwide [1]. *Erysiphe pisi*, causal agent of pea powdery mildew, has been described to cause damage in grass pea [2]. More recently, *Erysiphe trifolii* was confirmed to overcome *E. pisi* resistance genes in pea (*er1*), anticipating cases of powdery mildew disease outbreak [3]. Although the genetic bases of powdery mildew resistance in grass pea is still largely unknown, a similar situation of resistance overcome could be occurring in this species, given the phylogenetic proximity with pea.

The present work aimed to assess the range of disease reactions and identify sources of resistance against these two powdery mildew pathogens (*E. pisi* and *E. trifolii*) in a grass pea germplasm collection, representative of the worldwide diversity (180 accessions). Responses to infection by both powdery mildew pathogens were macroscopically evaluated, under growth chamber conditions, considering the observed disease severity (DS) and the infection type (IT) [4].

Differences on the resistance levels to *E. pisi* and *E. trifolii* were found among the grass pea accessions. Our results showed that inoculations with *E. trifolii* lead to higher DS when compared to *E. pisi*. A compatible interaction with no macroscopic signs of tissue necrosis (IT=3, 4) was frequently observed with both powdery mildew pathogens infections. Despite this compatible interaction, accessions with significant DS reduction, as compared to the susceptible control, were identified in response to *E. pisi* and *E. trifolii*, thus depicting an intense partial resistance phenotype.

Additionally, contrasting responses of the same accessions to both pathogens were observed. In this case, 25% of the accessions showing high resistance to *E. pisi* were susceptible to *E. trifolii.* Although to a lesser extent, the opposite situation was also observed. These observations, allied to previous reports of resistance overcome in pea, could be an indication that *E. trifolii* can overcome resistant factors effective against *E. pisi* also present in grass pea, or that an effective response against both pathogens requires the activation of different components in the resistance pathway. Despite the contrasting reactions in the evaluated grass pea germplasm, a set of accessions were identified as resistant to both pathogens, representing a potentially interesting resource for breeding programs.

Results here presented indicate that grass pea gene pool constitutes an important source of resistance against powdery mildew pathogens. Overall, these observations emphasized the importance that the identification of novel resistance sources and clarification of pathogens host-range have for the success of breeding programs. The genetic architecture controlling the partial resistance phenotype, once described, will be of great interest not only for *Lathyrus* spp. germplasm improvement but also for other related legumes species, due to their durable and stable nature [5].

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POSTER SESSION

8.19

Effects of biocontrol agents and elicitors on reducing *Orobanche crenata* infestation and enhancing agro-morphological parameters of two faba bean cultivars

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In Morocco, the parasitic weed *Orobanche crenata* represents a major biotic constraint to production of faba bean. Several control strategies were used but without unequivocal success. Using biocontrol agents to induce host plant natural defence against this parasite constitute a promising solutions. In this perspective, potential use of arbuscular mycorrhizal fungi (AMF), and elicitors on activation of faba bean systemic resistance against *O. crenata* were assessed on two contrasting genotypes. Thus, elicitors used in this experiments reduced *O. crenata* infection in both tested genotypes by 75% for Lobab (susceptible) and 66% for Giza 843 (tolerant). Where AMF reduced significantly *O. crenata* attachment and emergence on both genotypes. These reductions of infection were concomitant with an improvement of host development and/or yield. Moreover, both elicitors and AMF induced phenylalanine ammonia lyase (PAL) and peroxidase (POX) activities in faba bean roots, where the magnitude of induction were more obvious in infested plants than healthy one. These results demonstrate that inducing plant defence could be an important tool for controlling *O. crenata* in faba bean.

Metabolic profile of cowpea plants inoculated with different rhizobia strains and subjected to drought stress conditions

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Keywords: hydroponics, *Bradyrhizobium*, *Enterobacter*, legumes, rhizobia, BNF

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Cowpea (*Vigna unguiculata* (L.) Walp.) is a warm-season, multi-purpose, annual legume cultivated mainly at tropical and subtropical regions, but also in Southern Europe and the United States. Being one of the most drought tolerant legume has the ability to grow in places where the rainfall is irregular and irrigation facilities are missing [1]. As being a legume crop, cowpea, has the ability to fix atmospheric-N through rhizobium symbiosis. This symbiosis however, depends on the host plant genotype, the *Rhizobium* strain, N availability in the root zone, and the environmental conditions [2]. Taking into consideration all the above, an experiment was designed to study the behavior of some N_2-fixing systems under deficit irrigation. For this reason, cowpea plants were inoculated with three different *Bradyrhizobium* strains namely *Bradyrhizobium pachyrhizi* PAC48, *Bradyrhizobium* sp. VULI11 and a mixture of *Bradyrhizobium* sp. VULI11 with endophytic bacteria of the species *Enterobacter* sp. All plants were grown hydroponically under normal or deficit irrigation conditions. For the purpose of this study a commercial cowpea variety resistant to drought conditions was selected. Before the sowing, the seeds were soaked in solution of the above three different strains. As high N levels have negative effects on legumes nodulation, the nutrition solution added to plants contained 50% of the N requirements. The irrigations treatments were applied 20 days after sowing, when the plants reached the stage of 4 true leaves. At the flowering stage, shoot samples from the plants were collected to determine the impact of the different irrigation levels and inoculation with rhizobia on plant growth and the amount of atmospheric N fixed. To assess the levels of biological fixed N, the N^{15} natural method of abundance were used [3]. The results from BNF analysis indicated that the irrigation levels (normal or deficit) and the inoculation of plants with rhizobia had a significant effect on the total amount of fixed N. In particular, under normal irrigation conditions plants inoculated with the mixture of *Bradyrhizobium* sp. VULI11 with endophytic bacteria showed the greater amount of fixed nitrogen (49,92 kg N ha^{-1}) compared to the plants that were inoculated with the strain *Bradyrhizobium pachyrhizi* sp. PAC48 (20.89 kg N ha^{-1}) or VULI11 (25.52 kg N ha^{-1}). However, under deficit irrigation no statistical differences were obtained among the different strains (Figure 1). This clearly indicates that the strains used in this study were susceptible to drought conditions since the cultivar used in this study is drought tolerant. According to Zablotowicz, [4] BNF activity remained unaffected when cowpea plants inoculated with *Rhizobium* sp. strains grown under drought conditions and decreased when the plants were inoculated with *Bradyrhizobium* sp. strains under the same irrigation conditions. To further investigate the differences in the metabolism caused by the irrigation and the inoculants, GC/EI/MS metabolomics combined with bioinformatics analyses were applied. The results indicated that deficit irrigation decreased considerably the N-fixation ability of cowpea causing a general disturbance of the plants' metabolism. Considering the metabolomics analyses, the predominant factor for the observed discrimination was the amount of irrigation provided to the plants, followed by the different inoculants. Differences in both irrigation level and inoculants had a major impact on the biosynthesis of metabolites that play important role in amino acid biosynthesis, carbohydrate and lipid metabolism.

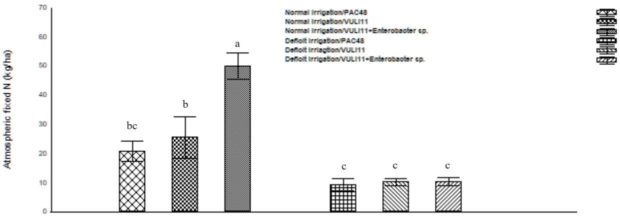

Figure 1. Effects of different irrigation levels and inoculation with 3 different strains on total amount of fixed N

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POSTER SESSION

8.21

Identification of fungi isolated from soybean seedlings – searching for potential pathogens influencing soybean emergence in Poland

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Keywords: soybean, fungal diseases, phytopathogens, *Fusarium* spp.

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Soybean [*Glycine max* (L.) Merrill] is an annual leguminous plant from the *Fabaceae* family. It originates from South-Eastern Asia, with evidence of soybean crops as early as 7000-6600 BC in areas of China. By the mid-1700s, they were introduced to the North American continent, and by the mid- to late 1800s, they had also arrived in South America and Africa. Currently, most soybean is produced in USA, Brazil, Argentina and Paraguay. Soybean cultivation area in Poland in 2017 was 9.333 ha and is still growing [1]. Soybean is a thermophilic plant which is easily damaged by frost, therefore cultivation it in Poland is very difficult. Weather conditions can affect the development of various diseases on soybean seedlings, of which the most dangerous ones are caused by soil-borne pathogenic fungi. Diseases of seedlings can be caused by a wide range of fungi including: *Fusarium* spp., *Phytophthora* spp., *Globisporangium* spp. (*Pythium* spp.), *Rhizoctonia solani*, *Phomopsis phaseoli* (*Diaporte phaseolorum*) [2], [3]. Most of these fungi occur world wide.

The aim of this research was the identification of pathogenic fungi responsible for lowering soybean emergence in Poland. Research material included seven soybean cultivars, which were not studied before in this context, namely cultivars: Annushka, Mavka, Violetta, Atlanta, Madlen, Lajma and Smuglyanka (the seeds of these cultivars were obtained from European soybean breeding company - Age Soya). Plant samples were collected in June 2017 and 2018 from the experimental field located in Makowiska (South-Eastern Poland). Collected material included seedlings with disease symptoms on cotyledons, leaves, stems, roots as well as ungerminated seeds. Seven individual plants/ungerminated seeds were collected from each of the tested cultivars. Fragments of plant tissues with disease symptoms were surface disinfected and placed to petri dishes with the mineral SNA medium. Then obtained fungal isolates were transferred to the agarose PDA medium. A total number of 470 pure fungal isolates were obtained within this study (181 and 289 isolates obtained in 2017 and 2018, respectively). Microscopic and molecular (PCR) methods were used to identify species for each of the isolates.

The most common fungi isolated from infected plants belonged to *Fusarium* spp. In 2017, *Fusarium* genus accounted for 92.8% of the isolates, while in the next year it reached 85.1%. Within all tested *Fusarium* spp., isolates the most frequently detected species was *Fusarium oxysporum* (67.4% in 2017 and 75.7% in 2018). Additionally, in 2018, the other identified *Fusarium* species included: *F. culmorum* and *F. avenaceum*. Less frequently detected fungi were: *Rhizoctonia solani* (8.3% of isolates), *Trichoderma* spp. (3.4%), *Cladosporium* spp. (1.7%), *Mucor* spp. (1%) *Alternaria alternata* (0.3%). Among the seven tested cultivars, in 2017, fungi belonging to *Fusarium* genus were the most frequently isolated from cultivar Annushka (76.9% of isolates), while in 2018 they were the most common among the isolates obtained from cultivar Mavka (97.1% of isolates). However, not all of the isolated fungi may be pathogens. In order to identify them among the isolated fungi, in subsequent study, soybean inoculation tests will be performed in controlled conditions using representative fungal isolates obtained here.

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8.22

Spatial dynamics of ascochyta blight caused by *Ascochyta fabae* Speg. in faba bean fields in Tunisia

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Keywords: *Vicia faba, Ascochyta fabae*, spatial pattern, mapping

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Ascochyta blight of faba bean, caused by *Ascochyta fabae* (teleomorph: *Didymella fabae*) is one of the most destructive disease of faba bean (*Vicia faba* L.) in Tunisia. spatial development of ascochyta blight in three faba bean fields located in Oued Mliz, Oued Beja and Mornag, were characterized during 2010-2011 and 2011-2012 using geostatistical analysis. Mass Disease Index (MDI) was assessed every two weeks in each quadra located around the central source of inoculum. Analyzing the spatial structure dynamics of the disease using Lloyed Index of Patchiness (LIP) and the mapping disease distribution at different dates showed that LIP was estimated by an exponential function that decreased through time in all sites and seasons. It dropped below 1 (limit value for aggregated distribution) after 12, 25, 30, 36 and 48 days at Oued Beja and Oued Mliz during both season and during the second season in Mornag in 2011-2012. After these periods, the disease distribution is randomly distributed. However, according to the severity mapping, disease distribution was aggregated even at the end of the epidemics indicating different temporal dynamics as the distance from the inoculum source changes.

Seedling vigour contributes to salinity tolerance in field pea (*Pisum sativum*) seedlings

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Salinity stress causes significant yield losses to field pea in cropping belts of Australia. Two pot experiments were conducted to assess the contribution of seedling vigour to salinity tolerance in semi-controlled environments in 2017 and 2018. The experiments were laid out in a split plot design with salinity treatments (control and salt treated) as a main plot factor and field pea genotypes as a sub-plot factor. The experiments included 147 advanced genotypes including check varieties. After 4 weeks of salinity treatment, visual scores of salinity stress were recorded based on a 1-9 scale (1=tolerant, 9=susceptible) from the salinity treatment. Seedlings were harvested from both the treatments to record dry weights. Dry weights from the control treatment (DWctrl) had significant ($p<0.01$) positive correlations with the dry weights from the salinity treatment (DWstress) ($r = 0.365$ and $r = 0.442$ in 2017 and 2018, respectively). DWctrl had significant positive correlations with the dry weight reduction due to salinity stress (DWredn) in 2017 ($r = 0.172$, $p<0.05$) and in 2018 ($r = 0.274$, $p<0.01$). The DWstress had strong and significant ($p<0.01$) negative correlations with DWredn ($r = -0.843$ and $r = -0.728$ in 2017 and 2018, respectively). The visual score of salinity stress showed strong and significant ($p<0.01$) negative correlations with DWsalt ($r = -0.853$ and $r = - 0.776$ in 2017 and 2018, respectively), strong and significant ($p<0.01$) positive correlation with DWredn ($r = 0.803$ in 2017 and $r = 0.561$ in 2018, respectively). However, the visual score of salinity stress had relatively weak but significant ($p<0.05$) negative correlations with DWctrl ($r = -0.178$ and $r = -0.351$ in 2017 and 2018, respectively). The consistent positive significant correlations between DWctrl and DWstress indicated that seedling vigour contributed to salinity tolerance of the seedlings although the more vigorous genotypes suffered more DWredn. Stronger associations of DWstress and visual scores with DWredn than the correlation of DWctrl with DWredn indicated that DWstress or visual scores or combination of them could be used in assessment of salinity tolerance of field pea seedlings. This could be achieved conducting the pot experiments with only salt treatment resulting in better use of resources.

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Molecular basis of drought stress tolerance in early vegetative stages of *Pisum sativum*

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Keywords: pea, drought, ABA

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A phytohormone abscisic acid (ABA) accumulates under drought stress conditions and plays an important role in the stress response and tolerance of plants. The drought stress experiment was conducted on 7 selected cultivars of field pea from the collection of the Institute of Field and Vegetable Crops, Novi Sad in controlled conditions in the phytotron chamber. The plants were irrigated at the optimal soil water content until the third pair of leaves began emergence. Soil drought was induced by a termination of irrigation up to 18% (moderate drought) and 9% substrate water content (severe drought). Control plants were irrigated at the optimal level of soil water regime (36% substrate water content).

ABA concentration was mesured by the Elisa method. The gene expression levels of three genes of aldehyde oxidase (*PsAO1, PsAO2, PsAO3*), which participates in ABA biosynthesis, were analyzed by semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) technique. Total RNA was isolated from stressed and non-stressed plant leaves. Arabidopsis 18SrRNA was used as an internal control.

An increased concentration of ABA under both stress conditions was noticed, in the conditions of severe drought significantly higher than in the conditions of moderate drought. Results confirmed the expression of three genes (*PsAO1*, *PsAO2* i *PsAO3*) needed for the synthesis of enzyme aldehyde oxidase (AO). Overexpression of the *PsAO2* and *PsAO3* genes was observed at different degrees, while expression of gene *PsAO1* did not indicate significant differences compared to control. As the expression of gene *PsAO3* had constantly been increased under drought conditions, it could be concluded that this gene is of primary importance for pea plants exposed to stress.

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How can grass pea overcome Fusarium wilt? Insights from a natural variation study

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Keywords: *Lathyrus sativus*, *Fusarium oxysporum*, GWAS

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Grass pea (*Lathyrus sativ*us L.) is an annual grain legume with high adaptability to unfavorable environments, which cultivation spread over three continents [1]. Beyond its recognized potential as a feed and food crop, is remarkably resistant to several important legume diseases [2], which may be useful in the breeding of related major legume crops [1].

Fusarium oxysporum (*Fo*) is a soilborne pathogen that causes devastating vascular wilt on a wide range of plant species [3]. It is considered as one of the most destructive disease in pea [4], lentil and chickpea [5], and it was recently identified as a threat for grass pea production in Portugal. The losses caused by this pathogen are expected to be more aggressive and widespread due to climate changes since *Fo* development is favored by warm and dry weather [6]. It is thus urgent then to identify new sources of resistance against *Fo*.

To identify new sources of resistance to *Fo*, 203 grass pea accessions, representing a worldwide germplasm collection, were screened for resistance under controlled conditions against *Fo* f. sp. *pisi* race 2. This isolate is the causal agent of fusarium wilt in pea [7] and has also been identified as virulent in grass pea. Each accession was inoculated in three independent experiments and a total of 15 plants per accession were tested, following a root dip protocol already established for pea [7].

Disease symptoms were evaluated by counting the number of yellow leaves per number of total leaves. This was done from the seventh until the thirtieth day after infection, on a 3-days interval basis. This counting scheme allowed calculating the area under disease progression curve (AUDPC). Using AUDPC as a susceptibility measurement, a wide and continuous range of responses, from highly resistant to susceptible, was identified among the present grass pea collection. Low AUDPC values were the most frequent, suggesting a quantitative nature of the resistance to *Fo* in our collection.

These will allow exploring the genetic basis of resistance/susceptibility of grass pea to efficiently use it on breeding against fusarium wilt. In order to achieve that, this germplasm collection was genotyped using Diversity Arrays Technology [8] and a total of 7,153 high-quality silicoDArT and 3,686 SNPs markers were generated. *Medicago truncatula* genome was used to infer the physical position of each marker since grass pea does not have a reference genome available.

Presently, with the objective to unravel the genetic control of fusarium wilt resistance in grass pea we are using a genome-wide association (GWAS) approach to search for genomic regions controlling fusarium wilt resistance. GWAS will be carried out on the adjusted means for AUDPC combined with the genotypic information from the DArT technology using a mixed linear model accounting for the genetic structure among accessions. With this approach, we aim to develop novel molecular tools to assist resistance precision breeding in grass pea and eventually in other related grain legume crops.

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Powdery mildew resistance QTLs revealed in chickling pea using an improved high-density genetic linkage map

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Keywords: *Lathyrus cicera*, *Erysiphe pisi*, *Erysiphe trifolii*

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Powdery mildew is among the most severe foliar biotrophic fungal diseases recorded in grain legumes [1]. Several accessions of *Lathyrus cicera* (chickling pea), a cool season legume crop, present interesting levels of resistance to this disease [2]. However, the underlying resistance mechanisms are still poorly understood.

Aiming to unveil the genetic basis and the molecular strategies of resistance against powdery mildew, a recombinant inbred line (RIL) population was developed from a cross between two *L. cicera* accessions with contrasting resistance phenotypes to powdery mildew disease (*Erysiphe pisi*) [2]. Recently, this population was used for the construction of the first genetic linkage map of *L. cicera* [3]. In the current work, the resolution of this map was improved by adding DArTseq based SNPs and SilicoDArT makers, obtained through Diversity Arrays Technology. The macrosynteny between *L. cicera* and *Medicago truncatula* was also re-investigated by comparing the order of markers in this *L. cicera* linkage map with their physical position in the *M. truncatula* genome (Mt4.0v1).

Phenotypic quantitative trait loci (QTLs) for *L. cicera* powdery mildew resistance were detected using this newly high-density integrated linkage map. The macroscopic responses to two powdery mildew pathogens known to infect chicling pea (*Erysiphe pisi* and *E. trifolii*) were evaluated for each RIL and parental lines in three subsequent inoculation assays under controlled conditions, using detached leaflets. Disease severity (DS), scored as the percentage of the leaflet tissue area affected by the disease, was used as the phenotypic metric. Results showed a continuous range of resistance-susceptibility responses within the RIL population for both pathogens under study.

The co-localization of QTLs identified for resistance to both pathogens will be discussed. Moreover, the function of genes underlying QTLs are being predicted by sequence alignment to *M. truncatula* genome.

This study extends considerably the available genomic resources in the *Lathyrus* genus, in a step-forward towards genomics-assisted precision breeding in *Lathyrus* spp. and its closest relatives.

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Effect of arachidonic acid of the fungus *Mortierella alpina* on growth of *Fusarium* fungi and their ability to synthesize zearalenone

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Keywords: *Fusarium*, Zearalenone, *Mortierella alpina*, arachidonic acid, inhibition

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The high danger of mycotoxins is that they exert toxic effects in extremely small amounts and are able to intensively diffuse into foodstuffs and cause various diseases that anything from acute skin damage to cancer.

Zearalenone (ZEN), one of the most important mycotoxin, which, when ingested with feed or food, can cause great harm to health to animals and humans [1], [2]. ZEN is a mycotoxin produced by numerous species of *Fusarium*, including *F. roseum, F. tricinctum, F. sporotrichioides, F. oxysporum* and *F. moniliforme.* In recent years, biological control of toxigenic fungi is emerging as an environment-friendly strategy. As the *Mortierella alpina* is a fungus with great antagonistic potentials for controlling pathogens possibly it can be used to limit the development and production of mycotoxins of pathogens.

The goal of this work was to determine the effect of the arachidonic acid (AA) isolated from mycelium of the M. alpina LPM-301 in colony formation and the biosynthesis of ZEN of phytopathogenic fungi *Fusarium tricinctum* F-RKM 0759 and *Fusarium oxysporum* F-RKM 0757nfected alfalfa cultivars.

As seen from table 1 The incubation of *F. tricinctum* with AA resulted in the inhibition of colony formation by 69% and incubation of *F. oxysporum* with AA reduced colony formation by 90%.

Table 1. Effect of AA of the fungus *M. alpina* on colony formation of phytopathogenic fungi

Object under study	percentage of inhibition (+) colony formation in:	
	F. tricinctum	*F. oxysporum*
AA preparation	+ 69	+ 90

The incubation of *F. tricinctum* with AA reduced colony formation by 69%.

To establish the mechanism of the AA inhibitory effect on the phytopathogenic fungi F. oxysporum and F. tricinctum, a chromatographic analysis has been carried out for the extracts from the culture liquid of the fungi, which were obtained by culturing them in the presence of AA (test group) and in the absence of AA (control group)

We used HPLC for analysis of ZEN in the extracts of the culture liquid of the *F. tricinctum* and *F. oxysporum*.

By analyzing the data and evaluating the effect of the AA preparation on the toxin of biosynthesis in phytopathogenic fungi F. oxysporum and F. tricinctum, we have found that when phytopathogenic fungi are grown on a medium with AA, they do not produce ZEN. When the fungi *F. oxysporum* and *F. tricinctum* were grown in the AA-free medium were found the ZEN in the culture liquid.

A number of authors who have noted that ZEN is one of the most common toxins synthesized by fungi of the genus Fusarium, which contaminate grains, silage, and animal feeds ([3]. The effect of the AA preparation on reducing the formation of zearalenone by fungi of the genus *Fusarium* makes it promising as a fungicide for protecting fodder crops from contamination by toxin-producing fungi.

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Legume plants and their associated symbionts in heavy metals contaminated sites

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Post-mining wastes are heavily contaminated with heavy metals like Zn, Pb, and Cd (calamine wastes) or Ni, Co and Cr (serpentine mine spoils) and also show water and nutrients deficiency, which make them unsuitable for growth for the majority of plants [1]. Nowadays special attention is paid on legumes plants and their symbionts that have been adapted to extremely high amounts of trace elements and thus are able to thrive in a metallicolous environment without phytotoxic effects [2]. Some legume species (e.g. *Lotus corniculatus*) are known as pioneer plants that can grow in heavy metals-contaminated soils. This ability to grow in such a demanding environment has been explained by their capacity to symbiotic interactions with nitrogen-fixing rhizobia and mycorrhizal fungi.

In our study, we investigated the response of chosen calamine and serpentine *L. corniculatus* population to heavy metal stress. The symbiotic bacteria were isolated from root nodules and an effective in nitrogen fixation isolates were subjected to phylogenetic analyses, which was followed by the characterization of heavy metals tolerance and plant growth promoting (PGP) abilities. Plant tests showed a positive effect of inoculation by isolated metal-tolerant rhizobia. *L. corniculatus* plants also showed high mycorrhization status on metal contaminated tailings. Structural adaptations of nodules for heavy metal stress conditions were also examined. It was suggested that both the specific characteristics of the habitats and their endosymbionts had a major impact on the growth of legume in the investigated sites.

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8.29

In vitro screening of mutant *Medicago sativa* genotypes for drought stress tolerance at germination stage

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Keywords: alfalfa, seed, PEG, drought, tolerant.

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Drought is one of the major abiotic stress factors threatening agricultural production all around the world. Perennial legume plant alfalfa (*Medicago sativa*) is the most important forage crop of moderate climate region. Along with deep root structure, mature alfalfa is generally considered as drought tolerant. However, alfalfa is especially sensitive to drought at germination, seedling and right after cutting. Various studies provided that poly ethylene glycol (PEG) can successfully be used to provide drought stress at controlled conditions. The mutant M_3 seeds were screened in a half strength root growth MS media containing 35% PEG under in vitro conditions to create drought stress environment in a root growth medium. Tolerant genotypes were determined in the screening and were transplanted to pots and then be grown in growth chamber. Candidate drought tolerant mutant genotypes were grazed at 5 cm cutting height when the first flowering buds visible and drought stress were applied for 24 days. Some morphological, physiological (Protein, MDA, APX and SOD enzyme activities) and molecular aspects (drought related gene expression levels) of drought response parameters were determined and were compared with control plants.

Screening field pea (*Pisum sativum* L.) for tolerance to high salinity conditions

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Field pea (*Pisum sativum* L.) is an economically important salt-sensitive crop that is utilized in rotation with wheat in semi-arid cropping systems; yet few studies have explored field pea response to high salinity conditions outside of germination testing [1,2,3] and even fewer have looked at tolerance to sodium sulphate (Na_2SO_4), the dominant salt affecting plant growth in the Northern Great Plains of the US. Saline soils (Electrical Conductivity (EC) > 4 dS/m) negatively impact over 4.3 million hectares in Montana, the leading state in planted and harvested field pea in the US. Despite its importance, no genes conferring salinity tolerance have been identified and relatively few QTLs and SNP markers have been reported for field pea [1]. In this study, 312 *Pisum* single plant (PSP) core collection accessions and 37 field pea genotypes were screened under high Na_2SO_4 conditions in germination and greenhouse seedling experiments.

Germination screening experiments were designed as randomized complete blocks with 4 replicates. 10 ml of H_2O (control) or 16 dS/m Na_2SO_4 (highly saline) solution was added to 100mm x 15mm plates with a double layer of Whatman^{TM} filter paper. Scarified seeds were sterilized in a 5% NaOCl solution for 20 minutes and rinsed with sterilized H_2O three times. Each plate received 20 seeds. The blocks were arranged in a growth chamber held at 25°C +/- 2°C. Experiments were conducted over a period of 8 days with the number of germinated seeds (radicle length > 1cm) recorded on days 3, 6, and 8. The mean percent of the control germinated was used to analyze tolerance.

Genotypes exhibiting low and high performance across the germination experiments were selected for initial greenhouse seedling screening. To determine the salinity tolerance threshold level for future screening, a concentration series experiment was constructed using a two-factor factorial design arranged in a randomized complete block with 4 replicates and 7 levels of Na_2SO_4 (0, 3, 6, 9, 12, 15, and 18 dS/m). Plastic pots filled with coarse sand media were sown with five seeds each and thinned to 3 plants per pot 3 days post-emergence (DPE). Beginning 10 DPE, all plots were watered daily with 100 ml of a nutrient solution containing a supplemental calcium source and all treated plots received an additional concentration of Na_2SO_4. The EC of the treatment solutions was increased 3 dS/m every 3 days to prevent plant shock. Salinity symptom scores were assessed on days 21, 28, 35, and 42 post-sowing using a visual growth response scale of 1-9 (healthy-dead). Final plant height, dry biomass weight, and score was used for genotype comparisons.

The data from the concentration series (Figure 1) showed the greatest difference in treatment response between the two genotypes at the 9 dS/m Na_2SO_4 level. These results and the inability to efficiently sow seed into field soils with concentrations above 8 dS/m, supported screening all 312 PSP accessions and 37 field pea genotypes at the 9 dS/m level. The phenotypic data from these seedling and germination experiments will be used in conjunction with SNPs available from the PSP accession database to conduct a Genome Wide Association Study (GWAS) and identify potential candidate breeding germplasm conferring high salinity tolerance.

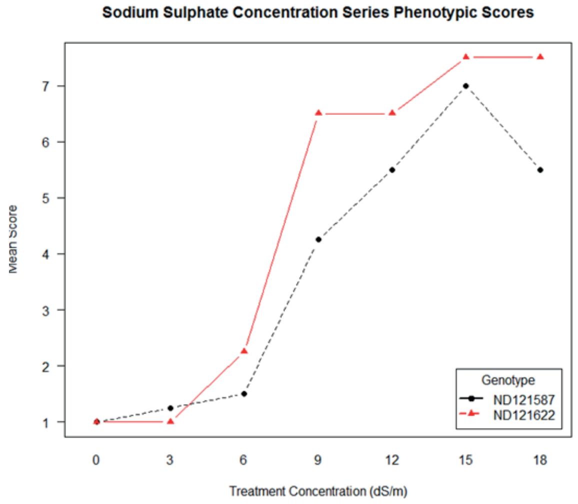

Figure 1. Mean phenotypic scores of two genotypes across Na_2SO_4 concentrations in a greenhouse seedling experiment. Score scale: 1-9 (healthy plant – dead plant)

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[3] Javid, M., Rosewarne, G.M., et al., *Plant Science*, 2015, 6.