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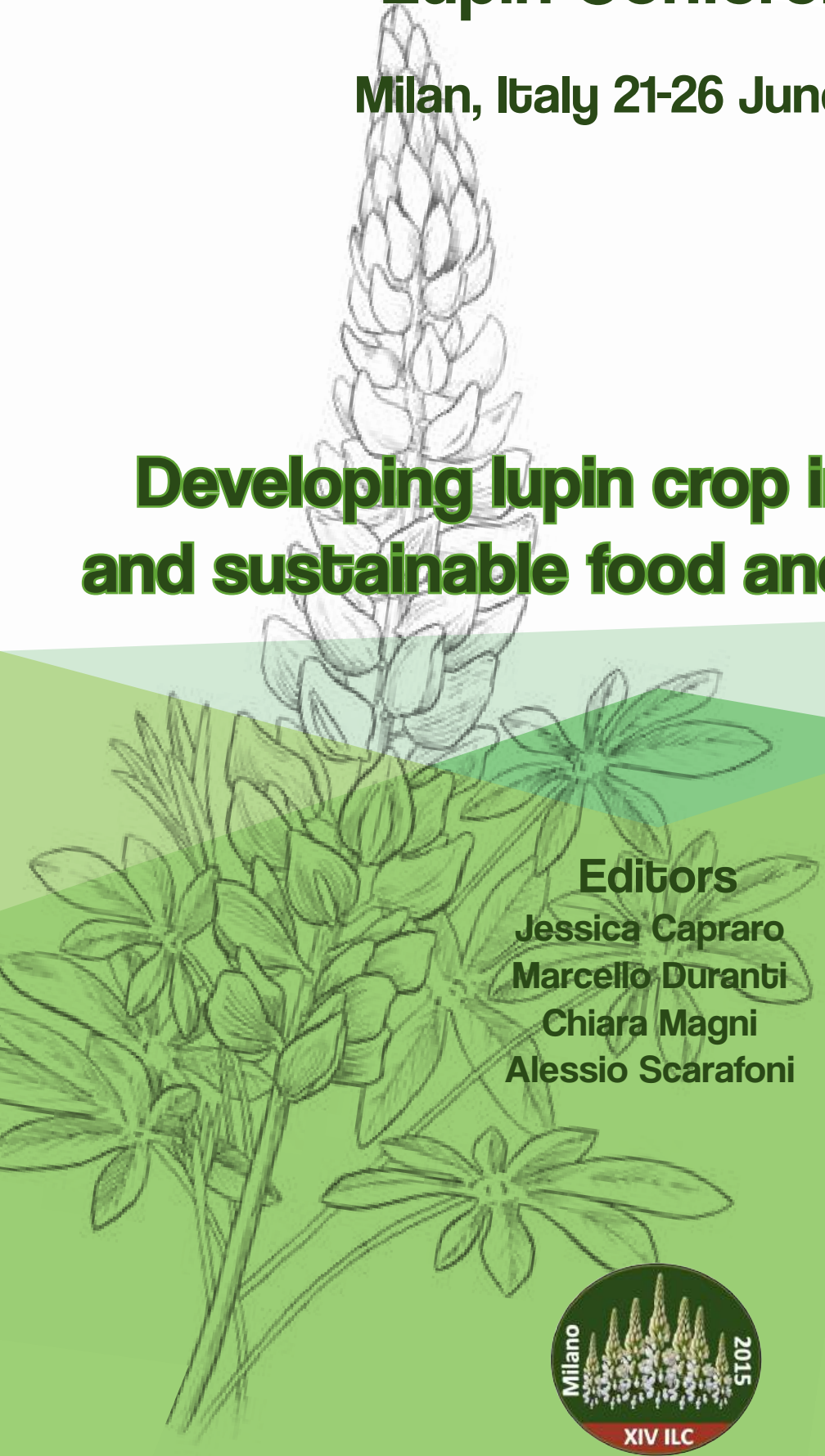

Developing lupin crop into a major and sustainable food and feed source

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XIV International Lupin Conference

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Preface

The XIV International Lupin Conference entitled: '*Developing lupin crop into a major and sustainable food and feed source'* takes place in Milan, Italy in the year of the World Exposition, EXPO-2015 '*Feeding the planet- Energy for life*'.

This choice, which breaks the traditional 3-year interval between the editions, has a clear rationale behind: lupin has been proved to have a great potential as a relevant protein crop in the last years and its positive contribution to human nutrition and health is no longer negligible. Indeed, the current frame, in relation to issues such as global developmental strategies, sustainability and food security, is extremely favorable to the development of alternative sources to animal foods and proteins. Undoubtedly, lupin is one of the richest natural sources of proteins for food and feed.

Therefore, the present edition of the Conference, which is the first held in Italy after wandering all over the world in the previous thirteen editions, acutely aims at addressing the broad theme of human nutrition and the role of lupin in it.

Nonetheless, novel outstanding advances, particularly in the areas of lupin genetics and genomics, have been achieved in the last years and months, and the information on lupin genome can definitely provide the necessary scientific background to all other researches and disciplines.

It seems that our small but enthusiastic lupin community is turning the corner and it is now ready to gather all available knowledge and experience to boost lupin cultivation and utilization. Notwithstanding, bottlenecks are still there; it is also for discussing them with the unique multidisciplinary approach of this Conference that the most prominent experts in the field worldwide meet regularly. At the same time, the attendance of young researchers has been fostered to allow new blood circulating in our community.

This Conference Proceedings book is the results of your contributions in the area and the Editors hope you will appreciate it, as well as the common effort to make it available, either in an electronic form or a printed version, already during the Conference.

Conversely, the Organizing Committee has strongly appreciated your attendance to the Conference, being aware that it is no longer easy to have funds to meet and travel.

Eventually, we wish to thank the ILA Scientific Committee members for their assistance and the few, but keen and farsighted sponsors, who are listed hereinafter.

The Editorial Board
Milan, Italy
June 2015

Technical note to the book
The oral contributions can be in the form of either one-page abstract or mini-paper, according to Authors' choice.

Lectio Magistralis

The evolving story of dietary proteins – from structural and functional nutrients to biopharmaceuticals: is lupin the superstar?

Cesare Sirtori

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A pharmacodynamic potential of dietary proteins, beyond their expected functional and structural properties, has been a major topic of nutritional studies in the last four decades. Initial clinical observations highlighted the potential for legume seed proteins to reduce cholesterol in blood. These observations, carried out mainly with soy, opened up the route to the recognition of active peptide components in these nutrients. Both soy and later lupin proteins proved to break down to small or large peptides, stimulating the expression of the low-density lipoprotein receptor (LDL-R) in human cells, thus reducing cholesterolemia. The field was ripe for the detection of other active peptides, *e.g.* angiotensin converting enzyme (ACE) inhibitory peptides for blood pressure regulation, particularly in lupin, but also in other legumes.

The question has arisen as to the mechanism/s whereby protein components can reach the general circulation. Stemming from the clinical observation that absorbed peptides can trigger allergic reactions, a number of mechanisms has been explored. A significant contribution has come from the observation of increased absorption of small and large peptides by the glucose-dependent insulinotropic polypeptide (GIP), thus uncovering a proton-peptide cotransporter-1 (PEPT1), which mediates the absorption of digestion-resistant peptides. These mechanisms allow, in particular, absorption of blood glucose regulatory peptides.

Aside from the activity related to direct absorption, dietary proteins may act by at least two further different mechanisms. They may act at the ileal "brake" system, suppressing both hunger and food intake. Finally, once absorbed, small peptides may undergo spatial remodeling, thus giving rise to "statin-like" compounds or compounds potentially resembling other therapeutic agents.

These and other investigations will promote a better understanding of the therapeutic potential of dietary proteins, particularly those from lupin, activating a large number of different pathways for the treatment of human diseases.

Oral contributions per session

Session 1

Lupin genome and beyond

Keynote lecture

Development of genetic and genomic resources for narrow-leafed lupin (*Lupinus angustifolius* L.) with a focus on the grain

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Research into the molecular mechanisms that govern growth, development and adaptation in species of the genus *Lupinus* has been hampered by the lack of detailed genomic information. Narrow-leafed lupin (NLL; *Lupinus angustifolius* [L.) is the main grain legume grown in Australia where it forms an important part of sustainable farming systems. The aim of our work is to build new genomic datasets for NLL. Thus over the last few years we have developed a suite of molecular and genomic resources including a large BAC library, large transcriptome data sets from multiple accessions, small RNA and protein datasets, reverse genetic resources and a comprehensive genome sequence for cultivar Tanjil[1,2]. We have used these resources to facilitate extensive marker discovery for this crop[3]. A major focus has been on the NLL grain and large numbers of seed storage protein genes have been identified and characterised and these are candidates for some of the exciting human health properties being asscoiated with lupins in the areas of obesity and diabetes[3].

Many of the genetic and genomic resources we have generated, once published, are housed on the lupin genome portal (http://www.lupinxpress.org) where they are made freely available. The lupin genome portal will have BLAST capabilities to query the genome and transcriptome datasets, a Gbrowse interface to assess the genome and variant calls and Cmap to query the genetic map. Together these resources, which are on pair with those available for other grain legume crops, will significantly improve and accelerate NLL breeding programmes.

References:

[1]Gao LL *et al.* (2011), *BMC Genomics,* 12, 521

[2]Kamphuis LG *et al.* (2015) *Plant Biotech J,* 13, 14-24

[3]Foley RF *et al.* (2011) *BMC Plant Biology*, 11, 59; Foley *et al.* submitted

Acknowledgements: This research was funded by a Grains Research and Development Cooperation (GRDC) grant (UWA00147).

Tags: Genomics, Transcriptomics, molecular markers, conglutins, lupin website.

Development of extensive gene-based molecular markers for narrow-leafed lupin (*Lupinus angustifolius* L.), using in-depth transcriptome sequencing of different tissue types

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Narrow-leafed lupin (NLL) 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. To date, none of the genes that control adaptive traits in NLL have been identified, whilst markers linked to these traits have been developed for marker assisted selection. Developments in genomic technologies now provide opportunities for researchers to identify adaptation genes and to understand how these interact with other genes and with the environment.
To this end we generated in-depth RNAseq datasets from five different tissue types, being roots, stems, leafs, flowers and seeds (Kamphuis *et al.,* 2015). These datasets were used to develop gene-based molecular insertion/deletion (indel) and SNP markers. From these transcriptome datasets we have also identified several groups of genes that are tissue specific. We have adopted high-throughput molecular marker techniques for assaying gene-based SNPs and indels in NLL, which we used to improve the reference genetic map with an additional 745 gene-based markers. These extensive transcriptome datasets, the novel gene-based molecular markers and improved genetic map will be housed on the lupin genome portal (http://www.lupinxpress.org), which will have BLAST capabilities to query the transcriptome datasets, a Gbrowse interface to assess the genome and variant calls and Cmap to query the genetic map. Together these resources will significantly improve and accelerate NLL breeding programmes, especially since NLL has only been 'domesticated' for little more than 50 years.

References:
Berger JD *et al.,* (2013) *Crop and Pasture Sci,* 64, 361-373
Kamphuis LG *et al.* (2015) *Plant Biotech J,* 13, 14-24

Acknowledgements: This research was funded by a Grains Research and Development Cooperation (GRDC) grant (UWA00147).

Tags: Genomics, transcriptomics, molecular markers, lupin genome portal.

Towards an understanding of the complex chromosome evolution of *Lupinus*

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State of the art and aim: Polyploidisation as a genomic event, resulting in multiplication of chromosome sets, has played a pivotal role in speciation and adaptive radiation, leading to the formation of new plant species. Lupins are considered to be paleopolyploids, but their variability in genome size (0.97 to 2.44 pg/2C DNA), chromosome number (2n=32 to 2n=52), basic chromosome number (x=5-8 and 13) and gene duplication (triplication) / gene loss illustrate complex genomic evolutionary history of the genus (Naganowska *et al.* 2003).

The significance of *L. angustifolius* as a crop plant and the relative wealth of data concerning its cytogenetic and genomic characterisation made it a model species within the genus *Lupinus* and a useful reference for studying genome evolution (Książkiewicz *et al.* 2015). The integration of the genetic and chromosome maps of *L. angustifolius* provides not only information about genome structure, but also establishes a solid basis for genetic and genomic research (Lesniewska *et al.* 2011).

The main objective of these studies was to investigate genome organisation at the chromosomal level in a targeted selection of lupin species. We surveyed the heterologous fluorescent *in situ* hybridisation with BAC clones (BAC-FISH) to provide unique information on genome divergence, evolution and relationships of 'Old World' lupins.

Results and discussion: The cytogenetic and genetic mapping of the *L. angustifolius* genome enabled us to identify and use BAC-based chromosome-specific markers. Thus we assigned the 20 linkage groups to appropriate chromosomes of its genome. Moreover, we used these BAC markers for comparative cytogenetic analyses of chromosomal rearrangements between *L. angustifolius* and several related wild species (*i.e. L. anatolicus*, *L. albus*, *L. cosentinii*, *L. digitatus*, *L. hispanicus*, *L. luteus*, *L. pilosus*). These selected BACs assigned to linkage groups (i.e. NLL 08, 11, 17) revealed chromosome changes among lupins. Furthermore, such a set of chromosome markers provided an opportunity to identify individual chromosomes in the genomes of closely related species. We also characterised a set of BACs with dispersed distribution in related species, and clones specific to the *L. angustifolius* genome. Thus we established the set of BACs that can be used as chromosome markers to describe the evolution of lupin genomes.

We showed for the first time that cross-species BAC-FISH is an efficient tool for investigating the divergence and evolution of lupins. Additionally, the chromosome rearrangements observed highlighted complex evolution events that have shaped the evolution of lupin genomes at the chromosome level.

References:

Książkiewicz M *et al.* (2015) *Plant Mol Biol Rep,* 33: 84-101
Lesniewska K *et al.* (2011) *J Heredity,* 102: 228-236
Naganowska B *et al.* (2003) *Ann Bot (Lond),* 92:349-355

Acknowledgements: This work was performed with the financial support of the National Science Centre, Poland (grant no. 2011/03/B/NZ2/01420 and N N301 391939).

Tags: linkage groups, BAC-FISH, chromosome rearrangements

Charting the impact of domestication on genome-wide diversity of narrow-leafed lupin

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State of art and aim: The taming of wild plants to become productive agricultural crops was a gradual process taking hundreds, even thousands, of years in most of our major crop species (Diamond 2002). It involved progressively accumulating domestication traits (such as removal of seed dormancy and reducing seed dehiscence) that made the plants increasingly more useful and productive to people. Narrow-leafed lupin (*Lupinus angustifolius* L.) is exceptional in that its domestication was rapid, starting and finishing within the 20th Century (Hondelmann 1984). It is quite an exception in the drastic decline in diversity present in narrow-leafed lupin crop varieties compared to their wild ancestors, a phenomenon common to most crop species (Berger *et al.* 2012). This phenomenon, known as the domestication bottleneck, was caused by heavy selection for individuals containing newly evolved domestication alleles. In major crops species analysed so far, reductions in diversity are most extreme near domestication genes but can occur anywhere in the genome due to random genetic drift or by heavy selection for productivity loci such as seed size or disease resistance. Little information of this nature is available for recently domesticated crops such as narrow-leafed lupin. Recent advances in genomic technologies now make it possible to survey the effects of domestication on genome-wide diversity in narrow-leafed lupin.

Results and discussion: A set of 151 wild accessions, 26 Australian varieties and 17 European varieties were subjected to high-throughput genotyping using 480 single nucleotide polymorphism (SNP) markers. These markers were anchored to the Tanjil genome sequence with genome-wide distribution, and polymorphic in transcriptome sequence datasets generated from eight wild accessions and eight Australian varieties. Of the 480 SNP markers, 470 (98%) gave clear genotyping results, which were used for subsequent analyses. Population structure analyses identified three major groupings within the narrow-leafed lupin germplasm tested: Group1 contained mainly Australian and European varieties (n = 45). Group2 contained mainly Southern European wild accessions (n = 40). Group3 contained mainly Iberian Peninsula and North African wild accessions (n = 51). The remaining 58 wild accessions were from across the whole geographic range of narrow-leafed lupin and showed evidence of admixture. Further analyses are underway to quantify the extent of variation within and among groupings, and to identify regions of the genome with markedly reduced genetic diversity associated with strong selection for domestication and productivity loci.

References:
Berger J *et al.* (2012) *Theor Appl Genet*, 124, 637-652
Diamond J (2002) *Nature*, 418, 700-707
Hondelmann W (1984) *Theor Appl Genet*, 68, 1-9

Acknowledgements: This research was supported by GRDC, UWA and CSIRO.

Tags: domestication, biodiversity, genomics, population genetics, SNP markers.

Comparative genomics of *Lupinus angustifolius* L. gene-rich regions

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State of the art and aim: Recently, a significant development of molecular genetic resources for the narrow-leafed lupin (*Lupinus angustifolius* L.) has been observed. These include genetic maps with gene-anchored markers, nuclear DNA libraries, a draft genome sequence aligned to the linkage groups, reference transcriptome assembly and cytogenetic markers tagging specific chromosomes. The aim of this research was to localize and analyze several *L. angustifolius* gene-rich regions (GRRs) using the resources mentioned above, together with those generated for other legume species. Complex approach involved: DNA fingerprinting, sequencing, genetic mapping and molecular cytogenetics, followed by bioinformatic analyses.

Results and discussion: The *L. angustifolius* genomic BAC library was screened with several gene-based probes, to track genes involved in symbiotic nitrogen fixation and fatty acid synthesis, as well as with SSR-probes to focus on gene-rich regions in general (Książkiewicz *et al.* 2013, 2015). BAC clones carrying particular sequences were isolated, fingerprinted and assembled into contigs. BAC-end sequence (BES) annotation allowed us to choose clones for sequencing, targeting particular genome regions. *L. angustifolius* reference genetic map (Kamphuis *et al.* 2015) was supplemented with new BES- and BAC-derived markers. Physical and genetic mapping was supported by multi BAC-FISH; new chromosome-specific landmarks were identified. BESs and BACs were aligned to *L. angustifolius* genome draft assembly, where they tagged several hundred sequences. Orientation of more than half of anchored scaffolds was identified by paired BESs. The *in silico* detection of coding regions revealed that the average gene density was higher than 10 genes/100 kb. Using genetic linkage distances and consensus band size data, the ratios of physical to genetic distances were calculated. Values obtained for gene-rich regions were below 150 kb/cM. The physical-to-genetic distance ratios supported the results of functional annotation. Comparative analysis of sequenced *L. angustifolius* GRRs revealed numerous syntenic links to the sequenced legume genomes (*Arachis duranensis*, *A. ipaensis*, *Cajanus cajan*, *Cicer arietinum*, *Glycine max*, *Lotus japonicus*, *Medicago truncatula*, *Phaseolus vulgaris*, and *Vigna radiata*). Some regions of the nuclear genomes retained their quasi-ancestral structures, visualised by identical order and orientation of microsyntenic blocks. The maximum-likelihood and Bayesian phylogenetic inferences provided novel evidences for ancient duplications and triplications in the *L. angustifolius* genome, which putatively have occurred after the divergence of ancestor lineage to *Lupinus*, *Arachis*, and *Glycine*.

References:
Książkiewicz *et al.* (2013) *BMC Genomics,* 14, 79
Kamphuis *et al.* (2015) *Plant Biotechnol J*, 13(1), 14-25
Książkiewicz *et al.* (2015) *Plant Mol Biol Rep*, 33, 84-101
Acknowledgements: Financial support: The National Centre for Research and Development project 244227 SEGENMAS. Ministry of Agriculture and Rural Development Nr 39/2015.

Tags: genome; synteny; sequencing; BAC-FISH; molecular marker; narrow-leafed lupin

The utility of RADseq data for species delimitation and phylogenetics: contrasting patterns among multiple *Lupinus* clades

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State of art and aims: Species delimitation and phylogenetic resolution remain pervasive problems across the genus *Lupinus*, especially for recently evolved clades where species boundaries are still poorly defined and species relationships are unresolved (Drummond et al. 2012). These difficulties present significant problems for documenting taxonomic diversity across the genus. For several large clades we still do not know how many species there are, nor how they are related. Establishing how many species there are and assembling a new taxonomic account are important priorities for lupin research. To address these problems we are assembling densely sampled (all species and multiple accessions of species) phylogenies using genome-wide DNA sequence data generated using nextRAD (SNPsaurus, Oregon, U.S.A). These new phylogenies are interpreted in relation to detailed morphological, geographical and ecological data in order to refine species delimitation and infer robust hypotheses of species relationships.
Results and discussion: So far we have generated nextRAD data for 475 accessions from across three clades (Florida unifoliolate, Old World and Andes). These data have the power to resolve species limits and relationships on an unprecedented scale. For example in Florida the RAD phylogeny provides evidence for delimiting species which is congruent with morphology, geography and ecology, including recognition of one or more cryptic species, despite the very young age of this clade (c. 1 MY). In contrast, in the Old World phylogeny, we have recovered deeper divergences which are congruent with previous species boundaries and hypotheses of relationships. Preliminary results for the very rapid and recent Andean species radiation (c. 85-100 species) show similar potential, offering great scope to finally resolve this large and taxonomically problematic group.

References:
Drummond CS *et al.* (2012). *Syst Biol* 61,443.
SNPsaurus Genotyping. 2015. SNPsaurus, OR, USA. Available at: http://snpsaurus.com/.

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Tags: RADseq, Genomics, Phylogeny, Taxonomy.

Session 2

Lupin genomics and advanced selection tools

Keynote lecture

Bridge sequencing technologies with crop breeding

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Keywords: Next-generation sequencing (NGS), genome sequencing, whole genome re-sequencing, marker-assisted selection (MAS), molecular plant breeding, *Lupinus angustifolius*

Introduction:

In recent years, next-generation sequencing (NGS), genome sequencing, and whole genome re-sequencing have been increasingly applied in plant research. Thousands of plant genomes have been sequenced and reported. However, there are few examples on how to apply these approaches to help practical plant breeding. We aim to examine the utility of these modern technologies on marker development for molecular plant breeding, using *Lupinus angustifolius* as an example.

Results and discussion

(1) Application of NGS as DNA fingerprinting for rapid marker development for MAS

Several methods utilising NGS platforms to sequence complexity reduced representations were established, including RADseq, and genotyping by sequencing (GBS). For major gene controlled traits, such as resistance to anthracnose disease and phomopsis stem blight, we applied RADseq for DNA fingerprinting on 20 representative plants (= 10 R, and 10 S) selected from a large RIL population. We successfully identified 38 and 33 markers closely linked to the two R genes, respectively. The markers were easily converted into cost-effective PCR-based SNP markers. The converted markers were tested on a large segregating population for genetic linkage confirmation, and then validated on breeding germplasm. SNP markers most closely linked to the R genes applicable to a wide range of breeding germplasm were selected, which have now been applied for marker-assisted selection (MAS) in the Australian national lupin breeding program based at DAFWA (Yang *et al.* 2012; Yang *et al.* 2013a).

The protocol of our above examples is the same as what we have successfully used in rapid marker development by MFLP (Yang *et al.* 2002, 2008; Li *et al.* 2010, 2011, 2012a, 2012b), except the MFLP method (Yang *et al.* 2001) was replaced by RADseq. We have demonstrated that NGS-based sequencing on complexity reduced representations shares the same principle as traditional gel-based DNA fingerprinting methods for sampling DNA markers throughout the plant genome, but with much higher efficiency and lower cost (Yang *et al.* 2012; Yang *et al.* 2013a).

(2) Application of NGS for rapid genetic mapping

Genetic mapping is a commonly used approach for marker-trait association discovery, which is particularly useful for QTL-controlled agronomic traits of interest. Genetic mapping using traditional DNA markers was very slow and expensive. For example, over a million dollars were invested in several research projects contributing to genetic mapping on lupin in Australia over the last 20 years, which produced three versions of a lupin map based on the same mapping population with less than 1,500 markers (Nelson *et al.* 2006, 2010). None of those markers these

maps were useful for MAS in lupin breeding, and the map did not have broad implication to lupin molecular genetics research, as the majority of the markers on these maps were anonymous without sequence information.

We applied RADseq in lupin genetic mapping, by which we constructed a dense genetic map consisting of 8,244 markers within one month. This NGS-based new map has several major advantages over previously reported lupin maps. Firstly, the marker number is several times higher than those previously reported, which also provides higher resolution landmarks for the lupin genome. Secondly, all the markers in the new map were sequence-defined, which can be easily and unambiguously transferred and interpreted in any lupin germplasm, and are useful for comparative genomic studies. Thirdly, 7,563 of the markers are SNP markers, which are compatible with high-throughput SNP-genotyping platforms for molecular breeding and genetics studies (Yang *et al.* 2013b).

Based on this new lupin map, we developed a "Lupin SNP Array" with the Fluidigm nanoflidic array genotyping platform, which contains 768 SNP markers evenly distributed across the lupin genome. This "Lupin SNP Array" is now being used in the Australian lupin breeding program as a key tool for rapid marker discovery by framework mapping for any marker population within *L. angustifolius*.

(3) Genome sequencing for developing functional markers

Functional markers based on genes of economic importance are very useful for molecular plant breeding. However, a plant genome may contain tens of thousands of genes. Lessons with lupin have shown that "gene-based markers" not based on target genes of agronomic interest (Nelson et al 2006) were not useful for MAS.

By using the whole genome shotgun (WGS) strategy with NGS, a draft genome sequence of lupin has been completed by scientists at DAFWA (Yang *et al.* 2003b). The lupin genome sequence has been deposited at the Genbank and available publically (website address: http://www.ncbi.nlm.nih.gov/bioproject?term=PRJNA179231). Gene annotation has identified large numbers of functional genes of agronomic importance, including carbohydrate metabolic genes and all the seed-storage protein genes, which provides the opportunity to develop markers influencing yield and seed quality. In lupin genome sequencing, we also discovered a candidate R gene for anthracnose disease, which is co-segregating on all the 190 RILs in a segregating population. Sequence-specific, PCR-based SNP markers were developed from this candidate gene, which offers >99% accuracy in the identification and selection of the R gene on MAS in lupin breeding (Yang *et al.* 2013b).

(4) Application of genome sequencing to develop cost-effective markers for MAS in plant breeding

Markers for MAS must be cost-effective, high throughput, amenable to screen large numbers of plant samples. Traditional markers (eg RFLP, AFLP, DArT) cannot be applied for MAS, because the genotyping procedures are too complex, let alone their high costs. Conversion of these markers into cost-effective PCR-based markers requires sequence information beyond the marker fragments for primer design. Unfortunately, it was often difficult to obtain flanking sequences before the sequencing era.

Now, with the reference genome sequence of lupin available to the public, any DNA marker can easily be converted to a cost-effective format to meet the MAS requirements. The is because a short piece of DNA of any marker in lupin can be applied to a BLAST search of the Genbank (NCBI) database, by which the corresponding scaffold in the lupin genome sequence assembly will be identified. Most of the scaffolds in our lupin genome sequence assembly are in the range from 5,000 bp to 50,000 bp in length, which provides ample sequence information for primer and probe design.

In Australia, molecular markers have been applied to large-scale MAS in the national lupin breeding program since 2002; and all the markers applied in MAS on lupin were developed by DAFWA using the DNA fingerprinting method of MFLP. In 2013, all marker work at DAFWA has moved from gel-based methods into SNP-based technologies following the purchase of the Fluidigm SNP genotyping platform and the LightScanner SNP genotyping platform. By using the draft lupin genome sequence, all previously established gel-based markers linked to 11 key genes of agronomic traits of interests to the breeding program were successfully converted into cost-effective SNP markers (Yang et al 2013b, 2015).

(5) Whole genome re-sequencing for developing diagnostic markers for MAS

MAS in molecular plant breeding requires high consistency between marker genotypes and plant phenotypes in a wide range of germplasm; and the markers should be able to screen progeny resulting from complex crosses involving multiple parents. Unfortunately, most molecular markers are not "perfect" lying within target gene sequences. Due to genetic recombination, cultivars showing desirable marker alleles may not necessarily possess targeted genes, and *vice versa*, which is the so-called "false positive" (Yang *et al.* 2008). To deal with this prevalent problem, molecular biologists need to undertake "marker validation" to determine which markers suit particular crosses, which not only increases the overall cost, but also slows down MAS application. The best solution for this plight is to develop "diagnostic markers" that can be applied in a wide range of breeding germplasm without marker validation.

Recently, we have re-sequenced the whole genomes of 12 commercial cultivars representing a high proportion of pedigrees in the Australian lupin breeding history. We found that "whole genome re-sequencing" is a great way to develop diagnostic markers for MAS through the following two avenues:

(**a) Developing diagnostic markers by genotyping markers on genetic linkage map.** We used the DNA sequences of the molecular markers in the sequence-defined genetic linkage map (Yang *et al.* 2013b) to BLAST search of the whole genomes of the 12 re-sequenced cultivars, and compiled a database of genotypes of the mapped markers against all the 12 sequenced cultivars. Then, we examine the markers on the map closing flanking the genes of agronomic traits of interest. Markers showing genotypes matching with plant phenotypes on all 12 sequenced cultivars were identified as "candidate diagnotic markers". These "candidate diagnostic markers" were then further validated on a large breeding germplsam, by which diagnostic markers applicable to wide range of crosses in lupin breeding were developed.

(b) Developing diagnostic markers by marker mining on geneome sequencing scaffolds. In lupin, we found that markers arising within a small scaffold sequence in the genome sequence assembly (eg, a 30,000 bp scaffold, which represents approximately 0.05 centiMorgan in lupin genome) can have a wide range of marker genotype variation on differenct cultivars (Yang *et al.* 2015). We demonstrated that non-diagnostic markers could be used to BLAST search the 12 whole-genome re-sequenced cultivars. Scaffold sequences bearing the non-diagnostic markers of the 12 re-sequenced cultivars were aligned together. Markers discovered from sequence alignment showing genotype matching with plant phenotypes on all 12 sequenced cultivars were identified as "candidate diagnotic markers", which were further validated on other breeding germplasm. By this approach, diagnostic markers were successfully developed applicable for lupin breeding (Yang *et al.* 2015).

Conclusions

In conclusion, the road of crop sequencing to breeding practice could be divided into the genome scale, population scale, and panel scale (Yang *et al.* 2015). The genome scale primarily focuses on the quality of the crop genome assembly, which is usually based on a single individual plant to avoid the problem of heterozygosity. Most of the genome sequencing studies stays at this stage, where markers useful for plant breeding cannot be developed. The second stage is the

population scale, where genetic variations among multiple individuals or cultivars were discovered. After population-based genomics, crop studies can then move to the panel scale where more attention is paid to precise assessment of crop phenotypes, and the further characterization of genotypes, which enables the capitalization of scientific research to deliver practical benefits to crop improvement and therefore the farming community (Yang *et al.* 2015).

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Lupin phylogenomics using transcriptome data

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State of art and aim: New World lupins comprise over 200 species thought to have originated in the past 5-10 Myr. They include lineages with estimated net diversification rates that rival the fastest speciating plant and animal groups elsewhere in the world (Hughes & Eastwood 2006). Due to the fast speciation rates and the scarcity of molecular markers, phylogenetic relationships within and between different New World lupin clades remain uncertain (Drummond 2008; Drummond *et al.* 2012), which hampers our understanding of the evolutionary history of this group.

Results and discussion: In this work, we use transcriptome data to resolve the phylogenetic relationships of over 50 species of New World lupins, focusing on the two fastest speciating lineages: the Andean and the perennial North American lupins. The RNAseq approach employed provides unprecedented amount of sequence data for each individual, but poses significant challenges for phylogenetic reconstruction. The methodology employed involved assembling a *de novo* transcriptome for each species and using clustering and phylogenetic algorithms to distinguish paralogous genes and assembly errors from true orthologous genes. This allowed us to identify ca. 6 thousand orthologous genes present in at least half the species analysed, which we used for phylogenetic reconstructions with either partitioned Maximum-Likelihood or coalescent-based phylogenetic reconstruction methods.
Phylogenetic trees recovered with the different approaches were in general very similar, exhibited high bootstrap support and with very few exceptions resolved conspecific individuals in monophyletic clades. We find that Andean and Mexican species form a monophyletic group in relation to all other New World lupins, and confirm the paraphyletic status of the perennial North American lupins. Within the Andean lineage, we find strong support for a clade of very high altitude species, and for the close relationship between the domesticated *L. mutabilis* and *L. semperflorens*. Relationships between other Andean species – including unidentified / undescribed species – are also strongly supported. The fully resolved and well supported phylogeny obtained in this work paves the way to further studies into the evolution of this genus, particularly in addressing the role of natural selection and demography in the speciation and adaptation of New World lupins.

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Tags: New World lupins, phylogeny, transcriptomics.

Breeding narrow-leafed lupin for seed quality traits to strengthen market acceptance

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State of art and aim: Lupins have great potential in higher value food and feed markets due to their unique seed quality. Wild, landrace or bitter breeding lines of *Lupinus angustifolius* (narrow-leafed lupin, NLL) seeds contain from 1-2% bitter, antinutritional alkaloids (Gladstones 1970; Wink 1987). The low alkaloid gene, *iucundus*, the sole source of for use in the Australian lupin breeding program, reduces seed alkaloid concentration by 100 fold down to less than 0.02%. Levels of alkaloids in whole seed of any newly released cultivar in Australia ('Australian sweet lupin') are required to meet the Australian and New Zealand Food Standard for lupins of not more than 200 mg/kg. However, very low alkaloid content of vegetative tissues results in increased susceptibility to aphid attack and transmission of aphid-borne viruses (Sweetingham et al., 1998). Low alkaloid in vegetation is indicated to result in lower resistance to disease and predation compared with 'bitter', wild germplasm (Wink 1990). Factors implicated in affecting alkaloid level variation in 'low alkaloid' NLL are nutritional stress (Gremigni et al., 2001), soil acidity and temperature during crop growth (Jansen et al. 2012). NLL has a whole seed protein content of 32% (Wolko et al., 2011), which is relatively high compared to other pulse crops such as peas, chickpea and lentil. We aim to breed for low, stable alkaloid levels, adequate to higher protein and high yield in cultivars.

Results and discussion: Preliminary results indicate that seed alkaloid levels increase on average by 8 fold when comparing sets of genotypes in winter-grown versus summer grown conditions or high versus lower post-flowering temperatures in controlled environments. Some genotypes display low and more stable alkaloids across both environments. A recombinant inbred line (RIL) population derived from two parents containing the low alkaloid gene '*iucundus*' but which differed in their average alkaloid contents between 0.003% (low parent) - 0.016% (high parent) produced a broad spread of alkaloid levels among RILs which were correlated with aphid susceptibility. There were differences in alkaloid levels of seed obtained from main stem versus branches and among seed size fractions of bulk-harvested seed. Data obtained are being used for a QTL analysis for marker assisted selection application for low, stable alkaloids in NLL breeding. It is hoped that future cultivars of lupins will possess seed quality attributes that may enhance their suitability to a range of end-uses and markets.

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Tags: *L. angustifolius*, seed alkaloid, seed protein, recombinant inbred lines

Detecting and exploiting white lupin genetic variation for gamma-conglutin

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State of art and aim: There is growing interest in lupin-based products to be marketed as functional foods or nutraceuticals. The gamma-conglutin protein fraction, in particular, has a proved ability to control glycaemia. Optimizing its industrial production, however, requires information on the extent of genetic, environmental and genotype-environment interaction effects that may influence its content in the lupin grain. If exploitable genetic variation for grain gamma-conglutin content exists, it is important to know whether grain protein content, or the proportion of protein represented by gamma-conglutin, is its main determinant. Apparently, there is no published information on these issues. Our study aimed to generate preliminary information for white lupin, as well as reporting the results of a selection program that included high grain gamma-conglutin content as a major breeding target.

Results and discussion: One line (line 7-50) selected for high gamma-conglutin content over three test environments, a second breeding line (line MB-38) with moderate gamma-conglutin content, and three cultivars adapted to Italian environments (Multitalia, Rumbo and Lucky), were grown in the subcontinental-climate site of Lodi and the Mediterranean site of Sanluri. We assessed gamma-conglutin content in the grain and the protein fraction, grain protein content, and grain yield. Grain gamma-conglutin content displayed large genotype variation ($P < 0.01$), ranging from 1.80 in line 7-50 to 1.47 in Multitalia, with no genotype-environment interaction. Consistent genotype response for this trait was confirmed by same genotype ranking between this set of environments and another set of three test environments. We found genetic variation ($P < 0.01$) also for grain yield, protein content, and proportion of gamma-conglutin on total protein, along with genotype-environment interaction for the first two traits. Simple and multiple regression analyses indicated that grain gamma-conglutin content of the genotypes was largely determined by the proportion of gamma-conglutin in the protein fraction ($R^2 = 0.89$). Line 7-50, however, combined high values of this trait and of protein content, along with sufficient grain yield to achieve high gamma-conglutin yield per unit area. The two sites displayed modest differences for conglutin content in the grain or in the protein fraction, while differing for protein content, grain yield, and gamma-conglutin production per unit area. The Mediterranean site showed lower values of these variables (while exhibiting higher grain oil content in earlier studies). Our results indicate that white lupin selection for higher grain conglutin content is feasable, convenient for this perspective use, and reliable across cropping environments.

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Tags: breeding, cultivar, gamma-conglutin, genetic variation, protein, white lupin.

Novel growth types with improved yield potential in narrow-leafed lupin (*Lupinus angustifolius*)

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State of art and aim: Narrow-leafed lupin provides a source of protein and fibre with exceptional functional properties, which may be used for a variety of purposes including the production of high-quality vegetable foods.
To keep lupin growing economically attractive to the farmer, further improvement of grain yield is mandatory. This may be accomplished by plant breeding. However, genetic variability of advanced breeding materials of narrow-leafed lupin is narrow. To generate novel and potentially valuable genetic variants we started a mutagenesis programme based on a variety of current elite cultivars such as cv. 'Boruta'.

Results and discussion: We selected a panel of aberrant phenotypes, some of which showed a more vigorous growth or higher branching as compared to cv. 'Boruta'. Some of these were propagated to stable mutant (M) lines of higher M generation and assessed for agronomically important traits. In a field trial over 6 environments (2 locations, 3 years) we identified M lines with significantly increased grain yields. For genetic analyses, these M lines were crossed to genebank accessions or backcrossed to cv. 'Boruta'. Segregation analysis suggested a monogenic-recessive inheritance of the underlying growth types.
As a next step, selected M phenotypes together with the wildtype are subjected to differential sequence analysis via RNAseq techniques. Sequences differentially expressed between mutants and wildtype will be used for devising SNP markers linked to the novel phenotypes.

Tags: narrow-leafed lupin, breeding, growth type, grain yield, molecular marker

Exploiting wild diversity for lupin improvement

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State of art and aim: Elite modern narrow-leafed and yellow lupin cultivars contain little genetic and adaptive diversity reflecting a short, fragmented domestication history (Hondelmann, 1984; Berger et al., 2013). To resolve this we suggest a return to wild germplasm that harbours much greater diversity. Australian lupin pre-breeding is doing with 2 distinct strategies:
Black box base broadening. The widely adapted narrow-leafed lupin cultivar Mandelup has been crossed with 8 distantly related Mediterranean wild accessions, and again back-crossed to Mandelup. This BC_2 population has been re-domesticated by selection for sweetness, white flowers and pod indehiscence, and is now being evaluated in multi-environment trials (MET) located along Australian rainfall gradients, and will be genotyped to facilitate MET QTL analysis.
Defining adaptive potential of wild material for targeted trait introduction. To investigate adaptive strategies, wild germplasm collected along Mediterranean rainfall gradients is being evaluated under well-watered conditions and terminal drought and a wide range of traits measured (Berger and Ludwig, 2014).
Results and discussion: Preliminary results indicate transgressive segregation for both flowering time and yield in BC_2 populations, auguring well for future METs. Our ecophysiological studies indicate that high and low rainfall environments have have selected for contrasting, integrated adaptive strategies. In both *L. luteus* and *L. angustifolius* long-season, high-rainfall habitats have selected for competitive traits where delayed phenology supports high above- and below-ground biomass production, leaf area, seed yield and number, but also high water-use, and early stress onset. Conversely, low rainfall ecotypes are characterized by drought escape/avoidance (e.g. early phenology, low biomass/water-use, late stress onset) which limits reproductive potential. Surprisingly, in *L. luteus* high rainfall ecotypes can reach lower critical leaf water potentials while maintaining higher relative leaf water contents than their lower rainfall counterparts, a drought tolerance capacity that appears to have evolved in response to intermittent self-imposed droughts driven by large biomass and water-use (Berger and Ludwig, 2014). Domesticated *L. luteus* and *L. angustifolius* have both been developed from low rainfall ecotypes, probably as a result of early selection pressure for timely ripening in the European summer and subsequent terminal drought escape in Australia. This strategy is ideal for short-season environments, but in *L. luteus* at least comes at the cost of drought tolerance, and is inappropriate for longer-season environments. We are currently investigating mechanistic differences between low and high rainfall ecotypes, and establishing the role of phenology in specific adaptation to deliver traits to widen the adaptive diversity of domesticated lupin.

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Tags: base broadening, adaptation, ecophysiology, *L. luteus*, *L. angustifolius*.

Potential for breeding white lupin for calcareous rich soils in the Netherlands

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State of art and aim: White lupin (*Lupinus albus*) can be an interesting crop for both organic and conventional farmers who are looking for leguminous crops to broaden their crop rotation scheme. Lupin is also of interest for food processors as a guaranteed GMO-free protein source for the replacement of soy. However, the available varieties ripen too late in the Netherlands. Furthermore, the largest areas suitable for arable farming are calcareous rich clayey soils. So, there is a need for early maturing calcium tolerant varieties. In 2010 and 2011, exploratory studies have been conducted with lines of white lupin from the Danish breeder Jørnsgård. The results were positive and the research was broadened in 2012 and 2013. In these years, also lines from the Dutch breeder, Van Mierlo from Globes Seeds, were included. In both years, field trials were conducted in four locations, mostly on calcareous rich clay soils with high pH. Measurements in both years included symptoms for calcium intolerance, soil coverage, plant length, earliness in flowering, earliness in ripening, yield and alkaloid levels. The goal was a broad evaluation of lines of white lupin for suitability for cultivation on clayey soils high in calcium content, in order to determine whether there is sufficient perspective for further breeder for both conventional and organic farming in the Netherlands.

Results and discussion: In total, 14 lines of a branching type, and 4 lines of a candle shape type (non-branching) were compared across four locations in the Netherlands during two seasons, 2012 and 2013. The branching lines came from the Danish breeder (Jørnsgård) and the non-branching lines from the Dutch breeder (Van Mierlo). The non-branching type has only one main branch with flowers and is supposed to ripen earlier and hence can be harvested earlier. The branching type has several branches with flowers, allowing it to close the canopy, also when plant density in the field is too low (because of harrowing or late night frost). Its disadvantage would be delayed ripening because of the difference in flowering between main and side branches. However, some early branching lines appeared earlier in ripening than the tested non-branching lines, although these early branching lines also appear to have lower yield potential. The comparison over four locations and two seasons showed clearly that soil quality and climate clearly influence plant growth. The branching type seemed somewhat more stable in growth than the non-branching type. There appeared to be no clear difference in yield potential between the two plant types. Together, these results show that it is feasible to develop a variety with a stable yield of 4 ton/ha (Nuijten and Prins, 2014). With a price of € 700/ton, this means sufficient return for growers. Of course, a higher yield is preferable. Various chain players have shown interest, for seed multiplication and processing. The market prospects for lupin for human consumption gradually increase. We see opportunities for breeding companies and processing companies to set up a production chain in the nearby future.

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Tags: White lupin, calcareous rich soils, selection, breeding, production chains

Session 3

Lupin cultivation and farming worldwide

Keynote lecture

Lupin and soybean global cropping evolution: lessons learned worldwide and in Argentina

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Lupin and soybean had a contrasting cropping evolution worldwide. Both crops are important sources of oil and protein. However, soybean has several orders of magnitude greater cropped area than lupins. Looking at possible causes of the dissimilar evolution in terms of agriculture and industrial aspects could lead to answers for promoting lupins. From the agriculture point of view, climate and soil played an important role in soybean expansion. The numerous varieties of this crop are adapted to a large number of soil and climates from light to heavy soils and from temperate to tropical climates. On the other hand, lupins require light soils and temperate climates to succeed. Also, the agriculture technology used with soybean in terms of crop breeding towards specific adaptation for soil and climate has led to a fast growing of the cropping area in the past few decades. A map for both crops can illustrate the original and current geographic distribution as well the potential areas of expansion. A particular case is analyzed for sweet lupin crops in Argentina. Lupins had a late start in the 90s and a quite slow expansion in Argentina. On the other hand, soybean started in the mid 50s and in the last two decades its expansion has been exponential. While lupin growing area is only few hundred hectares, soybean has reached 22 million hectares and it keeps expanding. Which were the factors leading to such different evolution in terms of cropping area? A climatic, agronomic and economic analysis is shedding some light into the subject and provides clues on how lupin can emulate soybean in terms of area expansion by profiting on soybean agro-technology and commercialization system already in place. First of all, lupin and soybean are not competing for the same area because one is a winter and the other is a summer crop; this means they are climatically different, so they can share the same area. Both are wide row crops so with a simple adaptation in seeding and harvesting practices, lupin can use the same soybean´s machinery. The already in place soybean transport system and commercialization channels can be used for lupin. To reach the same soybean path, it is necessary to accomplish some goals as it was done with soybean, including to establish a breeding program leading to better adapted varieties to soil and climate, to promote an agriculture extension mechanism to reach the farmer with news highlighting lupin advantages in crop rotation, to establish economic incentives such as tax breaks for lupin cropping, etc. Considering the above factors and the global demand of more protein-oil crops, lupin may have a promising future in many regions worldwide as well as in Argentina.

Australian sweet lupin production in Australia

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State of art and aim: This paper is aiming to discuss the drivers of lupin adoption by Western Australian farmers to help inform the science community of issues that may be addressed by research and development projects. Australian Sweet Lupins (*L.angustifolius*)have been a significant part of broadacre grain production in Western Australia since the mid 1970's. Principally grown for their rotational value (both nitrogen fixation & disease break) for wheat production and their high value as a ruminant animal feed. Australian Sweet Lupin production reached its peak in the 1990's averaging 1.064 million hectares per annum. This area has declined in the last 15 years with an average of 0.52 million hectares in the 2000's, and declining further to 0.29 million hectares per annum from 2011 to 2014.
There are 3 principal reasons for this decline;

1. The rise of herbicide resistant weeds. Successful control of grass weeds with selective herbicides drove widespread adoption of a lupin/wheat rotation in a large area of the Western Australian grain belt. The inability to easily control grass weeds, and an increasing difficulty in controlling brassica species weeds, has necessitated adoption of more difficult techniques, such as Integrated Weed Management (IWM), which do not favour tight rotations with lupins.
2. Herbicide tolerant Canola. The issue of resistant weeds became a 'solved' problem for Western Australian farmers with the widespread use of Triazine Tolerant canola up to 2010 and the increasing use of Glyphosate tolerant (Roundup Ready) canola since 2010. The success of controlling weeds with this technology, along with very profitable markets for canola, has exacerbated the disaffection of lupin production by WA farmers.
3. Climate variability. Australian broadacre agriculture is heavily exposed to variable seasonal rainfall. Starting with a drought in 2000, the past 15 years has seen Western Australia suffer 6 serious drought years, 5 very good years, and 4 average years. Drought years lead farmers to producing more drought tolerant crops, such as wheat, in the year following the drought as a 'risk management' tool to restore their financial position. Lupins area is often downsized in this recovery year scenario.

Results and discussion: Lupins are a relatively minor crop in Australian and global terms. Attracting research into the development of new chemistry for controlling grass and brassica species weeds is problematic. Industry funded research is required to encourage new chemistry discovery.
Drought tolerance of lupins through breeding and/or agronomic techniques to mitigate variable climate influences may sustain lupins as an important crop if yield variability can be minimised.
Market prices for Australian Sweet Lupins in the past 3 years has been high enough (ave AUD300-350/t) to produce a small movement to sowing more lupins.
Farmers react to higher profitability of any crop. Development of the Lupin Food industry is seen as a basis for sustaining attractive pricing for lupins into the future. The human food ingredient industry currently presents the greatest potential for demand and value growth for lupins.

Tags: lupin, food, climate, profitability, Integrated Weed Management, IWM, weeds

Sweet blue lupin (*Lupinus angustifolius* L.) as multipurpose crop: on-farm yield performance, different utilization options and smallholder farmers' perception in Ethiopia

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Abstract

Though bitter lupin is an old aged traditional crop, sweet lupins are new crops to Ethiopia. Recently sweet lupins are introduced to north western Ethiopia and are proved to be adaptive as multipurpose crop in the area. A series of on-farm trials under farmers' condition were conducted to evaluate their yield performance, feed and food value. The yield performance of three sweet blue lupin cultivars was evaluated in a randomized complete block design (RCBD) using farmer as a replication in three study districts (*South Achefer, Dera* and *Finoteselam*). The feeding value of sweet lupin grain for sheep was evaluated using independent t-test and the food value was evaluated using sensory test with inexperienced panelists. Among the three districts grain yield from the three cultivars was significantly higher ($P<0.05$) at South Achefer. At South Achefer cultivar Sanabor gave the highest grain yield (4.4t/ha) compared to the other two cultivars, however there was no significant difference among cultivars ($P>0.05$) in grain yield in the other two study districts. There was no significant difference in daily weight gain between animals fed on sweet lupin grain (52.8 g/day/sheep) and those fed on the standard protein supplement (68.8 g/day/sheep) used in the study area. The result of sensory evaluation showed that substitution of up to 75% of field pea with lupin for the preparation of traditional stew, *shiro wot,* has no significant difference ($P>0.05$) in appearance, texture, aroma and overall acceptability. For taste it has no significant difference ($P>0.05$) up to 25% portion sweet lupin blend with field pea. In addition to these results, farmers were very much interested in the absence of the bitter taste in these new sweet lupin cultivars. From the results of these on-farm trials we can conclude that sweet lupin could be used as an important multipurpose crop in the mixed crop livestock production system of the study area where protein is the limiting nutrient in human food and livestock feed.

Introduction

In Ethiopia white lupin is an old aged traditional pulse crop. However, its use as human food is limited due to its high alkaloid content. The crop is hardly used as livestock feed due to its high alkaloid content. To improve the value of the crop for livestock feed and human food a series of on-station experiments were conducted to evaluate the adaptability and nutritive value of sweet lupins in the traditional lupin growing area. The experiments were adaptation trial using several sweet lupin cultivars (Yeheyis *et al.*, 2012), an extensive laboratory evaluation of these cultivars (Yeheyis *et al.*, 2012) and a feeding trial on sheep using lupin seed as protein source (Yeheyis *et al.*, 2012). The results of the experiments showed that sweet blue lupins are adaptive and promising to be used as livestock feed and other purposes in the study area. However, these experiments were conducted under on-station condition managed by researchers. Therefore, on-farm trials were conducted to evaluate the yield performance and different utilization options of selected promising sweet blue lupin cultivars. Finally farmers' perception towards the newly introduced sweet lupin varieties was assessed. Hence, this paper summarizes the results of these series of different on-farm trials.

Materials and Methods

On-farm yield performance

The trial was conducted in 2012/13 cropping season in the North Western Ethiopia in three districts namely; South Achefer (10.21^0N 36.57^0E), Dera (11.42^0N 37.37^0E) and Finoteselam (10.84^0N 37.36^0E). The altitude for South Achefer, Dera and Finoteselam is 1983, 1900, 1935 m.a.s.l., respectively. For the experiment three sweet blue lupin cultivars namely Sanabor, Probor and Bora were used. The design was RCBD with five replications. During the experiment farmers were used as replication i.e., each farmer planted all the three varieties. The plot size was 10m*10m. Grain yield was estimated by harvesting the whole plot. Spacing was 7 cm between plants and 30 cm between rows. Plant height and number of pods per plant were calculated as the average of three plants sampled randomly.

Animal evaluation

This study was conducted only in Dera district. A total of twenty four yearling *Farta* sheep with initial body weight of 20.5±0.62 kg were used for the experiment. Twelve volunteer farmers were selected and participated in the feeding trial. All the experimental animals were dewormed against internal parasites. They were allocated randomly in to two feed treatment groups: Hay+ 300g/day/head Noug (*Gizotia abyssinica*) cake (T1) and Hay + 400g/day/head whole sweet lupin grain (T2). Hay was used as basal diet and was provided *ad libtum*. Sheep in each treatment group received their supplementary feed twice a day half in the morning and the rest in the afternoon. Water and salt was provided *ad libtum*. The feeding trial was conducted for 105 days.

Utilization for human food

Lupin and Field Pea flour preparation

The study was conducted in South Achefer district, North-Western Ethiopia. Sweet lupin grain was pre cleaned to avoid foreign materials, then slightly roasted. After cooled the roasted grain was splinted to remove the hull. Then the necessary spices were mixed with the splinted lupin and milled together in miller to get a fine flour for the traditional stew, *shiro wot,* making. Flour from field pea was prepared following the same procedure that sweet lupin was prepared. Then using the flour from the two crops five different blend proportion (BP) including control were formulated for the *shiro wot* making. The proportion of field pea:lupin flour for the five blend proportions was 100:0, 75:25, 50:50, 25:75 and 0:100 for BP1, BP2, BP3, BP4 and BP5, respectively.

Sensory evaluation and data analysis

The sensory test was conducted in Bahir Dar University, Ethiopia, following the procedure of Watts *et al. (*1989). A thirty-member, trained but inexperienced panelists consisting of students, researchers and faculty staff of the University were selected to rate the quality attributes. Following the procedure of Yost *et al.* (2006) appearance, Color, texture, flavor and overall acceptability were evaluated according to the 1–9 hedonic scale. The scale had verbally anchored with nine categories, as follows: like extremely, like very much, like moderately, like slightly, neither like nor dislike, dislike slightly, dislike moderately, dislike very much, and dislike extremely. Coded samples (samples of one blended cooked *shiro wot* at once with *injera*) were served for each panelist separately in similar plastic trays.The evaluation was repeated in the field by the farmers for each five sample. Finally the collected data from the on-farm yield performance and feeding trial was analyzed using SAS, version 9.2 and data from sensory evaluation was analyzed using SPSS version 20 statistical software.

Result and Discussion

On-farm yield performance

Among the three study districts seed yield from the three cultivars was significantly higher ($P<0.05$) at South Achefer (Table 1). This could be associated with the better soil fertility condition at South Achefer than the other two sites. At South Achefer cultivar Sanabor gave the highest seed yield (4.4t/ha) compared to the other two cultivars. However, there was no significant difference among cultivars ($P>0.05$) in seed yield in the two study districts. Plant height among the three cultivars was significantly different in South Achefer and Dera. However, number of pods per plant among the three cultivars in all locations was similar. In general the seed yield from the three cultivars in all locations is within the range values reported by Yeheyis *et al.* (2012) from on-station trial.

Table1. LSmeans (±SE) for plant height, pods per plant and seed yield from an on-farm trial of three blue sweet lupin cultivars at three locations (*South Achefer, Dera* and *Finoteselam*) in Ethiopia.

	South Achefer			Dera			Finoteselam		
Variety	PHT(cm)	PPP	SY(t/ha)	PHT(cm)	PPP	SY(t/ha)	PHT(cm)	PPP	SY(t/ha)
Sanabor	77^{a}±1.8	33±1.9	4.4^{a}±0.2	78^{a}±1.8	35±2.9	2.6±0.3	98±2.2	34±1.2	3.0±0.3
Probor	70^{b}±1.8	27±1.9	3.6^{b}±0.2	70^{b}±1.8	38±2.9	2.6±0.3	96±2.2	35±1.2	3.1±0.3
Bora	69^{b}±1.8	31±1.9	3.2^{b}±0.2	78^{a}±1.8	38±2.9	2.6±0.3	101±2.2	32±1.2	2.8±0.3

PHT=plant height; PPP=pods per plant; SY=seed yield. Means followed by different letters in a column are significantly different ($p>0.05$).

Animal evaluation

The difference in average daily weight gain and final body weight between the two treatment groups is not statistically significant ($p>0.05$). But relatively higher body weight gain and final body weight was obtained from T1 (Table 2). The variation could be associated with the higher crude protein and bypass protein content of noug cake compared to sweet lupin grain. The daily weight gain obtained from T2 was lower than the reports of Yeheyis *et al.*, (2012) (76g/day) for *Washera* sheep supplemented with sweet blue lupin seed. The variation could be associated with variation in sheep breed, basal feed type and the on-farm nature of the experiment in this study.

Table 2: LSmeans (±SE) for body weight change parameters of farta sheep fed on the two different supplement feeds.

Treatment groups	Initial weight(kg)	Average daily gain(g)	Final body weight(kg)
T1	20.8±0.62	68.8±3.48	26.8±0.31
T2	20.1±0.62	52.8± 2.50	25.9±0.31
Mean	20.5±0.62	58.8±3.13	26.4±0.31
CV (%)	12.3	13.6	14.2

T1: Hay + 300 g /day/head Noug cake; T2: Hay + 400g/day/head Sweet blue lupin grain

Utilization for human food

The result showed that up substituting up to 75% of field pea with lupin for *shiro* making has no significance difference ($P>0.05$) in appearance, texture, aroma and overall acceptability (Table 3). For taste it has no significance difference ($P>0.05$) up to 25% portion sweet lupin blend with field pea. In all the parameters except taste the blending proportion 0 - 75% sweet lupin had significant difference with that of 100% lupin made *shiro*. This indicates that there is an opportunity to substitute other legumes with lupin for both economic and nutritional benefit.

Table 3. Means (±SD) for the five sweet lupin and field pea blend subjected for sensory parameters

Blend type	Appearance	Taste	Texture	Aroma	Overall acceptability
BP1	7.0±1.3[a]	6.4 ± 1.9[a]	7.2±1.3[a]	7.3±1.4[a]	7.3±1.2[a]
BP2	7.2±1.2[a]	6.4 ± 2.4[a]	7.2±1.3[a]	7.4±1.4[a]	7.2±1.1[a]
BP3	7.2±1.5[a]	7.3 ± 1.6[b]	7.4±1.1[a]	7.0±1.3[a]	7.1±1.4[a]
BP4	7.1±1.4[a]	7.4 ± 0.8[b]	7.3±1.3[a]	6.9±1.7[a]	7.3±1.1[a]
BP5	5.7±2.3[b]	7.6± 1.1[b]	5.8±1.9[b]	5.5±2.4[b]	5.8±2.2[b]

BP1=100:0, BP2=75:25, BP3=50:50, BP4=25:75 and BP5=0:100 field pea:lupin flour blend. Means followed by different letters in a column are significantly different ($p>0.05$).

The bitter lupin is known in the area but its use as food is only as snack after long processing procedure to get rid of the alkaloid. However, after farmers got these sweet lupin cultivars in addition to snack they are using sweet lupin for the preparation of different types of food like the traditional stews, *shiro wot* and splinted *wot*. According to farmer panelists from the five samples delivered they preferred a blending ratio of 50% sweet lupin with 50% field pea. So, this shows that blending sweet lupin with field pea for *shiro* making up to 50% is very acceptable.

Farmers opinion about the new crop

According to the participant farmers the crop's important value is absence of bitter taste, palatability for livestock and its higher productivity. As a result farmers mentioned that the crop is being used for human food, livestock feed and as rotational crop for soil fertility maintenance. Thus, sweet lupin is becoming an important multipurpose crop in the mixed crop livestock farming system of the study area.

Conclusion

The seed yield of all the three cultivars was relatively good. Among the three cultivars Sanabor can be used for further scaling up of sweet lupin production in South Achefer district because of its higher seed productivity. However, in the other two districts all the three cultivars gave similar seed yield and hence all of them can be used as alternative cultivars for further use. From the sensory evaluation and animal evaluation trials it is possible to conclude that sweet blue lupin seed can be used for both traditional stew preparation and as protein supplement in the diets of sheep. Hence, compared to the local bitter lupin cultivar the newly introduced sweet lupin crop has higher value in the area and its acceptance by local smallholder farmers is very high.

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Germination of wild lupinus with ornamental features

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State of the art and aim: The germination percentage is one indicator of seeds viability and indirectly determines the quality of the future adult plant. Water absorption is the first step in the germination process and depends on the permeability of the seed coat and the internal seed characteristics. Seed germination is affected by the deterioration of seed coat and by the loss of cell membrane integrity that involves the leakage of electrolytes. Seed hardness and dormancy are the major strategies for wild species to survive in rough environment conditions. With the aims to verify the possibilities of propagating wild species of *Lupinus* as garden plants, germination experiments were performed to determine the most convenient method to be recommended to amateurs gardeners.

Results and discussion: Table 1 shows the germination percentage in Petri dish. Table 2 shows the total number of seedling in small pot with first ***nomophyl after 15 days*** of germination for each soil mixture.

Table 1. Germination responses to different scarification treatments (Means followed by same letters do not differ significantly – p>0.05)

	L. albescens Germination %	***L. arboreus*** Germination %	***L. gibertianus*** Germination %	***L. honoratus*** Germination %	***L. polyphyllus*** Germination %
Control	0a	5.8 a	0a	2.3a	3.6 a
Hot water	3.3b	18.2 b	5.6b	10.4b	22.1b
Sandpaper	87.4c	83.4 c	71.5 c	72.3c	98.2c
Pierce	84.3c	72.9 c	68.5c	69.7c	94.3c

Table 2. Total number of seedling with first *nomophyl after 15 days of sowing*

	L. albescens # of seedling	***L. arboreus*** # of seedling	***L. gibertianus*** # of seedling	***L. honoratus*** # of seedling	***L. polyphyllus*** # of seedling
(¾ sand-¼ plan.soil	9	9	8	9	10
½ sand-½ plan.soil	8	10	6	8	10

The results showed that the best scarification method was scratching the testa in a lateral side of the seed, with sandpaper. The species with the best performance was *L. polyphyllus* which had the highest number of germinated seeds, in Petri dish and on both soil mixtures. Instructions for germination of wild lupins are included in a booklet guide for lupin cultivation.

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The genus *Lupinus* in Mexico

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State of art and aim: Mexico is a secondary dispersion center of genus *Lupinus*. Between 60 and 100 species had been reported, most of them growing on highlands at altitudes between 2000 and 4000 m above sea level even if some species are adapted to sea level growing in the dunes of Baja California. By reviewing 14 international herbaria we determined the existence of 162 *Lupinus* species names growing in Mexico at altitudes from sea level to 4000m above see level (Bermúdez Torres, 1998). The geographical distribution of this genus in Mexico is very wide, going from Baja California coasts to Chiapas highlands (Dunn, 2001). However, most of them are represented in Central Mexico, in the New Volcanic Axis. This region is the point of confluence of the highlands Sierra Madre Oriental and Sierra Madre Occidental. In Mexico there are several groups working on different topics of genus *Lupinus*. This work aims to present a review on the work doing to understand the genus *Lupinus* in Mexico.

Results and discussion: Present review includes ca. 100 references on the genus *Lupinus* in Mexico and summarized systematic approaches, phytochemical and molecular characterization, pharmacological, clinical and toxicology studies and potential uses of Mexican species. Most studies (32%) are dealing with some possible uses of wild *Lupinus* for restoration, reforestation programs and bioremediation. 20% of the studies about phytochemical characterization of Mexican *Lupinus* species, specifically on quinolizidine Alkaloids (QA), flavonoids and saponins. Most of these chemical studies were done to evaluate possible applications of biological activities. Some clinical and pharmaceutical uses of QA extracts are documented, but also their use as insecticides and fungicides are discussed in these articles. Protein patterns and nutritional values are topic of a high number of research papers on Mexican *Lupinus*. Among all the published work only 10% of references deal with taxonomy of this genus. However, systematic of Mexican *Lupinus* is still not well understood, more than 150 species have been described, however, for a big part of them there is only one herbarium exemplar known, and furthermore, some species were not being collected since a century. Identification keys were elaborated for each regional floras, leading that one species may have several names. In this work we discuss about the possible causes for such a chaotic taxonomy and some strategies for integrative clarification will be proposed. Our work intents to recollect all this information into a global taxonomical data base for Mexican *Lupinus* species. This work will have to be enlarged to Central American species.

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Tags: Mexico, *Lupinus*, biological activity, chemical profiles, restauration

The role of two *lupinus* species in a mountain ecosystem in Córdoba, Argentina

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The mountains regions of Córdoba have diverse ecosystems which are affected by a constant deterioration caused by deforestation, advance of agricultural frontier, rural fires, exotic plants and expansion of urban areas (1). The mountain environment and specifically the watersheds play an important role in the water cycle and their deterioration may change the function and appearance of the landscape, accelerating biodiversity losses and culminating in desertification processes. Several species of *Lupinus* were cited as pioneers in the colonization of dunes (2), and in the recovery of areas affected by volcanic eruptions (3). The objective of this research was to survey the mountain areas of Córdoba, Argentina and to analyze the role of vegetation cover and its species in the protection of land or in the colonization of degraded areas.

Results: The results show that along with *Husperzia saururus* (Lam.) Rothm, *Lupinus buchtienii* Rusby, grows on skeletal and rocky soils on the top of Champaquí mountain (2,884 m). This is the first time to document the presence of this species in Córdoba, which is the southernmost distribution along the Andean Region. Figure 1a shows the specimen collected that has a narrow taproot and a prostrate and procumbent habit. Another species *Lupinus honoratus* CPSm., was found growing in slopes and hillsides of Pampa de Achala where there is a complex of different types of mosaic vegetation with grassland of tall grasses (*Stipa* and *Festuca*) and areas with lower plants and beautiful flower such as *L. honoratus*, *Glandularia peruviana* (L.) Small and *Nierembergia linariaefolia* Graham var. *linariaefolia*. The physiognomic landscape structure shows different geomorphologic elements and important anthropic disturbances. Herbaria records proved that several populations of *L. honoratus* were recorded in Pampa de Achala and Los Gigantes. Nowadays it is rarely found and can be considered an endangered species. Figure 1b, shows the plant in the natural habitat with well developed inflorescences and racemes bearing immature fruits.

Figure 1

Conclusions: The taproot system and the prostrate and procumbent structure of *L. buchttienii* as well as it capacity for nitrogen fixation are important features on soil protection and enrichment in the upper areas of the mountain region where are the beginning of the watersheds. Lupins and associated vegetation avoid extreme runoff and prevent flooding in the lowland. This type of environmental protection is needed to avoid the large flooding recorded recently (Feb., 2015) in the Sierras Chicas of Córdoba caused by excessive rainfall.

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Tags: mountain environment; endangered species; environmental protection; South American *Lupinus*

Endangered *Lupinus mariae-josephae* species: conservation efforts

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State of art and aim: A lupin endemism, *Lupinus mariae-josephae* (Lmj), singularly has been identified in the Valencia province, in Eastern Spain. This lupin thrives in alkaline-limed soils with high pH, a unique habitat for lupins, from a small area in Valencia region. In these soils, Lmj grows in just a few small, defined patches, and previous conservation efforts directed towards controlled plant reproduction have been unsuccessful. This lupin was thought to be extinct in Valencia until 2007, when it was discovered in a limestone patch. The reasons behind Lmj endangered status are presently unknown. This study will focus on the symbiosis between Lmj and rhizobia, and how this relationship might impact the population size of Lmj. We have previously shown that Lmj plants establish a specific root nodule symbiosis with bradyrhizobia present in those soils, and we reasoned that the paucity of these bacteria in soils might contribute to the lack of success in reproducing plants for conservation purposes.

Results and discussion: Greenhouse experiments using bait soil and Lmj trap-plants showed the absence or near absence of *L. mariae-josephae*-nodulating bacteria in "terra rossa" soils of Valencia outside of Lmj plant patches, and in other "terra rossa" or alkaline red soils of the Iberian Peninsula and Balearic Islands outside of the Valencia Lmj endemism region. Among the bradyrhizobia able to establish an efficient symbiosis with *L. mariae-josephae* plants, two strains, LmjC and LmjM3^T were selected as inocula for seed coating. Two planting experiments were carried out in consecutive years under natural conditions in areas with edapho-climatic characteristics identical to those sustaining natural Lmj populations, and successful reproduction of the plant was achieved. Interestingly, the successful reproductive cycle was absolutely dependent on seedling inoculation with effective bradyrhizobia, and optimal performance was observed in plants inoculated with LmjC, a strain that had previously shown the most efficient behavior under controlled conditions. Our results define conditions for *L. mariae-josephae* conservation and for extension to alkaline-limed soil habitats, where no other known lupin can thrive. In general terms, the singular conclusion is that symbioses impact the distribution of leguminous plant populations, especially endangered legumes, and this should help define future strategies for the conservation of native legume populations.

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Tags: Lupinus, endangered legumes, conservation, alkaline soils

Lupinus polyphyllus as a New Zealand rangeland pasture species

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Abstract
Several medium to long term sheep grazing pasture trials sown in multi-species mixtures, including *Lupinus polyphyllus,* are summarized. At low fertiliser rates of S and P it became the most productive and dominant species and corresponding grazing capacity. At the lower rates, and with grazing it tended to increase in abundance over time by reseeding, though passing to a grass phase as soil nitrogen levels built up.

Introduction
Lupinus polyphyllus is an adventive species to New Zealand from horticultural sources in the 1940's. It was its colonization of roadsides, wet areas, acidic, low P, high Al soils, eroding subalpine soils, and ungrazed areas, but often general absence across the fence in adjacent grazed land which first indicated its possibilities as a controlled sown forage species.

The studies summarized here are within the context of exploring the pasture development options for the cool temperate rangeland tussock grasslands of the South Island of New Zealand (Scott 2008, 2014; Scott et al. 2006; and references therein).

These studies have been based on the general concept of environmental gradients and species niche (Scott et al. 1995), and the experimental approach of sowing multispecies mixtures into a range of conditions desired (alternative land types, allowable fertiliser levels, animal type, possible grazing regimes, etc.) to find those correspondences. The four environmental gradients are: temperature in terms of latitude, altitude, aspect and slope; soil moisture in terms of rainfall, drainage, soil depth, and irrigation; soil fertility – either natural or applied; and the interaction of grazing and treading (=grazing management) with the growing points of species. Within those each forage species has a niche where it would be the most appropriate and productive species to grow.

The current assessment of the prime ecological niche for a range of pasture legumes within those four environmental gradients is given in Fig. 1. These derive from and overview of the literature and establishing trials under various conditions then sow the range of contending species as a single mixture within those, and then let time and experience show which is the most successful in each. In any subsequent application only the main contenders would be sown.

Methods
L. polyphyllus has been included in those pasture mixtures in ten medium to long term (5-35 years) sheep grazing pasture trials initially drilled at one location in an area of depleted hawkweed *Pilosella officinarum* infested native short tussock (*Festuca novae-zelandiae*) grassland with soil of pH ~5.2 and Al ~10-20%. Some are briefly described with the main features, as they relate to *L. polyphyllus,* uplifted from them.

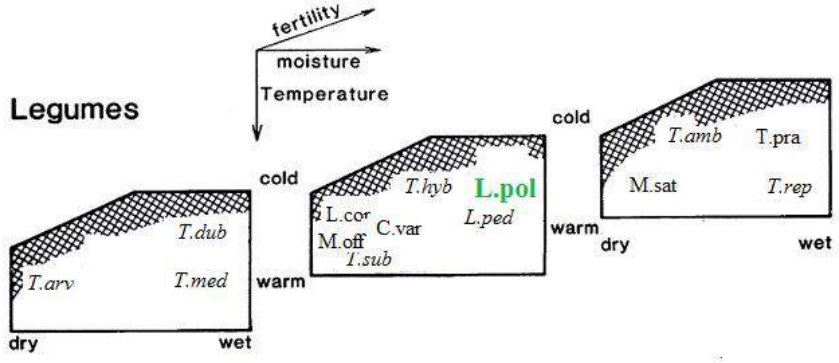
Trial A: a 25 species mixture with 58 treatment combinations of 5 fertiliser/growth levels (nil, 50,100, 250, or 500kg/ha/yr super-phosphate with irrigation in the 500kg), 2 stocking method (sustained grazing, or short term mob stocking); and 3 grazing pressures of each fertility (lax, moderate, hard).

Trial B: a 31 combinations of P and/or S rates of 0, 5, 10, 20, 50 and 100kg element/ha/yr with the same multi-species mixture. In both trials the fertiliser treatments were maintained for the first 20 years of the 35 years to date.

Trial C: an 8 species legume/grass mixture sown into either an area previously undeveloped, or had gone through a previous 5 year legume development phase, with treatments of ± prior

herbicide, spring versus autumn drilling, and use of nil or 150kg N/ha in first two years, superphosphate, and followed for 19 years .

Fig. 1. Dominant niche for pasture legumes in New Zealand in relation to environmental gradients of temperature, moisture, soil fertility and grazing. Rangeland area shaded. Upper case = lax grazing preferred, lower case = hard grazing tolerant. (Scott et al. 1995).



Trial D: a 10 species mixture under low and variable fertiliser levels and possible stock behaviour transfer patterns. The 22 year trial on sloping ground was sown with a mean annual fertiliser rate of 25 kg/ha superphosphate of different granulations under different grazing regimes.

Trial E: over-drilling of *L. polyphyllus* and *Trifolium hybridum* into already partially developed 5 year pastures pasture plots of 24 different grass and legume species, and followed by 4 different grazing regimes for 20 years.

Trial F: a 13 species mixture investigating periodic spelling to allow reseeding. The treatments over 22 years were: grazing every year versus every second year (even and odd); in summer, autumn or both; and at high or low stocking rates.

Trial G: 14 different legume species in individual 1.5 m wide strips and cross sown with similar strips of 14 different grass and herb species into cultivated soil with superphosphate for several years and the common plot grazed hard once or twice each year and followed for 20 years.

Results

Trial A & B: the general features were: the rapid initially sorting out of plant species according to the fertiliser level; an initial legume dominance; the nitrogen build up and gradual legume to grass transition – again both related to fertiliser level; the dominance of *L. polyphyllus* at low fertiliser rates; a slow change relating to sheep stocking levels and methods; the similarity in grazing capacity of the three intermediate fertiliser levels of Trial A; and still little change in subsequent non fertiliser decade.

Other features were: *P. officinarum* remaining dominant in the absence of fertiliser; the initial success of *T. hybridum*; the dominance and persistence of *L. polyphyllus* in the lower fertiliser treatments; the transition to *Dactylis glomerata* dominance for a period in the second decade at higher fertiliser levels following a legume phase; the slow vegetative spread of *T. ambiguum* clover to become the dominant species of the moderate and started from cultivated soil sown of into cultivated soil. and high fertiliser treatments in the second decade and later; the increase of *Arrhenatherum elatuis* in the moderate fertiliser treatments; and grazing management effects on pasture species selection was small relative to that of fertiliser rates.

L. polyphyllus became the dominant, and remained the dominant at the lowest fertiliser levels of the trial, but not without fertiliser. In that sense the 50 kg/ha/yr S fortified superphosphate is the most relevant treatment for discussing its potential role (Table 1). There was little discernible effect of grazing intensity on *L. polyphyllus* during the first decade but it decreased subsequently under the moderate, and more particularly the high stocking rate.

Table 1. Percentage of *L. polyphyllus* in Trial A, and relative grazing achieved.

	Years							Stock
	3	8	13	18	23	28	32	
Fertiliser								
0	29	13	13	11	4	+	+	9
50	27	45	32	8	17	4	3	37
100	23	40	14	7	5	2	1	35
250	27	17	15	2	4	2	+	34
500+I	1	0	0	0	0	0	0	100
At 50 kg/ha								
Low	35	55	55	32	22	5	4	2
Mod.	32	55	55	20	23	15	8	38
High	19	31	13	1	4	3	+	47

Trial B: the initial rapid sorting out as species was related to fertiliser P application rates; while the few direct measurements of pasture production were related to both S and P fertiliser rates; but that the grazing capacity, as measured by sheep grazing days achieved, was related to only S fertiliser rates. *L. polyphyllus* was initially moderately abundant across most S and P fertiliser combinations but subsequently increased in the high elemental S rates.

Analysis of cost of fertiliser components, transport to the rangeland and its spreading in those two trials, relative to gain in grazing capacity, indicated that the most efficient fertiliser regime was low fertiliser rates of ~50 kg elemental S/ha/yr, in the range where *L. polyphyllus* was the most successful species.

Trial C: The desire is for grass dominance in rangeland pastures for their better winter-holding feed capacity. Associated with that is the dilemma of whether to include the grasses in the initial sowing mixture or whether to have a separate grass introduction stage. In Trial C there was a marked legume establishment phase in the previously undeveloped area; and initial failure from competition to new plants in the previously developed area. For *L. polyphyllus*: initially co-dominant with *T. hybridum* in previously undeveloped block; almost uniformly across other treatments; increasing by seeding and rising to dominance in most of those treatments; with that increase from re-seeding establishment being in the presence of continued grazing.

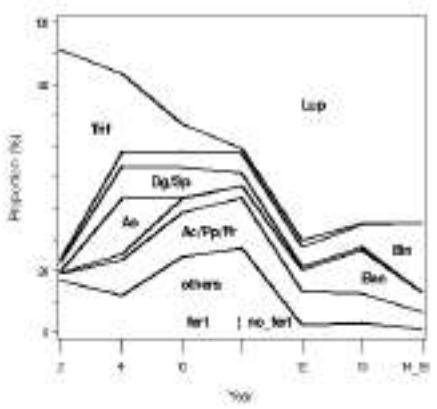
Trial D: *L. polyphyllus* was only introduced into this very low rate fertiliser trial as broadcast sowings in the 7th and 9th year in the presence of continued grazing treatments. It did establish and further increase from re-seeding in the presence of continuing grazing in the lower fertilised sections of plots, but seldom in the upper unfertilised sections.

Trial E: Investigating over-drilling *L. polyphyllus* and *T. hybridum* into previous swards of other species. There was an initial further legume dominant stage involving both legumes but a gradual rise to dominance of *L. polyphyllus,* though increasing least in a hard grazed treatment or the close sward of the *T. ambigum.*

Trial F: Spelling in some years to allow reseeding is advocated in many rangelands. The present trial showed little advantage in periodic spelling of a perennial species based pasture after adjusting for average grazing capacity over time. *L. polyphyllus* became dominant in all but the highest stocking rate plots and sheep were able to penetrate the bulk of second year stands.

Trial G: In this more recent trial, as in other trials, there was an initial legume dominant stage, initially by *Trifolium* species. Over time this dominance has changed to *L. polyphyllus* and continued to increase by self-seeding and displacing species in adjacent areas and under the continuing grazing regime (Fig. 2).

Fig. 2. Changes in species proportions over two decade. Lup = *Lupinus. polyphyllus*; Trif = *Trifolium hybridum, T. repens, T. medium, T. ambiguum;* Dg = *Dactylis glomerata;* Sp = *Schedonorus phoenix;* Ao = *Anthoxanthium odoratum;* Ac = *Agrostis capillaris;* Pp = *Poa pratensis:* Fr = *Festuca rubra;* Bin = *Bromus inermis;* and Ban = *B. tectorum, B. diandrus, B. mollis.*



*B*esides determining what is the most suitable species for a particular combination of environmental and management conditions, the use of a multi-species seed mixtures, can simultaneous give indication of suitable companion species under the same conditions.

Across these trials *L. polyphyllus* was the most successful species at low to moderate fertiliser inputs, and that in those conditions, the other contending legume species, in approximate descending order of merit were: *Trifolium hybridum, T. ambiguum, T.repens, T. medium, Lotus pedunculatus, T. pratense, L. corniculatus, Medicago sativa, Coronilla varia* and *Hedysarum coronarium.*

The corresponding accompanying grass species, again in descending order were: for the taller species *Arrhenatherum elatuis, Dactylis glomerata, Bromus inermis, Secale montanum, Phleum pratense* and *Festuca arundinacea;* and for the shorter under-story species *Festuca rubra commutata, Agrostis capillaris, Poa pratensis, Anthoxanthum odoratum, Holcus lanatus* and annual *Bromus* species.

Discussion

The trials described show that *L. polyphyllus* is a controllable sown forage pasture species. It out-performs and persist other contending pasture species in a combination of environmental conditions – particularly acid-, aluminium- moist soils under low allowable fertiliser rates.

The species, in its present form, could/does fulfil a number of pasture roles. One is as an initial N-fixer and organic matter producer in otherwise N-deficient situations. A second role is as a general rangeland over-sowing species with both N-fixing and stock diet components. The environmental gradient and species niche concepts, and the presently described trials, indicate that *L. polyphyllus* is probably one of the best species if only low fertiliser S and P inputs are affordable – though the resulting pasture production will only be commensurate with that fertility level and the response time longer. A third possible role is as 'special purpose' high input managed pastures as indicated by other papers at the conference.

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Liveweight gain of young sheep grazing perennial lupin-cocksfoot pasture compared with pure lucerne pasture

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Abstract
The liveweight gains of young sheep grazing on perennial lupin-cocksfoot pasture and pure lucerne pasture were compared under dryland (no irrigation) conditions at Lincoln University, Canterbury, New Zealand. This paper reports the results of the first year after establishment (July 2014 to April 2015). Total liveweight gain was 731 kg/ha for the lupin-cocksfoot pasture compared with 1146 kg/ha for the lucerne. The lupin-cocksfoot pasture yielded 6550 kg DM/ha whereas the lucerne yielded 9410 kg DM/ha. The average botanical composition of herbage offered to the sheep was 23% perennial lupin and 68% cocksfoot compared with 94% lucerne. Water use efficiency was higher for lucerne than lupin-cocksfoot when expressed in liveweight gain (3.1 and 2.1 kg/ha per mm of water used) and in herbage yield (24 and 18 kg DM/ha per mm). Overall the lupin-cocksfoot pasture was approximately 65-70% as productive as the lucerne pasture in the first year. These results support the use of perennial lupin-cocksfoot pasture as an alternative forage option in extensive grasslands where cultivation of lucerne is unsuitable.

Keywords: dryland, *Dactylis glomerata*, *Lupinus polyphyllus*, *Medicago sativa*, water use.

Introduction
As global industry competitors in meat and wool production, sheep farmers in the high country of the South Island of New Zealand (NZ) face the challenge of increasing efficiency and productivity. This challenge can be met, at least in part, by the cultivation of pasture species that are adapted to the difficult climatic and soil conditions. The expansion of the area planted in lucerne (*Medicago sativa*) is an obvious and widely adopted option (Anderson *et al.*, 2014). However, lucerne is unsuitable for many regions where low pH, high exchangeable aluminium, and low phosphorus in soils severely restricts its economic use. Other pasture legumes used in NZ are also unsuitable for these conditions. Therefore, alternative pasture options that combine different pasture species are required.
One possible opportunity for enhancing sheep production on high country farms may involve a system where cocksfoot (*Dactylis glomerata*) is grown with perennial lupin (*Lupinus polyphyllus* × *Lupinus* spp. hybrids). Cocksfoot is widely regarded in NZ as a persistent and productive grass that can persist under dry summer conditions and low soil fertility. A long term grazing trial and observations of wild populations in the South Island high country indicate perennial lupin survives the climate and has provided an ongoing source of forage over 30 years. It has been successful in trial plots and is adapted to low pH and low phosphorus soils (Scott, 2014). However, there has been little commercial sowing of perennial lupin in the high country.
This research was developed to enhance the potential of perennial lupin-cocksfoot pasture as a viable forage option for high country farms. The performance of Merino sheep grazing on perennial lupin-grass pasture was quantified on-farm at Sawdon Station, Tekapo (44°03'54"S, 170°29'22"E, elevation 677 m), using one of only a few commercial stands of perennial lupin in the district (Black *et al.*, 2014). To complement this investigation, a more intensive grazing study was established at Lincoln University, Canterbury (43°38'53"S, 172°27'24"E, elevation 9 m). The aim was to evaluate a perennial lupin-cocksfoot pasture relative to a conventional pure lucerne pasture in terms of their sheep liveweight gain, herbage yield and water use efficiency under dryland (no irrigation) conditions. This paper reports the results of the first year after establishment of the Lincoln grazing trial.

Materials and methods

An area of 2 ha was subdivided into three 0.52 ha blocks. Half of each block was randomly allocated a perennial lupin ('Blue' and 'Russell')-cocksfoot ('Kara') pasture or a lucerne ('Force4') pasture. The pastures were drilled into a cultivated seedbed on 5 December 2013. Each of the six 0.26 ha plots was fenced with permanent fences and supplied with stock water; they were divided into five equal "breaks" using temporary electric fences for rotational grazing within each plot. The soil was a Templeton silt loam with pH 6, Olsen P 17 mg/L and sulphate S 1 mg/kg.

In the spring of 2014, ewe hoggets (Coopworth breed, 11-12 months old) were brought on to the plots when the average herbage mass of the pasture was approximately 2000 kg of dry matter (DM)/ha. This occurred on 5 August for lupin-cocksfoot and on 15 September for lucerne. The average live weight of the hoggets at the start of grazing was 38.9 kg and 46.6 kg for lupin-cocksfoot and lucerne, respectively. The stocking rate, duration of grazing and interval between grazing for each break in the rotational grazing system were adjusted regularly between and within plots based on pasture growth rate and average herbage mass. All hoggets were shorn on 28 November 2014 and on 18 February 2015 they were replaced by new ewe lambs (Coopworth, 5-6 months old, average live weight 33.7 kg).

Sheep were weighed unfasted each day they were shifted to the next break in the grazing rotation (every 4-12 days). Liveweight gain was calculated as the change in mean live weight of two or three core animals per group since the previous weighing multiplied by the stocking rate.

Herbage mass was measured in each break every 2 weeks in winter (1 July to 5 August) and then each day the sheep were shifted, using a calibrated sward stick. Herbage yield was the change in herbage mass since the previous measurement. Breaks that were grazed during this interval were excluded from the yield calculation. Botanical composition was determined in each break before grazing by separating and drying quadrat samples. Herbage mass and composition were also quantified every 2-3 days for the duration of each grazing of one break per plot, to determine the acceptance of perennial lupin, cocksfoot and lucerne by the sheep.

Soil moisture was determined in one break per plot on 20 August and then each day the sheep were shifted, using a Time Domain Reflectometer (0-0.2 m depth) and a Neutron Probe (0.2-2.3 m). The amount of water used since the previous measurement, including during grazing, was calculated as rainfall minus the change in soil moisture content.

Results and discussion

The hoggets and lambs grazing on the lucerne grew faster ($P<0.05$) than those on the lupin-cocksfoot pasture (Figure 1A). Average daily gains for lucerne and lupin-cocksfoot were 303 and 211 g/day from the start of grazing to shearing on 28 November, 279 and 151 g/day from shearing to 18 February, and then 179 and 146 g/day until 24 April. The lupin-cocksfoot pasture had enough feed in early spring to start grazing 41 days earlier than the lucerne, but from then on the lucerne was able to support a higher stocking rate (average 19.1 and 15.0 sheep/ha, respectively). Therefore, the lupin-cocksfoot pasture produced more live weight in early spring, but its total liveweight gain was 731 kg/ha compared with 1146 kg/ha for lucerne (Figure 1B).

Spring pasture growth started at about the same time for lupin-cocksfoot and lucerne, in early September (Figure 1C). After that growth was similar for both pastures until mid-November when it started to decrease for lupin-cocksfoot and increase slightly for lucerne. Lucerne growth eventually decreased in mid-January and was about the same as lupin-cocksfoot growth over the following summer and autumn months. However the total herbage yield of the lucerne was 9410 kg DM/ha compared with 6550 kg DM/ha for lupin-cocksfoot ($P<0.05$).

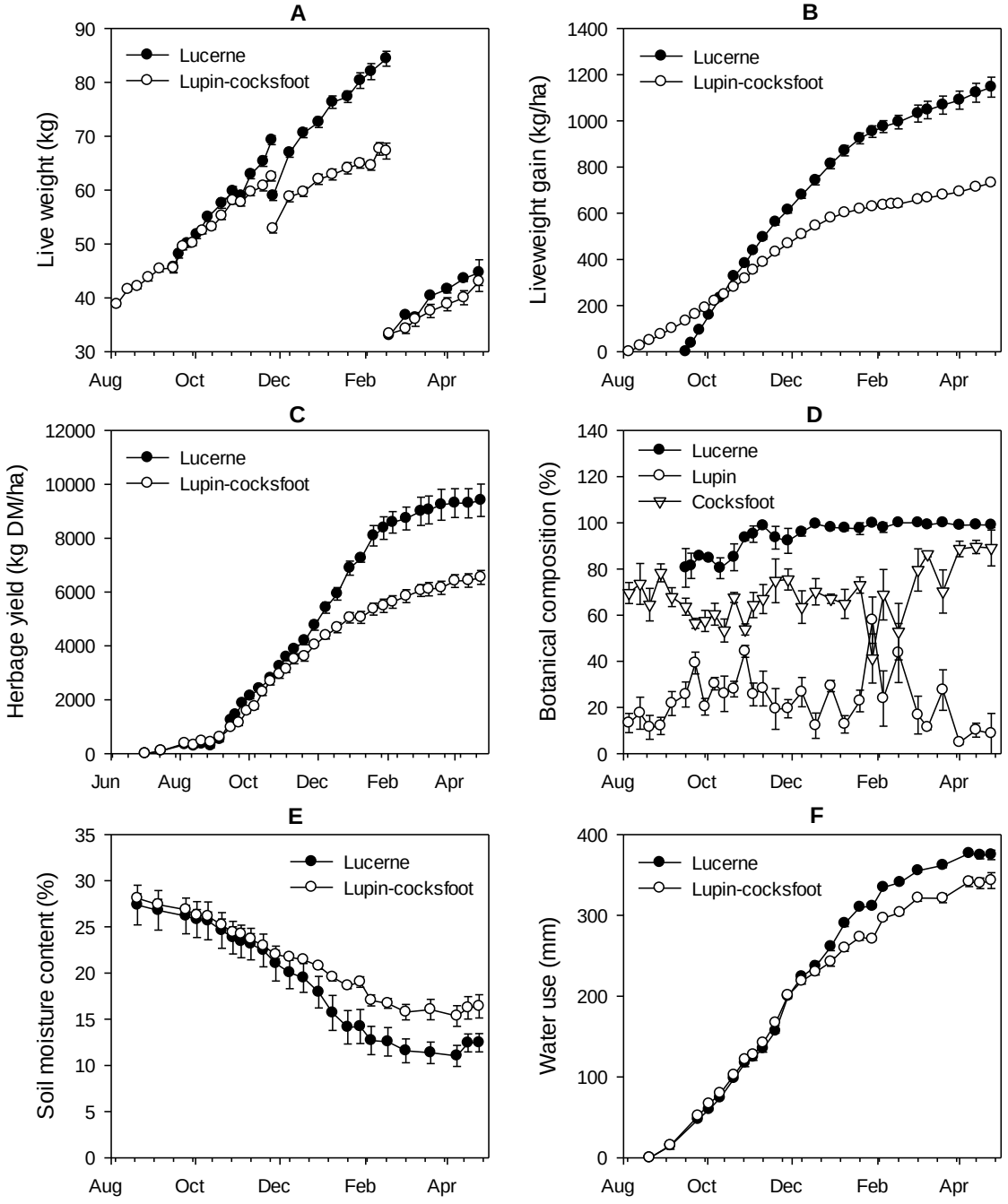

Figure 1. Seasonal changes in (A) live weight of young growing sheep, (B) liveweight gain, (C) herbage yield, (D) botanical composition of pre-grazing herbage, (E) soil moisture content to 2 m depth, and (F) water use of perennial lupin-cocksfoot and pure lucerne pastures in their first year after establishment (1 July 2014 to 24 April 2015) at Lincoln University, Canterbury, NZ. Error bars are SEM.

The average botanical composition of the herbage offered to the sheep was 23% perennial lupin and 68% cocksfoot compared with 94% lucerne (Figure 1D). The disappearance of herbage components during grazing indicated the sheep preferred to eat the leaves of cocksfoot before eating the leaves and petioles of perennial lupin, and preferred the leaves more than stems of lucerne. These results suggest the quality of the feed consumed was higher for the sheep grazing on the lucerne pasture than the lupin-cocksfoot pasture. Preliminary chemical analyses of the herbage support this conclusion.

The serial soil moisture readings indicated that the lupin-cocksfoot and lucerne pastures both extracted water to a maximum depth of 2 m. The soil moisture content to 2 m decreased to as low as 15.3% for lupin-cocksfoot and 11.0% for lucerne on 8 April (Figure 1E). When pasture growth started to noticeably decline (Figure 1C), soil moisture content was 22.9% for lupin-cocksfoot (19 November) compared with 14.1% for lucerne (19 January). From 20 August to 24 April, lupin-cocksfoot used 343 mm of water whereas lucerne used 375 mm (Figure 1F); rainfall was less than normal at 215 mm. Water use efficiency was higher ($P<0.05$) for lucerne than lupin-cocksfoot when expressed as liveweight gain per unit of apparent water use (3.1 and 2.1 kg/ha per mm) and as herbage yield (24 and 18 kg DM/ha per mm) over the same period.

Conclusions

The perennial lupin-cocksfoot pasture was about 65-70% as productive as the pure lucerne pasture in terms of liveweight gain of young sheep (64%), herbage DM yield (70%) and water use efficiency of liveweight gain (68%), under lowland conditions in a moderately fertile soil without irrigation in the first year after establishment.

Acknowledgements

We are grateful for the NZ Merino Company Ltd, Struthers Trust, Sinclair Cummings Trust and Alexander Agribusiness scholarships received by Ryan-Salter.

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Merino lamb and wool production from a commercial stand of perennial lupin (*Lupinus polyphyllus*) on a high country farm in New Zealand

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State of art and aim: Sawdon Station is a 7500 ha high country farm located near Lake Tekapo in the South Island of New Zealand. The farm produces fine Merino wool from about 4500 ewes for the global markets. Following the tradition of early pastoral run-holders, the Merino ewes are raised on a pasture-based system, generally comprised of native grasses and introduced pasture species grown in the difficult high country environment. This system is acceptable for producing fine Merino wool, which is shorn in September, and market weight lambs by February. However, as a global industry competitor in fine wool production, Sawdon Station faces the challenge of improving efficiency and productivity. This challenge is being met, at least in part, by using pasture legumes that are adapted to the environment. The area planted in lucerne (*Medicago sativa*) and perennial clovers has expanded in the more fertile areas, while perennial lupin (*Lupinus polyphyllus*) is cultivated in the lower-cost developments. Perennial lupin survives the climate and is adapted to acidic, low phosphorus soils. However, there has been little commercial sowing of perennial lupin in the high country; Sawdon Station is one of only a few farms using perennial lupin in the district. The objective of this study was to quantify the performance of Merinos grazing on a commercial stand of perennial lupin-grass (10 ha) on Sawdon Station over 4 years.

Results and discussion: The stand of perennial lupin-grass used in this study had persisted under sheep grazing, modest inputs of fertiliser and lime, and 600-650 mm of rainfall a year, for 8 years prior to commencement of measurements in 2011. Over the next 4 years, the stand carried an average of nine ewes/ha from October to May each year. The ewes lambed in October and the lambs were weaned and taken off the stand in February. On average, the annual lambing percentage was 112%, the weight of the lambs at weaning was 27 kg, and the amount of lamb liveweight produced was 344 kg/ha a year. The wool yield in September averaged 4.62 kg/ewe (greasy) with a mean fibre diameter of 18.5 μm. In comparison, a similar flock of ca. 200 ewes managed predominantly on lucerne and clover-based pastures on the farm achieved on average 105% lambing, 31 kg lambs at weaning, and 4.92 kg/ewe of 18.5 μm wool. The average standing biomass in the lupin pasture was 3.0 t of dry matter (DM)/ha at the start of lambing in October, reached 7.8 t DM/ha in December, and decreased to 3.5 t DM/ha in May. Results support the use of perennial lupin where lucerne fails to thrive on high country farms in New Zealand.

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Acknowledgements: The authors are grateful for funding from NZ Merino Company Ltd for year 1 of this trial and from Lincoln University for years 2-4. We are also grateful for the NZ Merino Company Ltd, Struthers Trust, Sinclair Cummings Trust and Alexander Agribusiness scholarships received by Ryan-Salter.

Tags: animal performance, grazing, high country, *Lupinus polyphyllus*, rangeland.

Session 4

Lupin….omics

Keynote lecture

Bioactive low-molecular weight compounds in lupins- potential health and anti-nutritional effects

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State of art and aim: Lupins seeds are rich in proteins and lipids but also contain several other components, which had evolved as means for defense against herbivores, microbes or competing other plants. Aim of this plenary is to discuss the composition of lupin seeds and the pharmacological, toxicological and anti-nutritional properties of their ingredients.

Results and discussion: Lupins seeds sequester a number of secondary metabolites of low molecular weight, including quinolizidine alkaloids, flavonoids, isoflavones, anthocyanins and other phenolics, oligosaccharides, phytin, and saponins (Wink 2011). In addition, some lupins seeds store peptides, such as protease inhibitors (a known anti-nutritional factor). Lupin alkaloids can be considered as neurotoxins affecting ion channels and receptors of neurotransmitters (Wink 2000, 2007) (especially nicotinic and muscarinic acetylcholine receptors). Potential pharmacological applications (Alzheimer, Diabetes) will be discussed. Isoflavones have structural similarity with the female sex hormone estrogen and therefore can be regarded as a phytoestrogen. Flavonoids, isoflavones, and other phenolics have antioxidant properties and might be relevant to influence health disorders caused by reactive oxygen species (ROS). Saponins and phenolics show antimicrobial activities. Data will be presented of a transcriptome analysis by Next Generation Sequencing on insulin secreting pancreatic cells treated with the quinolizidine alkaloid lupanine, which induces the release of insulin.

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Tags: secondary metabolite, quinolizidine alkaloid, isoflavones, phenolics, saponin, protease inhibitor, pharmacology, toxicology.

Gamma conglutin: extraction kinetics and adsorption isotherm studies

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State of art and aim: Lupin, a major rotation crop in Western Australia, has recently attracted interest as a food ingredient isolate, replacing legumes and animal proteins in human diet. Gamma conglutin, a unique lupin protein amongst legumes has already been credited for its blood glucose lowering effect (Magni *et al.* 2004). Most of the reported methods on purification of lupin proteins (Sironi *et al.* 2005) focus on extraction of total proteins via conventional alkaline extraction followed by acidic precipitation at isoelectric point, yielding major conglutins- alpha and beta. However, the more nutritional gamma conglutin remains in acid supernatant after isoelectric precipitation, which is considered as waste stream for many food industries. There are very few reports on isolation of gamma conglutin from lupin seed on analytical scale. These methods are not suitable for large scale preparations. Hence gamma conglutin needs to be exploited by implying high efficiency purification processes such as chromatography on commercial scale for value addition to the lupin crop.
This paper investigates all the key parameters for selective extraction of gamma conglutin enriched fraction from lupin (*Lupinus angustifolius*) seed flour, and presents isotherm & kinetic studies for gamma conglutin adsorption on ion exchange adsorbents for practical applications in preparative scale chromatography.

Results and discussion: The effect of all the key parameters such as pH of extractant, time of extraction, solid to solvent ratio, temperature of extraction and number of extraction stages for selective extraction of gamma conglutin enriched fraction on yield and purity is reported. In our preliminary studies, it is observed that while percentage of protein extracted is higher on alkaline side, gamma conglutin is prominently seen on acidic acid. Using one at a time parameter optimization technique, optimal conditions for obtaining highest yield of gamma conglutin enriched fraction from kernel flour on dry weight basis are obtained and presented. It is observed that extraction of gamma conglutin enriched fraction follows a second order rate kinetic model. Gamma conglutin enriched fraction obtained by following optimized extraction conditions is further then concentrated by ultrafiltration and lyophilized for further adsorbent screening. Adsorption isotherm of gamma conglutin enriched fraction is determined on five cation exchange resins in a batch system. For Langmuir isotherm model maximum binding capacity and dissociation constants are determined, whereas for Freundlich isotherm model, adsorptive capacity and affinity constants are determined. Kinetic data on batch uptake of gamma conglutin enriched fraction on different adsorbents is presented and critical parameters are determined. Both the isotherm and kinetic parameters are further used to select appropriate resin for gamma conglutin purification.

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Tags: lupin, extraction, gamma conglutin, chromatography, adsorption isotherm, kinetics

Quantification of BLAD in sweet varieties of *Lupinus albus*

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State of art and aim: During seed germination, an important aspect of *Lupinus* β-conglutin proteolysis is the accumulation of BLAD in the cotyledons, between days 4 and 12 after the onset of germination. BLAD is a 20 kDa polypeptide which exhibits chitosanase and *N*-acetylglucosaminidase activities, thus inhibiting fungal growth, during experiments conducted since the early 2000's. These results led to the development of a recent, broad-spectrum biological fungicide for plant disease control, known by its absence of toxicity to the environment and humans, an interesting characteristic given the consumer's increasing concern about food safety.

Although BLAD represents an alternative pathway for agricultural production and may increase interest on lupin production in Portugal, there is no evidence about the *L. albus* varieties concerning BLAD cotyledonary concentration, and therefore on the most promising varieties for BLAD extraction and fungicide production. To obtain this information, quantitative assays in seven sweet varieties of *L. albus* were implemented: Amiga, Energy, Estoril, Ludic, Misak, Multitalia and Rumbo.

Results and discussion: In this study, cotyledons from each variety, collected from plantlets aged between 4 and 12-days-old, were used for total protein extraction. Total globulins (β-conglutin and others) were isolated by a modification of Blagrove & Gillespie method (1978). BLAD was subsequently purified by anion-exchange chromatography in an AKTA fast protein liquid chromatography system and quantified.
Quantification assays demonstrated maximal accumulation of BLAD at 4-d-old *L. albus* plantlets, independently of the variety studied. The assays also showed that var. Energy had significant higher accumulation of BLAD (31.67 μg mg^{-1} wet weight) than Rumbo (25.67 μg mg^{-1}) and Misak (22.12 μg mg^{-1}), respectively second and third varieties with highest accumulation of the polypeptide.

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Tags: BLAD, *Lupinus albus*, quantification assays, seed germination, sweet varieties

Lupin storage proteins: targeted proteolysis and unforeseen functionalities

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State of art and aim: The frequent presence of twin and multiple arginine residues in the amino acid sequence of several storage proteins and the occurrence of a -R-R- endopeptidase in *Lupinus albus*, L. seed (Magni *et al*) support the hypothesis that these residues are cleavage-prone sites for selective degradation. Whether such specific cleavages take place in the plant kingdom leading to the liberation of biologically active peptides, as they do in mammalian cells (Becker KL), is not known yet. The 11S storage globulin binds to a metal affinity chromatography matrix and the role of two unusual stretches of contiguous histidine residues at the C-terminusof the 11S globulin acidic chains, was hypothesized. Being this region rich in R-R motifs too, the protein was incubated with the lupin seed R-R-endopeptidase to confirm the metal binding activity of this his-rich peptide (Capraro *et al*).

Results and discussion: Upon incubation with the enzyme, the loss of metal binding capacity paralleled that of the anti-his-tag reactive polypeptides. The recovered small proteolytic fragment was analyzed by mass spectrometry and N-terminal sequencing and found to correspond to the 24-mer region cleaved off at twin arginine residues and containing the natural his-tag-like region. Similarly, when lupin seeds were germinated for a few days, the his-tag containing 11S globulin chain was converted into a form devoid of such region, suggesting that this mechanism is part of the natural and complex breakdown of the storage proteins during germination. The liberation of a metal binding peptide at early stages of germination can play a number of physiological roles in the plantlet. This work supports the hypothesis of an orderly proteolytic deconstruction of storage proteins during germination rather than an extensive, disordered breakdown and also add preliminary insight on the potential physiological role of the transiently liberated peptide fragments. Moreover, the present findings open new perspectives in the identification of cryptic bioactivities related to seed ontogenesis and protection originating prior to complete breakdown with a a mammalian-like specific proteolytic event..

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Tags: 11S globulin, targeted proteolysis, cryptic bioactivities, twin arginine motifs, R-R endopeptidase

Proteomics as a potential biomarker of vigor and vitality of the long-term stored seeds of white lupin (*Lupinus albus,* L.)

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State of art and aim: The ageing of seeds is a natural and irreversible degradation process, which is characterized by loss of vigor and germination capacity. Reduction of the quality of seeds increases with the storage time. The rate of adverse changes in stored seeds are affected by high temperature and high relative humidity. The main cause of the durability reduction of the seeds is considered to be the harmful effects of free radicals. These effects are leading to defects in the structure and to the biosynthesis of the seed storage proteins (Kapoor *et al.* 2010; Gholami and Golpayegani, 2011). In the current study, the authors analyzed individual protein fraction profiles after 28 years of storage at -14°C and +20°C.

Results and discussion: White lupin seeds stored at -14°C germinated better in comparison to seeds kept at +20°C. Seeds stored at -14°C germinated 100%, while those stored at +20°C did not germinate. For seeds stored at -14°C, the content of 11S, 7S and 2S protein fraction were respectively to 2.8%, 13.6% and 0.8%. The content of protein fraction was slightly lower in seeds stored at +20°C and amounted to 3,1%, 12,8% and 0.6%. The SDS-PAGE gel performed for seeds stored in -14°C contained 18, 14 and 14 bands for the individual fractions, while the SDS-PAGE gel created for seeds stored in +20°C was contained 18, 13 and 15 bands, respectively. Many authors have observed a reduction in total protein content in terms of both quantity and quality, also changed the profile of the protein. For example, the total protein content of the yellow lupin seed aging fell by more than 50% (Piotrowicz-Cieślak *et al.* 2008). Lowering the temperature and humidity conditions of the stored seeds slows protein degradation processes significantly, thereby increasing the durability and vitality of seeds (Gholami and Golpayegani, 2011).

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Tags: white lupin, seed, vigor, vitality, protein content, storage

Activity of anti-oxidant enzymes of yellow lupin (*Lupinus luteus*, L.) which grows in soil polluted with oxytetracycline in different light conditions

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State of art and aim: Veterinary medicines are used in the breeding of farm animals (Kim, 2012). Oxytetracycline (OTC), which belongs to a group of tetracyclines, is distinguished by incomplete gastrointestinal absorption in animals, and is often excreted as an unmetabolized compound (Wu, 2011). Then, together with organic fertilizers, it finds its way into soil, where its toxicity can change depending on different light conditions. The presence of OTC in soils has a harmful effect on the organisms which live in this environment. It has been shown that a cultivated plant, yellow lupin (*Lupinus luteus* L.), is a species sensitive to the pollution of soil. The aim of research was the assessment of the effect of OTC present in soil on the selected morphological and biochemical qualities (peroxidase and catalase activity) of yellow lupin seedlings which grow in different light conditions.

Results and discussion: Oxytetracycline caused a modification of peroxidase activity, mainly in the roots of yellow lupin. An increase of the activity of the enzyme was observed in the range from 40 to 200 mg of OTC/kg of soil, both in the summer photoperiod (from 14 to 18 U), and complete blackout (from 19 to 22 U), while in the stems the peroxidase activity was at a similar level (6-7 U). A comparable tendency was observed with catalase. Also the experiments conducted by Liu (2012) showed a phytotoxic action of OTC. The osmotic pressure of lupin seedlings increased together with a growing concentration of the drug in soil, regardless of exposure conditions. Low concentration of OTC (up to 4 mg/kg of soil) brought about the growth of a fresh biomass of roots and stems, both in the summer photoperiod, and in the dark. Higher concentration contributed to the decrease of a fresh biomass of tested tissues. Dry substance of roots and stems grew independently of tested lighting variants. It has been found that OTC was phytotoxic to yellow lupin seedlings.

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Tags: oxytetracycline, lupin, photoperiod, peroxidase activity

Content of biogenic amines in yellow lupin (*Lupinus luteus,* L.) seedlings which grow in soil polluted with tetracycline

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Background and aim: Large-scale analysis conducted in the USA, Germany and Great Britain indicates the presence of a large amount of drugs in the environment. Due to good solubility sulfonamides can easily be absorbed by plants, and significantly curb their growth (e.g. rice and oat) (Liu *et al.*, 2009). The aim of research was to assess the effect of tetracycline present in soil in the following concentrations: 0.00005, 0.0005, 0.005, 0.05, 0.5, 5, 50 mM through the assessment of selected morphological and biochemical qualities on the roots and stems of yellow lupin seedlings.

Results and discussion: It has been shown that the highest analysed concentration of tetracycline (50 mM) curbed the elongation of roots (74%) and stems (88%) of yellow lupin seedlings. High concentrations of tetracycline not only modified the morphological qualities and osmotic pressure of lupin, but also caused the changes in the content of biogenic amines. Polyamines are an important component in plant responses to stress, and play a significant role in counteracting stress (Liu *et al.* 2007). In the control roots of lupin diamine putrescine 157 $\mu g.g^{-1}$ and triamine spermidine 29 $\mu g.g^{-1}$ were determined. The content of biogenic amines grew together with the increase of concentration of the drug in soil. The highest amount 388 $\mu g.g^{-1}$ of putrescine was noted in roots which grew in soil containing 5 mM of tetracycline. The highest amount of spermidine 140 $\mu g.g^{-1}$ was in roots which grew in soil polluted with the highest concentration of the antibiotic (50 mM). Similar growth tendencies of biogenic amines depending on the increase of the tetracycline concentration in soil were noted in the stems of lupin seedlings. Control stems contained 12 and 22 $\mu g.g^{-1}$, of putrescine and spermidine respectively. The highest content, 29 $\mu g*g^{-1}$ of putrescine was found at the 0.05 mM concentration of the drug. The greatest amount, 83 $\mu g.g^{-1}$ of spermidine was in stems exposed to 50 mM of tetracycline. Previous research (Adomas *et al.* 2012) showed that the direct effect of stress factors, including drugs, on plants results in accumulation of biogenic amines. Control roots contained 13 times more putrescine and slightly more spermidine. Roots, which had direct contact with pollutants, were more sensitive to tetracycline than stems.

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Acknowledgements: The present study was financially supported the project NCN UMO-2011/01/B/NZ/02646

Tags: tetracycline, yellow lupin, biogenic amines.

Session 5

Physiology and pathology of lupin

Keynote lecture

Lupins as a model for studies of translocation and systemic signaling

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A number of the Mediterranean species of the genus *Lupinus* share the feature that phloem elements only slowly occlude following a wound to their vasculature. The species include *Lupinus angustifolius*, *L. albus* and *L cosentinii* but not *L. polyphyllus* or *L. luteus*. Among the new world species *L. mutabilis* also bleeds phloem contents from a wound as well as spontaneously from floral abscission zones. This feature has permitted collection of phloem 'sap' from many sites on the shoots of intact lupin plants and has permitted detailed analysis of solutes in these exudates. White and narrow-leafed lupin have proved to be most suited to these studies as their phloem exudate can be collected from virtually any site on the plant, including from both upward and downward translocatory streams in stems, petioles, leaflet midribs, flowers and flower buds, pedicels, peduncles, the stylar tip and both sutures of developing fruits (Atkins *et al*, 2011). The inventory of solutes in lupin phloem exudates includes sugars, organic acids, amino acids, peptides, proteins. mRNAs, small RNAs including micro RNAs, alkaloids, cytokinins and other plant growth regulators as well as a spectrum of inorganic ions (Atkins *et al*, 2007). Coupling data from solute analysis together with data for the deposition or generation of solutes and the growth and development of component organs has permitted a dynamic picture of translocation. Initially economies of C, N and H_2O were developed for fruits and seeds, leaves and nodulated root systems but more recent data has been extended to economies of alkaloids and plant growth regulators. These 'economies' have identified specific sites and mechanisms of solute exchange that fashion particular components of the translocatory pathways to fulfill the nutritional requirements of individual organs. The documented presence of bioactive peptides and proteins and a diverse array of RNA species in the phloem translocation stream has raised the likelihood that these macromolecules are also translocated and their movement serves to alter gene expression in sink organs. As a consequence, a critical 'signaling' role for phloem has been recognized and developed (Rodriguez-Medina *et al*, 2011). Confirmation of the translocation of specific macromolecules is slowly accumulating and while gene targets for their postulated regulatory roles have been identified data unequivocally demonstrating such roles have yet to be gathered. However, there is no doubt that lupin species offer a unique resource among legumes to identify and describe the systemic signals that regulate processes like flowering and flower abscission, seed development, nitrogen fixation and root responses to nutrient supply and soil stresses.

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Tags: Phloem translocation, systemic signaling, phloem solutes and macromolecules

Bradyrhizobia with a distinct *nodA* gene nodulate the exotic *Lupinus polyphyllus* in New Zealand soils

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State of art and aim: Wild populations of perennial, horticultural lupins (*Lupinus polyphyllus* × *Lupinus* spp. hybrids, tribe Genisteae, hereafter *L. polyphyllus*) have colonised roadsides and riverbeds throughout the South Island of New Zealand (NZ). *Lupinus polyphyllus* has potential as a forage crop on acidic, low phosphorus, high aluminium soils in extensive high country grasslands in the NZ South Island. The objectives of this study were to 1) determine if *L. polyphyullus* is nodulated over a wide range of sites throughout the NZ South Island, 2) genotypically characterise rhizobia that nodulate *L. polyphyllus* in the NZ South Island on the basis of their 16S rRNA and *nodA* gene sequences and 3) determine if these rhizobia from *L. polyphullus* can nodulate three important, exotic Genisteae weeds in the NZ South Island (*Ulex europaeus*, gorse; *Cytisus scoparius*, common broom and *Chamaecytisus palmensis*, tree lucerne) and if rhizobia from gorse, common broom and tree lucerne can nodulate *L. polyphyllus*.

Results and discussion: *Lupinus polyphyllus* plants were heavily nodulated at all ten field sites sampled across the NZ South Island. Twenty two bacterial isolates from these nodules formed functional nodules on *L. polyphyllus* indicating that rhizobia that nodulate *L. polyphyllus* are widespread in the NZ South Island. Gene sequences identified all twenty two isolates as *Bradyrhizobium*. Twenty one isolates clustered together for both 16S rRNA and *nodA* gene sequences. The 16S rRNA sequences separated into four groups. Groups l and 2 (11 isolates) were most closely related to the *B. canariense* type strain (*B. canariense*^{T}, 99.58 – 99.75 % similarity) while groups 3 and 4 were most closely related to *B. japonicum*^{T} (99.57 – 99.83 % similarity). For *nodA* sequences, nineteen of the strains clustered together closest to, but clearly separate from (92.89 – 96.67 % similarity), *B. cytisi*^{T}. A representative isolate from *L. polyphyllus* produced functional nodules on gorse, common broom and tree lucerne. Also, a representative isolate from each of gorse, common broom and tree lucerne (all *Bradyrhizobium*) produced functional nodules on *L. polyphyllus*. Results are considered in relation to the possible origin of the bradyrhizobia nodulating *L. polyphyllus* in NZ, the possible requirement of an inoculum for *L. polyphyllus* in NZ South Island high country soils and the success of Genisteae species in establishing over wide areas of NZ.

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Tags: Lupin, *Bradyrhizobium*, Genisteae, nodulation genes

Physiological and biochemical responses of *Lupinus luteus* L. to levofloxacin

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State of art and aim: Drugs, which are used in human and veterinary medicine, are a new risk of environmental pollution. Worldwide, about 100,000 to 200,000 tons of antibiotics are used annually, out of which 82 tons are used in veterinary medicine (Kolar et al. 2014). Drugs, which are introduced into the soil and water, are not readily degradeable by microorganisms. Fluoroquinolones (FQ) induce phytotoxic and toxic effects for most plants and animals (Adomas et al. 2013). The aim of the current study was to assess the influence of levofloxacin on peroxidase activity in lupin (*Lupinus luteus* L.) seedlings. A number of different concentrations of levofloxacin were used in the laboratory analysis. Furthermore, the influence of levofloxacin on elongation of roots and shoots of leguminous seedlings was examined.

Results and discussion: The growth of lupin seedlings (*Lupinus luteus* L.) was analyzed after the application of different concentrations (0.01, 0.05, 0.1, 0.5, 1, 1.5, 2, and 2.5 mM) of levofloxacin after twelve days. The root and shoots growth was analyzed after twelve days. For the seedlings that grew in the soil without levofloxacin (control soil), the longest roots and shoots were of 81 and 71 mm respectively. The lupin seedlings growth was inhibited with increasing concentration of levofloxacin. The highest concentration (2,5 mM) of levofloxacin inhibited the elongation of roots and shoots by 62 and 46 % respectively, in comparison to the roots of the control seedlings. The peroxidase enzyme activity in lupin seedlings was studied by spectrophotometry after twelve days of treatment with increasing concentrations of levofloxacin. A rise in peroxidase activity was observed with increasing concentrations of the antibiotic in the samples from lupin seedlings. Phytotoxic impact of enrofloxacin (FQ group) and sulfamethazine (sulfonamides) on plants was observed by Piotrowicz-Cieślak et al. (2010) and Adomas et al. (2013). The research performed by Piotrowicz-Cieślak et al. on legumes treated with sulfamethazine at concentrations of 0.01, 0.1, 0.25, 1, 5, 15, and 20 mM showed a reduction in root length in comparison to the roots of the control seedlings. Reduction by fifty percent in the root length after three, seven and nine days was observed at a concentration of 10 mM. Phytotoxic impact of levofloxacin was higher than the effect of sulfamethazine. In the studies, the isolated activity of peroxidase in the seedlings that grew in the soil with increasing concentrations of the antibiotic, was tested. In the soil with levofloxacin (2,5 mM), the lupin peroxidase activity was higher than the peroxidase activity in lupin control seedlings. The study showed a negative effect of levofloxacin on the growth of lupin seedlings. The dynamics of seedling growth proved to be the best indicator of soil contamination with levofloxacin. Lupin is a good bioindicator of soil contamination with levofloxacin.

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Tags: lupin seedlings, levofloxacin, peroxidase activity, biotest

Assessing root variability, growth, morphology, and metabolite content, in a diverse germplasm collection of yellow lupin (*Lupinus luteus,* L.)

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State of art and aim: Plant breeders have continuously generated new better yielding varieties more adapted to local conditions and specialized to specific needs of the industry. This constant change has transformed modern varieties into modular entities where genome portions are moved to answer the needs of farmers and industry. However, this breeding effort has been mostly dedicated to increase shoot biomass and seed yields, leaving the study of roots, and their influence upon grain yield, in the rear seat of crop breeding [1]. Recent efforts have shown that by better understanding root physiology, morphology and genetics, significantly increases on seed yield could be achieved even under stress conditions [2; 3]. However, to manipulate root favorable variation, one must first study and understand how much of this variation exists and what percentage of it is genetic. *L. luteus* is a semi-domesticated large orphan crop with great potential to supply the much needed protein by the food industry of southern Chile. Lupins are mostly grown in the South Central part of the country, under a typical wet cold winter/hot dry summer Mediterranean Climate. Given that grain filling happens at the end of the raining season; an important amount of the water needed by lupin plants to fill their grain comes from the soil reservoir. Thus, breeding lupins with strong and deep roots should increase and stabilize seed yields, one of the hardest traits to breed in lupin crops. The generation of new varieties with aggressive roots, even at early plant stages, will definitely increase the odds of ending with high seed yields. The main goal of this research is to genetically analyze root natural variation, including root growth, morphology, and metabolite content, in a diverse set of *L. luteus* to facilitate the manipulation and introgression of favorable root traits into cultivated types.

Results and discussion: A total of 164 *L. luteus* accessions from several origins were included in the experiment. Seeds were sown in 1.2 m PVC tubes and only one plant per accession was grown in each tube. The experiment was set as a completely randomized block design with three replicates. Several root and aerial traits were meassured, including tap root length, shoot length, total fresh root weight, fresh root weight every 20 cm of soil depth, total root volume, total root dry matter, and root alcaloid and sugar content. Lupin accessions were genotyped using a set of ~300 molecular markers (SSRs, EST-SSRs, SNPs and INDELs). Association analyses uncovered several genomic regions associated to root morphology and alkaloid and sugar content. Currently, we are carrying out bioinformatic analyses to detect polymorphisms linked to the root trait associated genomic regions as an attempt uncover candidate genes responsible of root variability.

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Tags: root morphology, *Lupinus luteus*, association mapping.

Relevance of bacterial secretion systems Type III and Type VI in the *Bradyrhizobium-Lupinus* symbiosis

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State of art and aim: One of the most studied models in plant-microbe interaction is the symbiosis *Rhizobium*-legume. This symbiosis is highly specific and depends on several molecular signals produced by both partners. Some of these signals are bacterial proteins named effectors that are translocated into the plant cells by secretion systems similar to contractile nanomachines (injectisomes). The injectosomes puncture and deliver the effectors into the target cell. The two main injectiosomes are the secretion system type III (T3SS) and the secretion system type VI (T6SS). The genome of many rhizobia encodes T3SS and/or T6SS but their role in symbiosis is mostly unknown. The aim of this work is to study the symbiotic relevance of T3SS and T6SS of Bradyrhizobia that nodulate lupins that thrive in alkaline (*Lupinus mariae-josephae*) and acid soils (*L. angustifolius*) in the Iberian Peninsula.

Results and discussion: The analysis of the genomes of *Bradyrhizobium* strains: LmjC isolated from *L. mariae-josephae* and ISLU10 isolated from *L. angustifolius,* allowed the identification of one T3SS in the first strain and two T6SS in the second one. The genes of the secretion systems were grouped and those encoding structural components showed a high degree of conservation regarding the genes from other rhizobia. The strain LmjC is an efficient symbiont with *L. mariae-josephae* while it induces few ineffective and white nodules with *Lupinus angustifolius*. The T3SS of LmjC is encoded in a group of 30 genes. A mutant in the transcriptional regulator *ttsI*, essential for the activation of the T3SS, induces the formation of white nodules unable to fix nitrogen with *L. mariae-josephae* while *L. angustifolius* inoculation with the same mutant produces abundant red and effective nodules. These results suggest a key role of the T3SS of LmjC in defining the lupin host range of this bacterium.
The strain ISLU101 contains two clusters of genes involved in the formation of T6SS. One, T6SS-1, containing 17 genes showed a high degree of conservation regarding the genes of *B. japonicum* USDA110, a well known endosymbiont of soybean. The other, T6SS-2, contains 16 genes flanked by transposition sequences. This second gene cluster does not show high similarity to other rhizobia. Currently we are studying the role of these two T6SS in the symbiosis with *L. angustifolius* by generation of specific mutants.

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Tags: *Bradyrhizobium*, *Lupinus mariae-josephae*, *Lupinus angustifolius*, secretion system type III, secretion system type VI

Lupin species (*Lupinus albus, Lupinus angustifolius*) and soya bean (*Glycine max*) growth dynamic parameters affecting yield formation

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Abstract
Growth dynamic parameters affecting yield formation of Lupinus albus L. under the conditions of the Central Chernozem zone and *Lupinus angustifolius L.* and *Glycine max (L.)* Merr. under the conditions of the Non-Chernozem zone of Russia are presented and discussed.

Keywords*: Lupinus albus*, *Lupinus angustifolius*, *Glycine max*, growth and development, yield formation periods, photosynthetic parameters, seed and protein yield.

Introduction
White and narrow-leafed lupin cultivation in Russia is important due to high protein content. Early maturing cultivars (cvs.) of *Lupinus albus* Start, Gamma, Delta, Dega and others have been registered in Russia. Cv. Start was the first of them. These cultivars are adapted in Central- Chernozem zone where drought during vegetation often occurs. Narrow-leafed lupin is cultivated in the Non-Chernozem zone of Russia. Moscow region is a part of this zone. Early maturing cultivars of soybean are adapted in Moscow region, cv. Mageva is among them.

The crop yield is not the same each year. It was shown that the yield of soybean crop depends on pod and seed formation per m^2 and is controlled by dynamic photosynthetic parameters (*Board J.E., 2011, Carpenter A.C., 1997*). It is important to compare the data of these parameters they have been measured on the same stage of plant development in different experiments and weather conditions. It is difficult to indicate the same stage for various Grain legumes, their species and cultivars with different growth type. Lupin species canopy develops as a photosynthetic system from emergence till maturity. We suppose that this time of development may be divided into four periods (subsystems): I – emergence –beginning of flowering; II – flowering and pod formation; III – pod growth; IV – seed filling. Parameters of previous period influence the next one. Some of them control the number of pod and seed per m^2 and seed yield (*Gataulina G.G., Medvedeva N.V., 2011).*

The aim of study is to determine common and different features in crops dynamic pattern of yield formation and their variability depending of weather conditions of the region.

Material and Methods
Field experiment with cultivation of cv. Start has been reproduced for 12 years in Tambov region (500 km south from Moscow). Plot size was 25 m^2. Field experiment was carried out in 4 replications. The soil was leached chernozem (pH 6.2). The sowing was performed by the end of April. The density before harvesting was 35-40 plants/m^2. Leaf area indexes (LAI), wet and dry matter accumulation (WMA, DMA) were determined by sampling 15 plants from every plot at 15 days interval. Then Photosynthetic potential (Ph.P.) was determined as a sum of every day leaf area for the period by graph method. Crop growth rate (CGR) and Net Assimilation Rate (NAR) were calculated. Yield components were determined by studying 25 plants from every plot. Dynamic characteristics on average for every period, their correlation and coefficient of variation (CV) were determined. The study of narrow-leafed lupin (cv. Kristal) and soybean (cv. Mageva) has been conducted in Moscow region for 6 years (2007-

2012) using the same methods. Soddy podzolic soils (pH 5.0 -5.8) with 1.5 -2.5% organic matter are the main soil type here. The lupin sowing was performed in early spring (late April - first date of May), soybean – 10 days later. The density before harvesting was 40-50 plants m^{-2}.

Results and discussion

Periods of development and yield formation. There is no photosynthesis during periods of sowing-emergence and maturity. Development of vegetative growth, flowering, pod development and seed filling periods are shown in Table 1. Period II is the most complicated, variable and critical for seed yield formation. It is characterized by intensive vegetative growth and pod formation that determines the potential of pods/m^{-2}.

Table 1. Periods of development and yield formation of grain legumes and stages of soybean development
Borders (end of period): I – first flower on plant; II – end of flowering; III – full pod size on the high level of plant; IV – full seed, leaf yellowing and falling.

<table>
<tr><td rowspan="6">Sowing-emergence</td><td colspan="3">I</td><td colspan="4">II</td><td colspan="2">III</td><td colspan="2">IV</td><td rowspan="6">Maturity</td></tr>
<tr><td colspan="3">Vegetative growth</td><td colspan="4">Flowering and pod set</td><td colspan="2">Pod growth</td><td colspan="2">Seed filling</td></tr>
<tr><td colspan="3">Vegetative development</td><td colspan="8">Reproductive development</td></tr>
<tr><td colspan="7">Vegetative growth</td><td colspan="2" rowspan="2">Pod growth
Slow seed filling</td><td colspan="2" rowspan="2">Intensive
Seed filling</td></tr>
<tr><td colspan="4"></td><td colspan="3">Flowering and pod set</td></tr>
<tr><td colspan="12">Soybean stage development</td></tr>
<tr><td>V1</td><td>…</td><td>V5</td><td>R1</td><td>R2</td><td>R3</td><td>R4</td><td>R5</td><td>R6</td><td>R7</td><td>R8</td></tr>
</table>

Dynamic parameters, seed and protein yield. White lupin canopy as a photosynthetic system functions effectively during periods II (flowering and pod set) and III (pod growth). 70-75% of DMA is accumulated at this time, which is about 40% of a total period from emergence till maturity. CGR during period II is 2–4 times higher compared to the previous period (Table 2).

Table 2. Dynamic parameters of *Lupinus albus,* cv. Start development per periods (average for 12 years)

Parameters	Period of photosynthetic activity				
	I	II	III	IV	I-IV
Duration, days CV, %	36 11	20 13	18 23	20 12	96 10
Leaf area index (LAI), average per period CV, %	0.7 28	3.2 31	3.5 39	1.7 36	2.1 30
Photosynthetic potential, (Ph.P) $10^3 m^2$ d ha^{-1} CV, %	260 37	630 31	705 53	405 45	2000 38
Dry matter accumulation (DMA), t ha^{-1}per period CV, %	1.70 36	2.50 36	3.00 52	0.30 50	7.50 35
Crop growth rate (CGR), kg ha^{-1} d^{-1} CV, %	47 35	125 37	167 52	20 50	78 34

The same was proved for narrow-leafed lupin grown under condition of Moscow region (Table 3). Data was also collected for soybean. Parameters of period III were decreased for soybean (Table 4). Soybean could not realize the potential possibilities under stressful for this crop conditions of Moscow region. It was shown by *Board J.E. et al.*(*2011*) for soybean that stressful factors (high temperature, drought) decrease duration of period and dynamic parameters value. Variation of parameters during period II for white and narrow-leafed lupin in connection to the weather change is 30-36%. CV of CGR for soybean

is 2 times higher compared to lupin. The maximum value of wet matter accumulation for studied crops occurred at the end of the III period when pods on upper level of plants reached their maximum weight. Lupin CGR in period IV is rather small (8-9 times less than in previous period). There was no visible DMA in this period for soybean. It is explained by leaf yellowing and falling, so photosynthesis decreased. Rapid seed filling is mainly due to redistribution of nutrients from vegetative plant parts. The number of pods and seeds formed per m^2 at the end of period II determines the potential seed yield. Pod and seed abortion can occur during period III. It depends on the weather conditions, Ph.P., and CGR value of the previous period II (*Gataulina G.G., Sokolova S.S,* 2013).

Table 3. Dynamic parameters of narrow-leafed lupin, cv. Kristal development per periods under condition of Moscow region, average for 2007-2012

Parameters	Period				
	I	II	III	IV	I-IV
Duration, days	36	13	13	14	76
CV, %	8	8	27	52	15
Leaf area index (LAI), average per period	1.06	2.21	1.98	0.41	1.40
CV, %	38	31	49	93	33
Photosynthetic potential, (Ph.P) $10^3 m^2$ d ha^{-1}	360	290	250	115	1015
CV, %	58	37	66	98	45
Dry matter accumulation (DMA), t ha^{-1}per period	1.26	2.69	2.36	0.29	6.60
CV, %	42	36	51	50	33
Crop growth rate (CGR), kg ha^{-1} d^{-1}	35	207	182	21	87
CV, %	33	28	38	39	18

Table 4. Dynamic parameters of soybean, cv. Mageva development per periods under condition of Moscow region, average for 2007-2012

Parameters	Period				
	I	II	III	IV	I-IV
Duration, days	35	20	21	15	91
CV, %	6	8	31	48	14
Leaf area index (LAI), average per period	2.04	3.15	2.44	0.97	2.15
CV, %	43	40	43	76	41
Photosynthetic potential, (Ph.P) 10^3 m^2 d ha^{-1}	705	570	540	215	2030
CV, %	49	52	64	94	51
Dry matter accumulation (DMA), t ha^{-1}per period	1.41	2.20	0.76	-	4.57
CV, %	29	52	60		60
Crop growth rate (CGR), kg ha^{-1} d^{-1}	40	110	36	-	60
CV, %	26	58	47		42

Weather greatly affects several dynamic parameters and seed yield. Drought during the critical period II (flowering and pod set) reduces 1.5–3 times DMA, CGR, seed yield and yield components (Table 2, Table 5). CV of these parameters for similar conditions was 2-3 times less than experimental average. The narrow-leafed lupin and soybean differed greatly in the length of periods and photosynthetic potential (Ph.P) value. Average for soybean total Ph.P was 2030 10^3 m^2 d ha^{-1}. It was 2 time less for narrow-leafed lupin. It could be explained by longer vegetation of soybean in comparison with lupin.
Net assimilation rate (NAR) for narrow-leafed lupin was much higher than for soybeans.
It could be explained by particular structural features of lupin leaves: bigger thickness of lupin leaflets and their heliotropism. The leaf surface of 1 g lupin leaves is 21 cm^2, for soybean it is 50 cm^2. NAR of narrow-leafed lupin was on the average 6.9–7.5 g m^{-2} per day, while for soybean it was 3.0-3.1.

Table 5. Effect of weather on white lupin seed and protein yield and yield components

Yield and yield components	Average	Favorable condition	Water stress (5 years)
Seed yield, t ha^{-1}	3.08	4.44	1.82
CV, %	42	12	19
Protein yield kg ha^{-1}	1190	1710	720
CV, %	40	12	18
Pods m^{-2}	246	360	160
CV, %	40	20	9
Seeds m^{-2}	800	1120	455
CV, %	45	34	14
1000 seeds weigh, g	390	375	400
CV, %	9	9	9

Seed yield of narrow-leafed lupin and soybean (2007-2012) was 1.85 and 1.40 t ha^{-1} (CV – 27 and 37%) respectively. Pods/m^{-2} were 660 and 590 (CV - 28 and 30 %), seeds/m^{-2} were 1600 and 1310 (CV - 21 and 28 %). CGR for period II strongly correlates with a number of pods, seeds m^{-2}, and seed yield. Coefficient of correlation (r) was respectively for narrow-leafed lupin 0.74, 0.65, 0.97, and for soybean 0.91, 0.87, 0.71. *Board J.E., Modali H.(2005)* found out for soybean that higher seeds m^{-2} were related to increased CGR from R1 to R5 stages and the production and duration of leaf area.

Conclusion

Grain Legumes canopy as a photosynthetic system functions from emergence till maturity and may be divided into four periods (subsystems): I – emergence–beginning of flowering; II – flowering and pod formation; III – pod growth; IV – seed filling. Studied Grain Legumes differed by the length of periods, the Ph.P., CGR value and other characteristics.

Crop canopy as a photosynthetic system functions effectively during periods II (flowering and pod set) and III (pod growth). 70-75 % of TDM is accumulated at this time, which is 40% of a total period from emergence till maturity. CGR during period II is 2–4 times more than in previous period.

Value of Ph.P, TDM and CGR during the critical period of flowering and pod set (period II) strongly correlates with a number of pods and seeds per m^2 and seed yield, and can be used as a criterion for predicting seed yield. These parameters may be effective in controlling yield formation.

Weather greatly affects several dynamic parameters and seed yield of lupin species and soybean. Drought during the critical period II (flowering and pod set) reduces TDM and CGR by 1.5–3 times.

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Keynote lecture

Improving yield stability in lupin – breeding for anthracnose resistance

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State of art and aim: Anthracnose, caused by the fungus *Colletotrichum lupini*, represents the most important disease in lupin cultivation worldwide. To obtain high and stable yields it is necessary to plant resistant cultivars as there are no possibilities to overcome the fungus via pesticides.

Results and discussion: Sets of plant genetic resources of sweet narrow-leafed and yellow lupin were assessed in their susceptibility to anthracnose. In both lupin species breeding lines were identified which displayed a qualitative resistance, each governed by a dominant gene as revealed by genetic analyses of segregating F2 families and their F3 progenies.
Field tests performed over six environments (2 locations, 3 years) with the resistant narrow-leafed lupin line confirmed its high and stable level of anthracnose resistance. The underlying gene was designated *LanrBo*.
Different resources were used in narrow-leafed lupin for developing molecular markers for the resistance, namely, (I) anchor markers already mapped in *L. angustifolius*, (II) sequence information drawn from model genomes and (III) sequence information from differentially expressed *L. angustifolius* cDNA derived from RNA-Seq. Linkage analysis identified a set of markers bracketing the *LanrBo* gene, with the closest bracketing markers showing a joint recombination of less than 1 %.
Linkage analysis allowed us to locate *LanrBo* into the same linkage group, NLL-11, as was previously done by Australian researchers in the case of resistance gene *Lanr1* from cv. Tanjil. Further experiments will have to be performed to decide whether *LanrBo* and *Lanr1* are allelic or reside at distinct loci in *L. angustifolius*.

Tags: narrow-leafed lupin, yellow lupin, *Colletotrichum lupin*, anthracnose, resistance, molecular marker

A long journey to the achievement of tolerance to *Colletotrichum lupini* in spring *Lupinus albus*

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Summary
White Lupin, *Lupinus albus*, is the species with the highest yield, nevertheless after *Lupinus luteus* it has been the most affected by *Colletotrichum lupini*. This fact had as a consequence the partial abandonment of this crop, especially in Europe.
On the other side in Chile, where mostly winter varieties are sown, and where tolerant varieties were developed, the European spring varieties kept showing their susceptibility.
Based on tolerance sources of winter origin from the Azores and Australian lines, with Ethiopian resistance bases (Adhikari, K. et al 2005) we obtained resistant and at least tolerant varieties, which were selected under controlled conditions in growing chambers during at least two successive generations. As controls we used the susceptible "Amiga" and the resistant "Pecosa". Afterwards this relative resistance or tolerance was confirmed in Chile and on six European locations during 2014, being partially repeated this year.

Keywords
Lupinus albus, Colletotrichum lupini, Anthracnose, tolerance, segregating population.

Introduction
Anthracnose by *Colletotrichum lupini* is considered by different authors (Yang et al 2004; Riegel, R. et al 2008) as the most important disease of the diverse lupin species. So it has almost eliminated the cultivation of the species *Lupinus luteus* and *Lupinus albus* in Europe (Römer, P. 1996; Cowling, W. et al 1999).
As it was indicated by Weimer in 1943 and 1952, the intensity of the infestation is strongly influenced by climate and humidity. This condition complicates the selection of tolerant, partially resistant or resistant material under from year to year changing field conditions.
Under field conditions in Gorbea (39º06´88"S. 72º41´29"), Araucania Region, Chile, diverse cultures and promising lines with enhanced tolerance or resistance were identified (v. Baer, E. 2008) and eleven promising genotypes selected.
Starting with this material a series of hybridizations was undertaken, trying to accumulate tolerance and resistance characters. Under tolerant plants we understand those which show a slight infestation and do not transmit the disease in severe form to plants of the same genotype.
For the selection different methods were used, the most effective was the inoculation at cotyledon state under growth chamber conditions, assuring the character of tolerance or resistance at least during two following generations.
This work produced two promising lines, BLU 17 and BLU 18, which were sown 2014 in Portugal, Spain, France, Germany, Suisse and Czech Republic.
The results show that only in Rostock, Germany, a slight attack without plant destruction was observed.

Material and methods
Inoculation and evaluation
It was not easy to arrive at a trustworthy method that rendered the same results every season.

At the beginning we worked with infected agar plates on which we made the seeds germinate, observing for the more resistant individuals a halo or area where the growth of the fungus was inhibited (Hashagen, U. et al 1999). This method allowed a first selection of a segregating population. The inconvenience was that some seeds presented latency and the resistance reaction could not be observed.

Description of the seed screening method

Selection of the material to evaluate, in order to then put fungi inoculate of stock A-10 in the center of each agar plate at a 1cm-distance of the seed.

The plates went into covered glass dishes and were incubated and evaluated after ten days under growth chamber conditions at 25 ºC and approximately 75% of humidity. Positive and negative controls were use.

TABLE 1.- Evaluation scale for tolerance with the seed screening method.

MARK	EVALUATION	SYMPTOMS
9	Highly susceptible	Infection as soon as the mycelium contacts plant tissue
8	Very susceptible	Fast infection, the root contacts the mycelium, local necrosis
7	Susceptible	Slow infection, first brown areas where the mycelium grows
6	Slightly susceptible	Slow infection, healthy radicle that gets the infection later
5	Slightly tolerant	Slow infection, no observable necrosis in ten days
4	Typically tolerant	Necrosis healing and building secondary roots
3	Tolerant	Slow infection, necrosis heal and plantlets continue their development
2	Hyper sensible	Infested root is replaced by secondary roots
1	Highly tolerant	No infection

DESCRIPTION OF THE METHOD THAT USES PLANTLETS IN GROWTH CHAMBER

Anthracnose stock A-10 of high virulence was used (FIGURE 1). Using a growth chamber for the material to analyze, per pot with sand as substrate four seeds were sown 1.5 to 2 cm deep. After ten days under controlled light, temperature and humidity conditions, when the plants opened their cotyledons and produced their first true leaves, they were inoculated with stock A-10 mentioned above in form of a spore solution of a concentration of $1 * 10^6$ (one million) spores per cubic mm.

The center of each cotyledon was punctured with a disposable hypodermic needle, and then a quantity of 10 micro liters of spore solution was deposited on each puncture. Afterwards the pots were incubated for 10 days under temperature of approximately 20 ºC and 70% humidity, and the Resistance Screening performed.

Figure 1: Anthracnose on lupin caused by the fungus *Colletotrichum lupini*. **A.** Symptoms on the leaf. **B.** Symptoms on the stem, abundant spore production by the fungus. **C.** Pod with acervuli **D.** Conidia of *Colletotrichum lupini* A-10.

TABLE 2. Evaluation parameters for the Anthracnose Test with the method of Plantlets in Growth Chamber.

Mark	Parameters	Infestation Grade
1	no attack	Resistant
2	1-2 mm necrosis	Resistant
3	2-3 mm necrosis	Susceptible
4	4-5 mm necrosis	Susceptible
5	Over 50 % cotyledon damage	Highly susceptible
6	Over 50 % of infected plants	Highly susceptible
7	Dead plants	Highly susceptible
H	Hyper sensitivity	-

FIGURE 2. Evaluation of plantlets regards to infestation grade **A.** Resistant plant **B.** Highly susceptible plant.

Results and discussion

With more advanced populations, F3-F5, we had the best results with the inoculation of plantlets under controlled conditions, achieving the shift of the segregating population progressively in direction of more tolerant and resistant individuals.

Harvest of one plant yielded for sowing four pots with three grains each, allowing for replications if necessary and for sowing the rest on the field.
Next season the plants of the chosen populations were selected and the same process repeated. Experience showed that it was necessary to repeat for two generations the cultivation in pots.
The lines so selected were sown isolated and the harvest was used for yield tests at two locations in South Chile (Cajón and Gorbea). The two best lines were sown simultaneously in South Chile and Europe (Portugal, Spain, France, Germany and Czech Republic).
The two selected lines were the following:
BLU 17-12 cross: (TWxCH304)(Lolita x Az 129). 9602-B-mult.143-011.
BLU 18-12 cross: Az 129 x (97B054/p27). =5-29-B mult. 149-011.

FIGURE 3. Resistance increase through three generations.

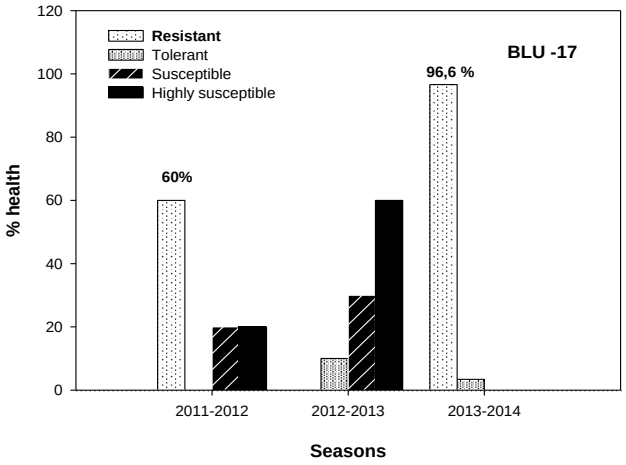
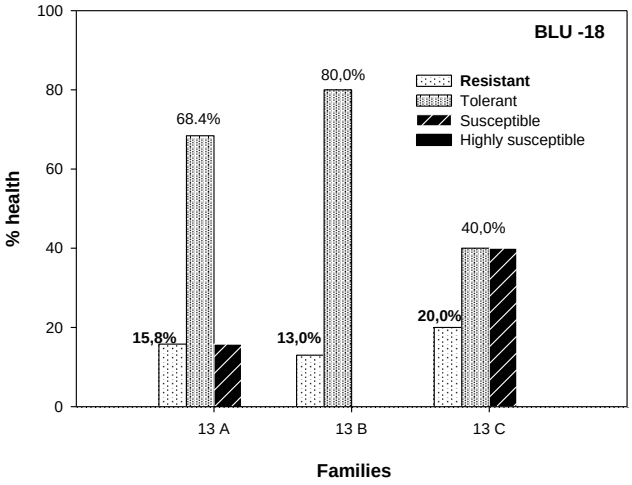


FIGURE 4. Segregation of sister lines.



In comparison with "Amiga" both varieties were one week later regards to total maturity and showed increased resistance to *Colletotrichum* but also a better resistance to *Fusarium*.
The lowest yield was obtained by the variety "Amiga" due to a heavy attack of *Fusarium* (Table 4).

TABLE 3. Yield in Ton/Ha at two locations in South Chile.

Variety	Cajón	Gorbea	Average	%Dry Base Protein
"Amiga" Standard	3,45	3,62	3,54	40,6
BLU 17	4,52	5,52	5,02	41,7
BLU 18	4,15	5,55	4,85	40,4
Sowing date	Aug.10	Aug.13		

Table 4. Yield in Ton/Ha in Gorbea, South Chile, Season 2014-2015.

Variety	Gorbea	(%)Dry Base Protein
"Amiga" Standard	2,27	33,7
BLU -17-12	5,14	41,8
BLU -18-12	3,97	40,8
"Energy"	3,95	37,4
"Ludic"	4,12	37,3
Sowing date	Jul.17	

Conclusions

- As the results show, selection of genotypes with increased resistance or tolerance starting with segregating populations is effective combining the seed screening method as preselection with the plantlet method under growth chamber conditions, and following confirmation on field trials under different climate conditions.
- In order to assure a better health it is necessary to breed three selection generations under controlled conditions
- The foregoing statement means that we are in presence of a polygenic selection
- As first breakthrough two new varieties with increased field resistance have been obtained.

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Session 6

Lupin for foods and ingredients

Keynote lecture

Improving sensory and functional properties of protein isolates from lupins (*L. angustifolius*, L. cv. Boregine) by solvent extraction

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Abstract
Protein isolates from lupin seeds show excellent techno-functional properties but also exhibit green and beany flavor notes that limit their use as food ingredients. The treatment of lupin flakes with solvents can reduce such unwanted flavors but can lead to a reduction of the techno-functional properties due to a possible damage of the protein structure. For the present work lupin seeds were de-hulled and the kernels were flaked and extracted with hydrocarbons (n-hexane, iso-hexane), alcohols (ethanol, iso-propanol) and supercritical carbon dioxide (sc. CO_2 extraction at 285 bar and 800 bar) to remove lupin oil. After aqueous extraction and isoelectric precipitation of the proteins, the extraction yield and functional as well as sensory properties of the protein isolates were studied. After treatment with n-hexane and iso-hexane the overall sensory acceptance was in a medium range and the protein yield was the same as for proteins without treatment, which showed lowest overall acceptance. By the use of alcohols the sensory properties were similar to the hydrocarbon samples but the yield decreased to 36% with ethanol as solvent and 38% with iso-propanol. The highest protein yield was reached after sc. CO_2-extraction of the flakes at 285 bar with a value of 48%, which is 6% higher than the yield of the untreated lupin flakes. Also the sensory properties and the overall acceptance showed the highest value after extraction with sc. CO_2. For cost reasons the treatment with sc. CO_2 should be carried out at a reduced pressure of 285 bar. Protein solubility and emulsifying capacity were in a similar range for all samples. The study demonstrated that solvent treatment led to protein isolates with improved sensory profiles, high overall acceptance and excellent techno-functional properties.

Keywords: Lupin protein isolates, solvent treatment, techno-functional properties, flavor.

Introduction
Plant proteins are becoming more important for food producers and consumers due to their high nutritive value and their promising techno-functional properties. Lupin flours, protein concentrates, and isolates can be applied in different food systems such as bakery products, salad dressings, lupin pasta, ice cream, and sausages (Wäsche et al. 2001, Drakos et al. 2007, Kohajdová et al. 2011, Eisner 2014). Yet the application of lupin products in food is limited, largely due to their green and bean-like flavor as has been shown in previous investigations (Bader et al. 2009). Since some unwanted flavor attributes are likely to emerge from fat degradation the treatment of lupin raw material with organic solvents or sc. CO_2 could influence the sensory properties and therefore could increase the use lupin protein isolates for human nutrition. As some organic solvents such as ethanol can damage the proteins, the influence of the solvent treatment on extraction yield and functional properties has to be investigated.
In general, lupin proteins exhibit good protein solubilities, good emulsifying, and moderate gelation properties compared to soy proteins (Sousa, 1993 and Wäsche et al., 2001). However, no studies have been published on the influence of de-oiling of the lupin flakes with different organic solvents or sc. CO_2 extraction and on the functional and sensory properties of lupin proteins. Nevertheless, attempts are increasingly made on implementing this material as a rich protein source. Thus, studies on both the application as well as sensory and functional properties are highly needed and will be of high relevance in future developments.

The aims of the present study were to determine the effect of de-oiling on the properties of lupin protein isolates. Extractions with n-hexane, iso-hexane, iso-propanol, ethanol, and supercritical CO_2 were compared regarding protein yield that can be reached with aqueous extraction after solvent treatment of the lupin flakes. Furthermore, the techno-functional properties (protein solubility, emulsifying capacity) as well as the flavor and the overall acceptance of the corresponding lupin protein isolates were investigated. As properties of sc. CO_2 are strongly depending on pressure and temperature the treatment with sc. CO_2 was carried out at a constant temperature of 50 °C and two different pressures at 285 bar (a) and 800 bar (b).

Results and Discussion

Lupin flakes of *L. angustifolius* cv. Boregine were de-oiled with a variety of different organic solvents including n-hexane, iso-hexane, ethanol and iso-propanol as well as with sc. CO_2 at two different pressures (285 bar and 800 bar). Subsequently, the de-oiled flakes were subjected to aqueous protein isolation at pH 7.2 to determine protein yields, functional and sensory properties of the corresponding isolates in relation to isolates received from untreated lupin flakes (Bader et al. 2011).
As displayed in Table 1, all isolates had protein contents of higher than 90%. The highest protein yields were obtained after extraction of sc. CO_2-de-oiled flakes at 285 bar with 48%, whereas the protein yields decreased to 36% and 37% after de-oiling with ethanol and iso-propanol, respectively. All isolates exhibited excellent protein solubilities and emulsifying capacities, which suggests the high application potential of lupin protein isolates in a wide range of food products.

Table 1. Properties of protein isolates from L. angustifolius cv. Boregine after treatment with different organic solvents compared to a non-treated reference (Data: Oviedo 2009).

Protein isolates from treated flakes	**Protein-content** Mass.-%	**Protein-yield*** Mass.-%	**Protein-solubility**** Mass.-%	**Emulsifying-capacity** mL oil/g prot.
not treated	92.2	42.2	92.9	740
treated with n-Hexane	97. 3	42.7	95.6	745
treated with iso-Hexane	94.8	42.5	90.5	720
treated with Isopropanol	96.5	37.9	93.7	725
treated with Ethanol	96.8	36.1	97.6	710
treated with sc. CO_2-285bar	97.9	48.4	100	720
treated with sc. CO_2-b-800bar	96.1	43.6	96.4	725

* Yield of dry isolate based on the mass of protein in the raw material

** Solubility at pH 7

Besides high protein yields and excellent functional properties, the overall flavor and thus a high consumer acceptance of the isolates is mandatory for the utilization in food products. Thereby, the overall flavor and characteristic attributes like bitter, astringent or beany were determined in a trained sensory panel with 3% (w/w) aqueous solutions of the different lupin protein isolates. The overall acceptance of the different lupin protein isolates is shown in Figure 1.

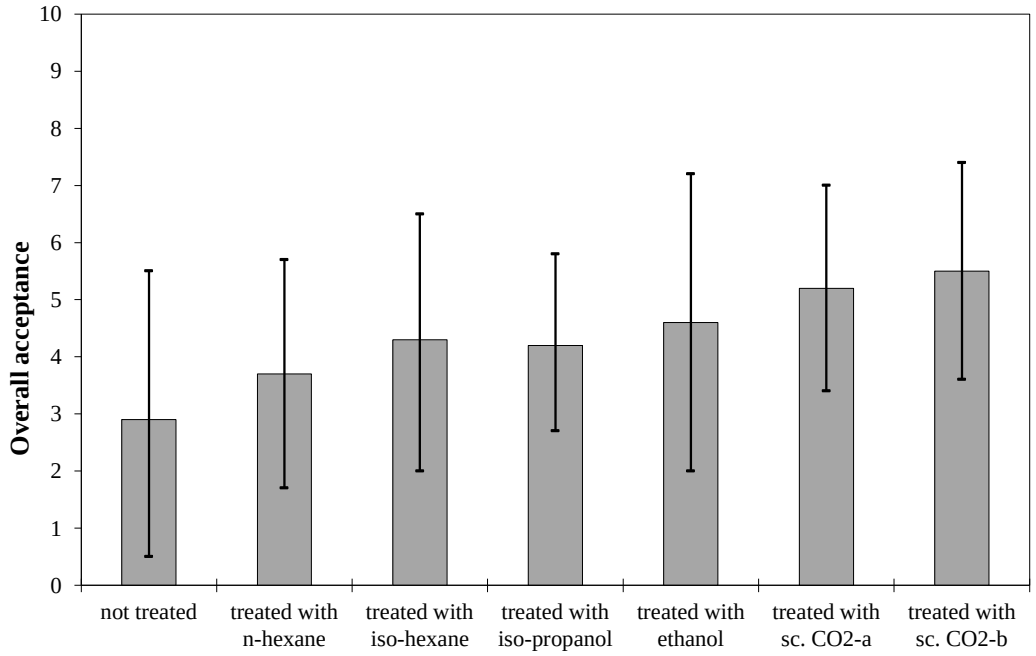

Figure 1. Overall acceptance of lupin protein isolates from *L. angustifolius* cv. Boregine (0 = dislike; 10 = loving) after treatment with different solvents (Eisner 2014).

Protein isolates from sc. CO_2-de-oiled flakes revealed the highest overall acceptance followed by the isolates produced from the other de-oiled flakes. The lowest acceptance was found for the isolates from untreated, full-fat lupin flakes, displaying the effects of flavor compounds derived from fat oxidation or fat accompanying substances in off-flavor formation. Therefore, the extraction with sc. CO_2 improves the overall acceptance of lupin protein isolates by the joint removal of unwanted odor-active substances and oil. However, these results were not statistically significant due to polarizing effects of the different attributes. In detail, de-oiling with sc. CO_2 resulted in decreasing beany, cardboard-like and bitter flavors, whereas de-oiling with alcohols (ethanol and iso-propanol) only reduced beany notes (Figure 2). For n-hexane and iso-hexane only minor reductions in beany and bitter flavor were obtained in relation to the untreated lupin flakes (Figure 2).
Altogether, these results show the high potential of sc. CO_2 extraction for improving the flavor of lupin protein isolates, while maintaining their excellent functionality.

Conclusions

The present study showed the high potential of sc. CO_2 extraction for improving the flavor of lupin protein isolates, while maintaining their excellent functionality. Lupin protein isolates with enhanced flavor properties are important for their application in a wide range of products including vegetarian or vegan sausages, dairy-like products (lupin milk, yoghurt, cream cheese) as well as bakery products. This enables food producers to develop new tasty vegan or vegetarian products with lupin proteins having a high consumer acceptance.

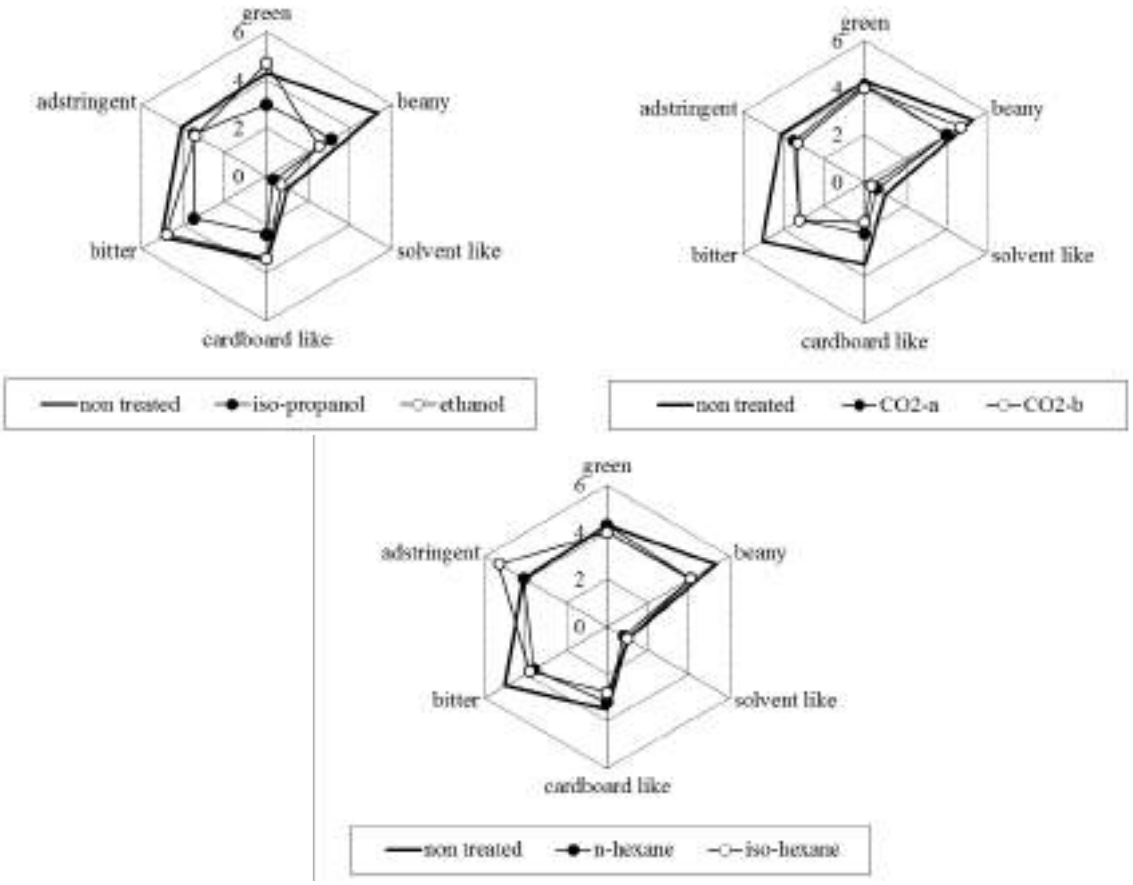

Figure 2. Sensory evaluation of isolates from *L. angustifolius* cv. Boregine in a 3% aqueous solution concerning the most relevant attributes (Eisner 2014).

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Using a multi-actor approach to getting lupins 'back on the menu' in The Netherlands: involving breeders, farmers, food and feed industry and consumers

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Abstract
The first initiatives aimed at increased lupin production in The Netherlands came from two high(er) value chains: the organic sector striving for regional feed production and the food industry looking for alternatives for soy-based products. As regional production is an added value for both market chains, an additional price could be afforded to compensate for the yield gap that developed over the years. Although this proved to be a good starting point for turning the downward spiral for lupins into an upward spiral, the barriers for a successful development turned out to come from many additional angles: a lack of knowledge on successful production among farmers, the absence of agro-chemicals allowed in the cultivation of lupins, the lack of infrastructure for collecting, drying and cleaning the lupins, the fear of food-producers to introduce a new and unknown allergenic ingredient to the production sites and the lack of knowledge among consumers were some of these additional barriers that needed to be addressed. To overcome these barriers, The Louis Bolk Institute started to cooperate with a great number of actors involved. In cooperation with food technologists and foodproducers new lupin based products were developed broadening the market for lupins grown for human consumption. Cooperation with a farmer union ensured that the allowance for necessary agro-chemicals was initiated and political support was organized at a regional and national level. Finally social organizations aimed at stimulating the public and political debate on protein transition were involved to get lupins better known among consumers and politicians as a new and healthy protein source. As a result of the involvement of all these different actors we are slowly seeing a rise in acreage and consumption of lupins in The Netherlands.

Keywords: lupin, multi actor approach, human consumption, the Netherlands

Introduction
Like in most other European countries the cultivation of grain legumes in The Netherlands has decreased dramatically since the end of the 20th century as a result of unfavorable crop-subsidies and cheap import of soy. With the continuous reduction of acreage, the whole market chain turns into a downward spiral: low acreages means low-breeding activities, low-breeding activities means less progress in yields while other crops do progress, creating an even larger yield gap between grain legumes and other competing crops (eg. cereals and oil-seed rape). In addition, the feed and food industries focus on cheap, imported grain legumes, mainly soy, added to the downward spiral. The negative effects of the disappearance of the grain legumes not just from the agricultural landscape but also from the diets are manifold and have come to such levels that it has started to attract the attention of politics (both EU and national). A large dependency to imported soy, making EU vulnerable especially with upcoming markets like China, the disappearance of biodiversity in the landscape, causing a reduction in insects and birds and increasing health issues related to the high animal product intake in large parts of society. This could mean a turning point for grain legumes in Europe in general and of lupins in particular. Especially lupins combine unique properties making them attractive for the modern day consumer: high in protein, low in starch and high in fiber like components. However, getting lupins back on the menu for both farmers and consumers means that work has to be done at all the different levels of the market chain simultaneously involving many different actors.

Results and discussion

The initial market opportunity

The start of our work with grain legumes came from the need in the organic sector to close nutrient cycles on a more regional level. A whole range of legumes were tested, among which L. angustifolius and L. luteus. The main attitude towards lupins was that the seeds were very interesting from a nutritional point of view, but yields were too low to be economically feasible. In 2007 however interest in lupins grew from the food industry that wanted to know whether a more regionally organized supply chain for lupins would be feasible. Field trials indicated that there were great varietal differences, but with the right variety and an additional price for regionally produced lupins, production could be done locally. But progressing from these early field trials to building a viable market chain meant we needed help.

Farmers, seed companies and farmer unions

To get farmers interested in new crops like lupins, means that you have to start with the more innovative farmers. But even for them there are numerous barriers. Getting enough sowing seeds of the right variety in The Netherlands is already one of the basic things. As the cultivation of grain legumes in general and lupins in specific is very underdeveloped in The Netherlands, seed companies only have a very limited amount of varieties available. For lupins they often have just one variety which they can supply and the knowledge on the suitability of this variety for feed or food purposes is limited. As only a few of the sweet varieties of lupins are sweet enough to be used for human consumption the Louis Bolk Institute started to organize the ordering of seeds from varieties that had proven to be suitable in our field experiments. We started to cooperate with a farmers union (NAV Dutch Arable Agriculture Union) that made inventory plans on how much acreage would be sown for the different processors. Also seed companies (Van Dijke Semo, Innoseeds) were involved in organizing the logistics.
But seed supply wasn't the only farmer obstacle. Since most of the grain legumes have largely disappeared from the agricultural landscape since the 80's and 90's in The Netherlands, knowledge on how to grow them has been largely lost for current generation of farmers. To revive this knowledge The Louis Bolk Institute started to organize farmers meetings together with the NAV and to make cultivation guides to support the farmers and contracters. For conventional farmers the problem was not just a lack of knowledge, but also a lack of agro-chemicals available for lupin-cultivation. The national body for allowance of agro-chemicals (CTGB) did not recognize lupins as a harvestable crop, but merely as a green manure. No herbicide or fungicide was allowed in the cultivation of lupins, forcing conventional farmers to reduce weeds mechanically with weed-harrows or hoeing equipment. However as weed control in all other crops is done chemically, a lot of the conventional farmers do not have hoeing or harrowing equipment and do not know how to use them properly. So cultivation guidance also included farmers to get themselves re-acquainted with this.
At the same time the NAV used it's influence at a policy level to get some of the necessary agro-chemicals accepted in the cultivation of lupins.

Food industry and food innovation

The first initiative for regional lupin production came from a start-up company in meat substitutes: Meatless. Setting up local production gave the producer several advantages: more control and assurance over the supply chain (less risks of contamination) and an added value to the product that he wanted to market. An extra advantage of this control over the supply chain was that a producer could ask for specific varieties or growing conditions for the lupins, something that cannot be gained from an anonymous market. A local supply chain meant that for farmers agreements could be made ahead of the growing season: acreages to be grown and pre-set prices. The agricultural union NAV started to play a role in coordinating these agreements, making collective ordering of sowing seeds possible and assuring prices for which the farmers were able to grow the lupins locally. They also started to coordinate the collection, drying and cleaning of the seeds by a specialized company (Blonk). However, for cultivating and processing lupins economically the amount of food-producers needed to grow. Fortunately the first initiatives created a lot of media attention, triggering additional producers to get interested in lupins.

Especially another start-up company: The Vegetarian Butcher created a lot of publicity around lupins. In addition to this the Louis Bolk Institute started to involve a food technologist (MFH-pulses) and the main producer of lupin flours and bits and grits (LI Frank) to help create new food stuffs based on lupins: low-carb bread, lupin ice-cream. Another innovation company (Color and Brain) created a new technology for creating meat substitutes based on lupin flour getting a large producer of vegetarian products interested in adopting this new technology (Vivera). Lupins started to be known more and more for their unique qualities of high protein, low starch and high fibre like components, perfectly fitting the modern day consumer. Small initiatives started to promote the use of the whole bean as well (powerpeul.nl and lupinfood.eu) making the beans and their flour available to the general public. Slowly the market opportunities for lupins as a new 'super food' are increasing.

Breeders

Creating a market for lupins with companies interested in locally grown lupins is a good start, but is not enough. The early developments in locally cultivated lupins are still very fragile. Lupins can only been grown locally if the producers pay an additional price for these lupins. The gap between world market prices and local prices is still quite big, which in the long run wouldn't hold. There is a need for more locally adapted, more stable producing varieties. Again however we find ourselves in a deadlock situation. For breeders to invest in a new crop, they need to be assured with sufficient acreages of cultivation. As these acreages are not there yet, they are very reluctant to engage. Without these new and improved varieties however, acreages are not going to grow that fast or maybe even not at all, creating a catch-22 situation. In order to make progress in breeding The Louis Bolk Institute started a small breeding program for white lupins (L. albus) while at the mean time starting cooperation with established breeding companies like Saatzucht Steinach to work out new and innovative ways to come to locally adapted varieties for the Dutch climatic conditions, meeting the criteria needed for human consumption. Also the cooperation with small and independent breeders in The Netherlands and in Denmark is important as they can make

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Conclusions

Trying to get grain legumes back on the menu for both farmers and consumers is something that is not done easily. Obstacles include: few available suitable varieties that perform well under Dutch conditions, insufficient seed supplies of these suitable varieties, insufficient knowledge on how to grow lupins, no admittance for the use of agro-chemicals, a small and fragile developing lupin food industry and little logistical infrastructure for collection, drying and cleaning. By involving a lot of different actors in the process we are trying to overcome most of these obstacles in creating innovative and locally based market chains. Farmer unions, breeders, food technologists, small and large food producers and policy makers all have to be involved to turn the tide for lupins as the food trend.

Optimizing lupin production for human consumption in The Netherlands

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Abstract
In a period of 7 years (2007-2013) 25 varieties of lupins were tested on different soil types in The Netherlands, including 20 varieties of *L. angustifolius*, three varieties of *L. albus* and two varieties of *L. luteus*. These varieties were obtained from various seed companies in Germany, Denmark, Poland and the UK,. It seems that relatively small differences in climatic conditions between The Netherlands (northern-european sea climate) and the regions where most of the varieties are bred (north-eastern land climate in Germany or middle European sea climate in the north of France) already result in varieties being less adapted to the growing conditions existing in The Netherlands. An important result is that, although breeding efforts have been focusing on developing low-alkaloid varieties for both feed and food, only a limited number of varieties meet the strict norm of less than 0,02% of alkaloid needed for food. Apart from variety effects, several different crop management factors have clear effect on both yield and grain-quality (alkaloids). Yields appeared to be higher on clayey soils than on sandy soils, although for *L. angustifolius* free calcium levels need to be sufficiently low to avoid having a lack of inoculation and chlorotic reactions. No yield effect was found with the application of K or S, but alkaloid levels tended to be lowered by the application of KSO_4 in soils low in potassium. Early sowing seems to increase the yield potential of both *L. angustifolius* and *L. albus* although early sowing can also cause reduced emergence and increased weed pressures. The application of fungicides did increase the yield of most lupin varieties considerably even in years that fungal-pressure appeared to be low.

Keywords: lupin, varieties, cultivation, crop optimization, human consumption, the Netherlands

Introduction
Lupin has always had the interest of the feed industry due to its high levels of protein, exceeding that of other grain legumes like peas and faba beans. The biggest drawback for lupins, however, has always been the lower and unstable yields making it less attractive for farmers to grow. Recently, the interest of the food industry in lupins has developed not just due to the high protein levels, but also due to high levels of fiber-like carbohydrates linked to improved colon health and a better mouth-feel for the modern-day consumer. Especially upcoming markets like that of meat replacers, plant-based dairy products en low-carb/high protein diets see a lot of potential in lupins as a main ingredient offering new opportunities for the cultivation of lupins in Europe in general and The Netherlands in particular. Due to this increased attention to lupins, The Louis Bolk Institute has been involved in numerous projects aimed at optimizing the production of lupins for human consumption since 2007, for both organic and conventional agriculture. These projects included variety screening and cultivation optimization, looking into the effects of soil type, fertilization (N, K and S), sowing date and fungicide treatment. These projects were conducted in close cooperation with both farmers and food industry. Apart from yield and protein content, an important aspect was to study how lupins with a sufficiently low alkaloid level (<0,02%) could be produced.

Results and discussion
The first demand for investigating regional lupin production for human consumption came in 2007 from a start-up business Meatless in the south-west of Holland. They wanted wanted to use lupins for producing high quality meat replacers. Instead of using imported lupins from Australia they wanted to know whether it was possible to grow lupins regionally. Apart from sufficient yield, sufficiently low alkaloid levels (<0,02%) were an important characteristic for the lupins. In the years that followed we have been testing many different varieties, on different soil types in The Netherlands.

Varieties

Starting from 2007 25 varieties have been tested for yield and yield quality in The Netherlands as a monocrop. Varieties were obtained from Denmark (B. Jørnsgard and S. Jørgensen), Germany (Saatzucht Steinach and Saaten Union), England (Soya UK) and Poland (Hodolwa Roślin Smolice). In table 1 the most frequently tested and most promising varieties are listed.

Table 1. Yield index and alkaloid content of 10 varieties tested in The Netherlands in 2007-2013

		Variety	No. of years tested	Average yield index	Yield index (alkaloid content) 2007	2008	2009	2011	2012	2013
L. angustifolius	non-branching	**Primadonna**	1	99						99
		(S. Jørgensen, Dk)								(0,019%)
		Haags Blaue	4	80			113	58	49	101
		(SZ Steinach, D)					(0,009%)	(0,010%)	(0,013%)	(0,024%)
		Boruta	6	76	83	84	68	58	65	99
		(SZ Steinach, D)			(<0,01%)	(0,010%)	(0,009%)	(0,006%)	(0,010%)	(0,013%)
		Iris	6	100	100	100	100	100	100	100
		(B. Jørnsgard, Dk)			(<0,01%)	(0,015%)	(0,009%)	(0,003%)	(0,004%)	(0,017%)
	branching	**Boregine**	3	85	72	89	94			
		(SZ Steinach, D)			(0,015%)	(0,037%)	(0,014%)			
		Sanabor	4	66			74	41	51	99
		(SZ Steinach, D)					(0,009%)	(0,013%)	(0,020%)	(0,046%)
		Regent	1	99						99
		(HR Smolice, Pl)								(0,009%)
L. albus branch.		**Dieta**	3	65				69	54	73
		(Soya-UK, UK)						(0,005%)	(0,007%)	(0,017%)
		Volos	3	61				51	57	76
		(Soya-UK, UK)						(0,009%)	(0,012%)	(0,019%)
				100 = t/ha	**4,4**	**4,3**	**2,7**	**2,7**	**3,1**	**4,2**

The criteria for selecting suitable varieties were yield, yield stability, earliness in ripening and alkaloid levels. For earliness we were looking for varieties to ripen before mid-september as risks of bad harvesting conditions grow rapidly after that. Alkaloid levels need to be below 0,02% to be used for human consumption. Some varieties were only tested for one or two years, as they didn't meet one of the criteria mentioned. L. angustifolius varieties Arabella, Probor and Boregine and L. albus variety Feodora were too late in ripening at least in some of the years tested. L. luteus variety Erantis and L. angustifolius varieties Galant, Boregine, Sanabor, Sonet and Haags Blaue proved to be unreliable in alkaloid contents to meet the criteria for human consumption in some of the years tested. A lot of varieties also turned out to be poorly adjusted to the fungal diseases dominating in The Netherlands making them unreliable in yield potential (eg. Haags Blaue, Boruta, Sonate, Vitabor, Haagena, Bojar and Dalbor). The best yielding variety and most reliable in sufficiently low alkaloid contents over almost all years tested turned out to be a Danish variety Iris from B. Jørnsgard.

Soil type

In 2008 and 2009 different varieties were tested on two different soil types: acid sandy soils and calcium poor clayey soils. Average yields turned out to be 16% higher on clayey soils than on sandy soils in 2008 and 67% higher in 2009. The most likely explanation seems to be that the better moisture supply on clayey soils assures a higher and more stable yield over the years than sandy soils.

Table 2. Yields of L. angustifolius varieties on sandy and clayey soils in 2008 and 2009

	2008			2009		
	Sand	Clay	Clay/Sand	Sand	Clay	Clay/Sand
Variety	t/ha		%	t/ha		%
Viol	2,9	3,6	122%	1,8	2,9	162%
Haags Blaue				3,0	4,0	134%
Boruta	3,6	3,7	102%	1,8	3,3	182%
Iris	4,3	4,8	113%	2,7	3,4	126%
Vitabor	1,8	2,6	143%	1,4	3,2	225%
Sanabor				2,0	4,0	201%
Boregine	3,8	3,7	99%	2,5	3,8	153%
Probor				2,0	3,1	156%
			116%			**167%**

Potassium fertilization and soil type

Based on the work of Gremigni etal. (2001) experiments were started in 2008 on the effect of potassium on alkaloid and yield in lupin.

Table 3. Effect of K_2SO_4 fertilization on yield and alkaloid levels of L. angustifolius (cv. Boregine) on sandy and clayey soils in 2008

	Yield		Alkaloide	
Fertilization	**Clay**	**Sand**	**Clay**	**Sand**
	t/ha		*ppm*	
0 kg K_2O eq./ha	3,6	3,9	317	500
100 kg K_2O eq./ha	3,5	3,5	267	450
200 kg K_2O eq./ha	3,7	3,8	317	367

Clay soil: 167 mg K/kg soil and pH 6,4 Sandy soil: 35 mg K/kg soil and pH 5,2

A sweet, but relatively high alkaloid variety Boregine was fertilized with three levels of K_2SO_4 on a clayey soil with high levels of naturally available potassium and on a sandy soil with low levels of naturally available potassium. On both soil types no yield effects were found due to the fertilization, however alkaloid levels decreased on the sandy soil when fertilized with potassium, but no decrease was found on the clayey soil. This seems to indicate that when levels of naturally available potassium in the soil are high, no effect can be expected from additional potassium fertilization. In the years that followed we have further investigated the effects of potassium fertilization on both yield and alkaloids. We have been looking into the form in which potassium can be administered: as K_2SO_4 or as KCl. The effect of lowering alkaloid levels is more consistent with K_2SO_4 than with KCl. In some varieties (eg the variety of L. albus: Dieta) KCl even seems to be elevating the alkaloid levels rather than decreasing them. If the effect of lowering alkaloid levels should therefore be subscribed to potassium or sulphur still remains point of study.

Sowing date

In 2012 we were able to compare yields of a great number of varieties sown mid-March vs. mid-April. Although one variety showed a yield increase when sown later, the average yield loss due to late sowing was 16% with a maximum yield loss of 38% for the variety Sanabor. We intended to replicate this experiment, but the weather in spring 2013 did not allow us to do so.

Table 4. Yield effect of early and late sowing of 5 varieties of L. angustifolius and 2 varieties of L. albus in 2012 on sandy soil

	Yield (t/ha)		Yield
	16-mrt	21-apr	reduction
Haags Blaue	3,1	2,1	31%
Boruta	3,5	2,4	31%
Sanabor	2,6	1,6	38%
Sonate	1,2	1,5	-19%
Iris	3,1	2,9	5%
Dieta	3,2	2,4	25%
Volos	3,0	2,9	4%
			16%

Fungicides

In the years 2011-2013 we tested the effect of fungicide treatment on yield for different varieties of L. angustifolius and L. albus. The fungicides used were 1,2 l/ha Caramba (72 g/ha metconazool) en 1,6 kg/ha Signum (427 g/ha boscalid and 107 g/ha pyroclostrobin).

Table 5. Yield effect of the use of fungicides in the years 2011-2013 on sandy soils

	2011			2012			2013		
	Yield (t/ha)		Yield	Yield (t/ha)		Yield	Yield (t/ha)		Yield
	no-fung.	fung.	increase	no-fung.	fung.	increase	no-fung.	fung.	increase
Haags Blaue	1,6	1,9	24%	1,5	1,9	26%	3,4	4,2	22%
Boruta	1,5	1,9	21%	2,0	2,2	10%	3,7	4,1	12%
Sanabor	1,1	1,0	-8%	1,6	1,7	6%	3,3	4,1	26%
Iris	2,7	2,8	3%	3,1	3,2	4%	3,7	4,2	13%
Dieta				1,6	1,9	18%	2,5	3,0	24%
Volos				1,8	2,2	24%	2,7	3,1	17%
Av of first 4 varieties			**10%**			**11%**			**18%**

The fungicides were only applied to the crop in the early podfilling stage in 2011 and 2012. However in 2013 the application was done early in the early flowering stage and a second time in the podfilling stage. Although fungal pressures were higher in 2011 and 2012 than in 2013, the early and repeated fungicide treatment have resulted in considerable yield increases in nearly all varieties whereas in 2011 and 2012 some varieties only showed minor yield increases due to the fungicide treatment.

Conclusions

Due to extensive field testing, lupin production for human consumption in The Netherlands is getting more and more feasible. Although the varietal choice for suitable lupin varieties with guaranteed sufficiently low levels of alkaloid is still limited low alkaloid levels does not seem to exclude good production levels. The most suitable variety so far under Dutch conditions seems to be a Danish variety Iris. Early sowing, clayey soils and fungicidal treatments all seem to increase the yield potential considerably. Fertilizing with K_2SO_4 does not affect the yield but seems to decrease the alkaloid levels in most years, especially on soils low in naturally available potassium. As KCl sometimes has the opposite effect it still remains a question whether it is the potassium or the Sulphur that is responsible for that.

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The barriers to the commercialisation of lupins in Europe

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State of art and aim: The UK currently imports 3 million tonnes of soya and soya products per annum with most of it (2.8 million tonnes) being used directly in the animal feed industry. In total, soya accounts for around two-thirds of the supplemental protein fed to all livestock in the UK, and this figure is almost identical in the rest of the European Union. On paper, lupins would be the perfect solution to the problem to Europe's over-reliance on imported soya and European-grown lupins could theoretically replace much of this imported tonnage. Despite the efforts by various seed companies across Europe, the area of lupins grown in Europe remains relatively small, and broad uptake of the crop remains as elusive as ever. The author is a director of Soya UK, which is one of the few seed companies producing larger tonnages of certified lupin seed in Europe. This paper examines the various reasons why lupins have so far failed to take off as a major crop, and what the propects are for commercial breakthrough.

Results and discussion: Whilst the UK has a well-established area of lupin production, that area has remained fairly static for the last 10 years at an annual area of between 2000Ha and 3000Ha. Uptake has been much slower than may have been expected given the steady rise in the price of other protein sources within the same time frame . In addition, almost all of this area is grown on livestock farms with 80% of it being grown in mixture with cereals (usually spring triticale), and cut as forage. The remaining 20% will be grown as a pure stand of lupins and cut with a combine harvester, but will be retained on the farm for home-feeding as a high protein feed. As a result, almost no lupins are traded in the UK and there has been little or no uptake from the arable sector with the current area almost entirely being grown on livestock units.
The author has attempted to identify and address the factors that have been holding back the uptake of lupins. Major factors identified include;

- Confusion over the true feed value of lupins, and their feeding performance in comparison to established protein sources like soya.
- Confusion over the best type of lupin to grow, which are the best varieties to grow, and which geographic areas are most suitable for each type.
- Agronomic questions, including a lack of effective herbicides making weed control difficult.
- Lack of uptake from the feed sector. This has meant there is no volume market for lupins in the UK and has restricted lupins to relatively low volume niche markets.
- Inconsistent yields and quality. This has undermined confidence in the crop among both growers and potential end-users.

The author has also provided a speculative view of the future propects for lupins in Europe and what can be done to take lupins to the next level of commercial uptake. Recent changes to the oilseeds and grain markets have favoured lupins, as have other factors including changes to the EU support mechanisms. Lupins propects are more positive in 2015, than they have looked for some time, but there is much work to be done in developing new markets.

Tags: lupin, markets, commercialisation

Application of lupin protein isolates

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State of art and aim: For many years, lupins have been used as food in a minimally processed form, such as cleaned whole seeds, grist or flour due to their high nutritive value and functional properties. However, the constant research effort demonstrated a huge number of potential food application of lupin protein concentrates and particularly isolates as a functional ingredient wherefore, lupin protein products are finding increasing usage within the food industry. The functional properties that receive the most attention when characterizing isolated lupin proteins are solubility, emulsifying, foam activity, water/oil absorption, gelation, and more recently the capacity to replace fat. The relationship between technofunctionality of the main lupin proteins and their molecular features (size, conformation, composition) have already been reviewed extensively (Duranti et al., 2008). Besides inherent molecular properties, several studies demonstrated that particularly different processing steps (e.g. milling, de-oiling, extraction, precipitation, drying) have an effect on the functional properties of lupin proteins (e.g. Bader et al., 2011). Additionally, further purification and modification procedures as well as the food matrix itself play a decisive role (Lampart-Szczapa et al. 2006).

Results and discussion: An overview of various process conditions on the functional and sensory properties of lupin protein isolates and their application possibilities will be demonstrated. In particular, the potential of fermentation will be highlighted. Dairy like products such as cream cheese with attractive sensory properties can be obtained by the production of a lupin based milk equivalent and the appropriate fermentation and technology regime with selected lactic acid bacteria. The typical legume-like off flavours like *green-grassy* or *pea-like* can be reduced or even partly removed. Another potential application aspect of lupin protein isolates is the development of palatable meat substitutes. The texturization can be achieved either by conventional thermoplastic extrusion or by high moisture extrusion cooking yielding products with a fibrous texture that resemble muscle meat. Finally, a lupin protein isolate with a specific fat-like or rather creamy texture was produced by a salt-induced extraction followed by a dilution precipitation. The effects of replacing fat by this lupin protein isolate on the sensorial properties of low-fat food will be demonstrated using truffle fillings as an example. Therefore, an up-to-date survey is provided on the potential use of lupin isolates in food systems, Diary like products, meat alternatives and low-fat products.

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Tags: lupin protein isolates, application, diary like products, meat substitutes, fat replacement

Enzymes in narrow-leafed lupin (*Lupinus angustifolius* L.) – Their inactivation and contribution to off-flavour formation

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State of the art and aim: Due to the increasing demand for dietary fibre preparations and growing health consciousness of many consumers, lupin kernel fibre (*Lupinus angustifolius* L.) is gaining in importance as a nutritionally valuable food ingredient. However, the broader application of this product is limited by "green" and "bean-like" off-odours (Bader et al., 2009), which have similarly been described for pea and soybean preparations. The formation of volatile compounds responsible for these off-flavours is most likely attributed to the conversion of unsaturated lipids by endogenous enzyme activities, in particular, by lipoxygenase (LOX), peroxidase and lipase (Rackis et al., 1979; Stephany et al., 2015).
Hence, our objectives were to elucidate the activity of these enzymes in the course of seed ripening aiming at the identification of a suitable indicator of heat inactivation. Furthermore, the effect of hydrothermal enzyme inactivation on the formation of relevant odour-active compounds during storage over a period of 1 year should be evaluated quantitatively.

Results and discussion: Contrary to peroxidase and lipase, the activity of LOX increased during seed development, thus most likely having the highest impact on aroma compound formation in mature seeds. Lupin LOX was classified as type-1 LOX due to its substrate specificity for free fatty acids, whereas trilinolein and β-carotene were not oxidised. After physicochemical characterisation, LOX inactivation was monitored by applying various hydrothermal treatments (70 - 100 °C at 100% r.h.). Thereby, inactivation of LOX was faster for lupin seeds than for de-oiled flakes, which is reflected by a lower activation energy of 60.5 kJ/mol for lupin seeds compared to 78.0 kJ/mol for the flakes.
To elucidate the effect of LOX inactivation on odorant formation during technological processing, lupin kernel fibre was prepared from untreated and heat-treated (10 min at 100 °C and 100% r.h.) lupin flakes. After selecting seven predominant odorants deriving from the oxygenation of fatty acids (hexanal, (*E*)-non-2-enal, pentanoic acid, hexanoic acid, γ-nonalactone, (*E*,*E*)-deca-2,4-dienal and β-ionone), these volatiles were quantitated using stable isotope dilution assays (SIDA). Thereby, the formation of undesired carbonyl compounds in hydrothermally treated lupin fibre was found to be higher than in untreated samples, indicating the importance of non-enzymatic pathways on off-flavour generation.
In conclusion, the present results provide important information for a process development to gain sensorially improved lupin fibre suitable for the application in a variety of foods.

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Tags: *Lupinus angustifolius* L., lipid oxidation, endogenous enzymes, hydrothermal treatment, stable isotope dilution assays (SIDA).

Malting process of lupin to improve nutritional properties

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State of art and aim: Germination is a very complex process which leads to the changes in the profile of proteins, lipids, carbohydrates and other components. It is also known to modify flavour and reduce oligosaccharides, which cause flatulence and abdominal discomfort while consumed. The objective of the study is to optimise malting conditions of *Lupinus angustifolius* seeds to reduce oligosaccharides content and to obtain malted lupin flour with improved nutritional properties.

Results and discussion: Malting process of hulled lupin seeds was monitored at room temperature (22°C) and samples were analysed after soaking and after 12, 24, 48 and 72 hours of germination. Samples were then separated to be dried using three different methods: freeze-drying, drying in the cabinet oven at 50°C and at 50, 60, 70 and 80°C to simulate malting conditions. At each point analysis of moisture, colour, shoot lengths and oligosaccharides was conducted. The moisture content of raw seeds was 8.5% and it increased to 55% after soaking, and was gradually increasing until 76% after 72 hours of germination. The drying of germinated seeds was conducted in a cabinet dryer with air flow and was optimised to dry the seeds to ~5% moisture at 50°C, and ~2% moisture content at 80°C. 96% of seeds were found to be viable showing good sprouting and vigour. The shoot length was monitored of the time of germination and the first shoots were observed after 12 h of sprouting and they achieved the length of 11 mm after 24 h and 47 mm after 48h. The colour of malted flour changed from yellow to green and had a green pea aroma. High Performance Liquid Chromatography (HPLC) with RI detector was used to analyse the major oligosaccharides and sucrose content in germinated flour. The concentration of verbascose, stachyose, raffinose and sucrose decreased with increasing germination time. Malting process of lupin seeds improved nutritional properties of lupin flour and reduced oligosaccharides content in malted lupin flour. Future studies will involve more analysis of nutrients and flavour changes in lupin. Malted flour will be a useful ingredient for the food industry.

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Tags: Lupinus angustifolius, malting, germination, oligosaccharides

Biotechnological solutions for wheat-lupin bread safety and quality characteristics improvement

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State of art and aim: There is a growing interest in industrial exploitation of new protein sources such as plant proteins to broaden the range and variety of foods (Guillamon et al. 2010). Legumes represent, together with cereals, the main plant source of proteins in the human diet. The main interest in lupin for foods is related to a high protein content of approximately 35–45% compared to soybean (Sujak et al. 2006). Lupin products are valued for their GMO free status, functional food properties, nutritional and health benefits and seem to be particularly promising as a source of an innovative food ingredient for the food industry in Europe. Foods based on lupin protein are gaining attention from consumers because of their possible role in the prevention of cardiovascular disease as well as in reduction of blood glucose and cholesterol levels. They are considered to be a good source of lysine, and are generally poor in the sulfur-containing amino acids (Gulewicz et al. 2008).
Furthermore, lupin exhibits useful techno-functional properties allowing its use as an ingredient in the production of several palatable food products, such as biscuits, pasta and bread. For instance, the supplementation of wheat flour with high-protein legume flours improve the nutritional quality of baked goods, also lupin does not contain gluten thus it could be used as a functional ingredient in gluten-free foods.
The aim of the study was to investigate the acrylamide formation during production of wheat bread supplemented with lupin flours fermented by *Lactobacillus* and *Pedioccocus* strains. Additionally, bread texture, sensory characteristics and overall acceptability were analysed.

Results and discussion: The use of fermented lupin resulted in a lower specific volume and crumb porosity of bread on average by 14.1% and 10.5%, respectively, while untreated lupin lowered the latter parameters at a higher level (30.8% and 20.7%, respectively). The addition of lupin resulted in a higher by 43.3% acrylamide content compared to wheat bread (19.4 μg/kg d.w.). Results showed that acrylamide was significantly reduced using proteolytic *L. sakei* and *P. pentosaceus* strains for lupin fermentation. Although the bread supplemented with lupin spontaneous sourdough had the lowest level of acrylamide (15.6 μg/kg d.w.), but it has the malodorous flavour and was unacceptable to the consumers. The lactofermentation could increase the potential use of lupin as a food ingredient while reducing acrylamide formation and enriching bread with high quality proteins.

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Tags: wheat – lupin bread, lactic acid fermentation, safety, quality.

Determination of quinolizidine alkaloids in lupin flour by LC-MS/MS A promising novel way of analysis

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State of art and aim: Quinolizidine alkaloids in lupin flours and lupin-containing food products, are regulated at a maximum of 200 mg/kg product, because of their suspected adverse effects on human health (Koleva *et al.*). Quinolizidine alkaloids are typically measured by GC-MS, GC-NPD or GC-FID (Resting *et al.*, Reinhard *et al.*). These methods have a long history of use and extensive databases are available to the researcher to assist in the identification of known or novel alkaloids. Main disadvantages of GC-based methods are the relatively complex and time-consuming sample preparation procedures required, including liquid-liquid partitioning (LLE) or solid phase extraction (SPE) techniques. Challenging matrices may require additional derivatization and polar or high-molecular weight metabolites may be lost due to limitations of the GC columns used. In principle, many of these disadvantages can be circumvented by using LC-based methods, most notably LC-MS/MS. Surprisingly, thus far very little work has been conducted in this area and in practice no LC-MS methods are available that can be used for the routine analysis of quinolizidine alkaloids. Our aim was to develop a fast, efficient and reliable LC-MS/MS method for the detection and quantification of quinolizidine alkaloids in lupin flours and products.

Results and discussion: As no LC-MS methods have been published to date, the method was developed from scratch. Using commercially available analytical standards and lupin seed extracts, a mass spectral database comprising 75 quinolizidine alkaloids was built by collecting fragmentation spectra under positive electrospray (ESI) conditions. For each compound two or more precursor-product ions were identified for incorporation in a multiple reaction monitoring (MRM) method. HPLC conditions were optimized for a Waters Acquity BEH C18 reversed phase column. In the final 15-min method an ammonium formate – acetonitrile gradient was used, allowing baseline separation of all relevant isomeric pairs (e.g. lupanine/isolupanine; sparteine/isosparteine). Quinolizidine alkaloids could be extracted in high yield (>90%) and repeatability (<10%) from the lupin matrix by using an acidified mixture of methanol and water. Due to the high sensitivity (LOQs <1 mg/kg) and selectivity of the LC-MS/MS instrument, the only clean-up required was filtration through an 0.45 μm membrane filter in the vial. The method was used to quantitate a set of lupin flours intended for food or feed applications from different origins (Australia/Germany/the Netherlands). An average content of 350 mg/kg (range 75-1314 mg/kg) was determined for the sum of lupanine, isolupanine and 13-hydroxylupanine.
The pros and cons of the novel method with respect to identification and quantification of lupin alkaloids will be discussed and its potential for applications as a straightforward analytical method in food and feed analysis. Its usefulness in the detection and identification of novel high molecular weight quinolizidine alkaloids will be highlighted as well.

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Tags: Quinolizidine alkaloids, lupin seeds, analysis, LC-MS/MS.

Session 7

Lupin: nutrition and human health

Keynote lecture

Can lupin consumption reduce risk factors associated with metabolic syndrome: current evidence and future studies

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State of art and aim: Lupin is gaining international interest as a source of high protein, high fibre food ingredients with potential to protect against metabolic syndrome. Metabolic syndrome is a cluster of cardiovascular disease risk factors including obesity, insulin resistance (risk factor for type 2 diabetes measured by blood glucose and insulin concentrations), dyslipidaemia (blood lipid abnormalities) and hypertension (high blood pressure). The aim of this paper is to review the current evidence from placebo-controlled, blinded, randomised human clinical trials investigating the effects of consuming foods, containing lupin flour, kernel dietary fibre or protein fractions on risk factors of metabolic syndrome.

Results and discussion: *Obesity*. Post-prandial studies in healthy adults on the effect of adding lupin flour or kernel fibre to foods provides some evidence for elevated short-term satiety effects. However longer term studies investigating effects of incorporating lupin flour containing foods into the diet on body weight have so far been equivocal. *Type 2 diabetes*. Short-term studies investigating the effects on post-prandial blood glucose and insulin levels of addition of lupin flour (non-diabetics and type 2 diabetics) and kernel fibre (non- diabetics) to carbohydrate-rich foods and of isolated lupin protein γ-conglutin (non-diabetics) provides evidence of beneficial effects for type 2 diabetes management. A longer term study is now underway in those with type 2 diabetes to confirm these potential benefits. *Dyslipidaemia*. Dietary intervention studies have reported beneficial effects on blood cholesterol of both lupin kernel fibre (one study in men with normal to mildly elevated cholesterol) and isolated protein (in hypercholestolaemic adults). However, long term consumption of lupin flour-containing foods appears to have little impact on blood lipids, at least in those without elevated baseline cholesterol levels. *Hypertension*. Dietary intervention studies on the effects of lupin flour-containing foods on blood pressure (in those overweight and obese) have provided consistent evidence of cardiovascular disease protective blood pressure lowering effects. *In conclusion*, the limited number of studies reporting the effect of lupin foods on metabolic syndrome risk factors have revealed some potentially health protective effects. Further evidence from long-term human studies in those with metabolic syndrome such as the obese, insulin resistant/type 2 diabetic, hypercholestrolaemic and hypertensive is now required to substantiate the metabolic benefits of lupin consumption.

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Tags: blood pressure, cholesterol, glycaemia, lupin, metabolic syndrome, obesity.

The health benefits of lupin in cardiovascular prevention: ten years of successful investigations

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Abstract
There is now a growing interest for lupin in human nutrition owing to the increasing number of experimental and clinical studies showing that it provides useful benefits in cardiovascular protection. This paper presents the main results in literature in the area of dyslipidemia and hypertension prevention and provides some data on the mechanism of action. By adding lupin protein isolates to the diet of different animal models of hypercholesterolemia, such as rat, rabbit, hamster and pig, significant decreases of total and non-HDL cholesterol are observed. These results are confirmed by clinical trials, with the limitation that only those involving hypercholesterolemic subjects and based on improved lupin foods give statistically significant total and/or LDL-cholesterol reductions. There are also evidences in favor of a hypotensive activity of hydrolyzed lupin protein. This body of information suggests that lupin may become an ingredient of innovative functional foods for cardiovascular protection.

Keywords: dyslipidemia, hypertension, statin-like, ACE-inhibitory.

Introduction
The interest for sweet lupin is continuously growing stimulated by its flexibility in food preparation as well as by the increasing knowledge of the health benefits provided by its consumption. Although different lupins are used as food, only *Lupinus albus* (white lupin) and *Lupinus angustifolius* (narrow-leaf lupin) have been investigated for their bioactivity. Different potential health benefits of lupin have been investigated, particularly in the area of dyslipidemia, hyperglycemia, and hypertension prevention. Starting from 2004 (Sirtori et al., 2004), our group has investigated the hypocholesterolemic and hypotensive effects and provided main information on the mechanism of action (Lammi et al., 2014). These activities are the center of this paper.

Results and discussion
Prevention of dyslipidemia. The studies in this area received a great impulse from the approval of the health claim on soy protein and cardiovascular prevention (FDA, 1999) and the demonstration that the protein itself is a main hypocholesterolemic ingredient in soybean (Consonni et al., 2011; Duranti et al., 2004).

Animal studies. In a first paper (Sirtori et al., 2004), rats fed a cholesterol-rich diet containing 20% casein were treated for 14 d with a lab-prepared total protein extract from white lupin *versus* the vehicle (control). The lupin-treated rats showed significant decreases in total cholesterol (-22.7%) and low-density-lipoprotein cholesterol (LDL-C, -30.2%) *versus* the control. A few years later, the possibility of performing biological studies was increased by the availability of large amounts of a purified protein isolate (W-LUP) from white lupin (D'Agostina et al., 2006), containing mostly legumins + vicilins, prepared by the Fraunhofer Institute IVV (Freising, Germany). The cholesterol-lowering activity of this material was successfully assessed in the hypercholesterolemic rat model, total cholesterol (-11.2%) and LDL-C changes (-38.7%) were observed (Bettzieche et al., 2008). The potential anti-atherosclerotic activity was tested instead in a rabbit model of atherosclerosis (Marchesi et al., 2008). In this model, cryosection analyses of the carotids indicated a significant reduction in focal lesion progression in the W-LUP group *versus* the casein group (-37.4%; $P < 0.05$). Finally, the cooked flour and a total protein extract from white lupin have been tested in hamsters again *versus* casein (Guadagnucci Fontanari et al.,

2012): the lupin diet not only induced significant decreases of total cholesterol and non-HDL-C, but also reduced the level of liver steatosis (level 1) *versus* the control diet based on casein (level 4). The researches on narrow-leaf lupin protein isolate (NL-LUP) are more recent and confirm that also the protein of this species possesses hypocholesterolemic properties (Bettzieche et al., 2008; Parolini et al., 2012).

Human studies. Table 1 reports the results of the clinical studies on lupin. These nine studies may be divided in three groups depending on the different lupin ingredients investigated. Surprisingly, only one study was performed on white lupin (Nowicka et al., 2006), whereas all others were on narrow-leaf lupin.

Table 1. **Lipid responses in clinical studies evaluating how lupin food consumption modifies lipid metabolism**. Abbreviations: T-C = total cholesterol; HDL-C = high-density-lipoprotein cholesterol; LDL-C = low-density-lipoprotein cholesterol; UNCON = uncontrolled; PARA = parallel design; CROSS = cross over design; SB = single blind; DB = double blind. Significant changes *versus* initial values are in bold, when they are significant also *versus* the control they are labelled with an asterisk (*).

Reference	T-C control value (mmol/L)	T-C change (mmol/L)	LDL-C control value (mmol/L)	LDL-C change (mmol/L)	HDL-C control value (mmol/L)	HDL-C change (mmol/L)	LDL-C / HDL-C change
1) Studies based on model foods from whole grain							
Nowicka et al., 2006	6.2	-0.42*	3.98	-0.32*	1.52	-0.07	
Hodgson et al., 2010	5.35	0	3.34	-0.04	1.39	+0.06*	
Belski et al., 2011	5.18	-0.10	3.29	-0.09	1.33	+0.01	
2) Studies based on model foods added purified fiber							
Hall et al., 2005	5.36	-0.22*	4.36	-0.19	1.26	+0.02	-0.15*
Fechner et al., 2014	6.55	-0.42*	4.08	-0.33*	1.53	+0.03	-0.29*
3) Studies based on model foods added purified protein							
Weisse et al., 2010	5.65	-0.50	3.61	-0.31	1.75	-0.08	-0.11
Sirtori et al., 2012	7.08	-0.30*	4.86	-0.15	1.45	-0.03	-0.02
Bähr et al., 2013	6.54	-0.12	4.38	-0.26	1.46	+0.04	-0.29
Bähr et al., 2015	6.41	-0.28	4.16	-0.15*	1.40	-0.05	-0.02

Group 1. An uncontrolled study on smokers (Nowicka et al., 2006) based on a lupin drink gave significant decreases in total cholesterol and LDL-C *versus* the same values measured during the preceding low lipid diet. The decrease was larger in highly hypercholesterolemic patients. The others were blind studies on model foods containing whole kernel flour (Hodgson et al., 2010; Belski et al., 2011). Both studies, involving only subjects with normal or borderline initial cholesterol levels, were essentially inactive on cholesterol.

Group 2. These studies have investigated purified narrow-leaf lupin fiber included in different foods (Fechner et al., 2014; Hall et al., 2005). Both studies produced significant decreases of total and LDL-C.

Group 3. In all these studies, the tested material was the lupin protein isolate NL-LUP. In two cases, this material was included in dietary bars and the control bars contained casein (Sirtori et al., 2012; Weisse et al., 2010). In the first study on subjects with a very moderate hypercholesterolemia (Weisse et al., 2010), the lupin treatment significantly decreased the lipid parameters compared to the baseline values, but similar improvements were observed also in the control group. In the second study on hypercholesterolemic subjects (Sirtori et al., 2012), the lupin bar gave a significant decrease of total cholesterol *versus* the control bar; LDL-C was, instead, essentially unchanged in both groups. In the third study, in which NL-LUP was incorporated into a drink, LDL-C was significantly lower at the end of the

study *versus* the baseline value, however, this decrease was non-significant *versus* the control (Bähr et al., 2013). Better results were obtained in the fourth study (Bähr et al., 2015), where the patients received a portfolio of different food items containing NL-LUP, very similar to normal foods. LDL-C was significantly lower at the end of the study *versus* the control, whereas both total and LDL-cholesterol were significantly lower at the end of the study *versus* baseline values. Possibly, the better efficacy of this study may be explained with a relevant improvement of the compliance of the tested products. The fact that subjects with higher cholesterol are more sensitive to treatment with lupin foods is not surprising. In particular, a meta-analysis on soybean has shown that the square of the initial serum cholesterol is the main significant predictor of the observed changes of total and LDL cholesterol concentrations observed after soy protein consumption (Anderson et al., 1995). The same phenomenon takes place also while consuming other grain legumes (Arnoldi et al., 2015).

Prevention of hypertension. Another main area of interest is the prevention of hypertension.
In vivo studies. Only one study (Pilvi et al., 2006) has investigated the potential hypotensive effect of lupin protein, by using the Goto-Kakizaki rat model, which develop hypertension when fed a salt-rich diet (6% NaCl). The protein sources in the diet were either a white lupin or a soy protein isolate. At the end of the two-week treatment, the systolic blood pressure (SBP) was 18.6 mmHg lower in the lupin group and 12.0 mmHg lower in the soy group than in the control group (casein). In addition, both lupin and soy treatments have normalized the vasoconstriction observed in the NaCl-fed control group, whereas only the lupin treatment improved the impaired endothelium-dependent vasodilatation (Pilvi et al., 2006). Some studies on humans, mainly with other primary end-points, reported also positive effects on blood pressure. In particular, in an uncontrolled study on subjects with moderate hypercholesterolemia and hypertension (Nowicka et al., 2006), the lupin treatment significantly reduced also SBP and diastolic blood pressure (DBP). The changes were more evident in hypertensive subjects. Moreover, two long-term randomized controlled studies showed that the consumption of foods supplemented with narrow-leaf lupin flour produces small but statistically significant decreases in blood pressure *versus* the control foods (Belski et al., 2011; Lee et al., 2009) and significant pressure decreases were observed in the recent study focused on narrow-leaf lupin fibre (Fechner et al., 2014).
In vitro studies. A possible explanation of the mild hypotensive activity observed either in human or animal studies after lupin consumption is that the proteins are cleaved in the gastrointestinal apparatus generating hypotensive peptides previously encrypted in the parent protein sequence. Since some food peptides are able to inhibit the activity of the angiotensin I converting enzyme (ACE, EC 3.4.15.1), which plays an important role in regulating blood pressure in the renin-angiotensin system, also white lupin and narrow-leaf lupin as well as yellow lupin (*Lupinus luteus*) have been investigated for their ACE-inhibitory activity. Total protein extracts from these seeds were digested with different proteolytic enzymes and the ACE-inhibitory activity was measured (Boschin et al., 2014a; Boschin et al., 2014b). In general, the most effective peptide mixtures were obtained with pepsin (mean IC_{50} value of the three species 186±10 μg/mL). Although there are not yet direct experimental evidences, on the basis of these results, the hypothesis that the formation of ACE-inhibitory peptides may explain the hypotensive effects observed *in vivo* appears feasible.

Conclusions

Available experimental evidences, both in animals and humans, indicate that lupin may provide some useful health benefits in the area of hypercholesterolemia and hypertension prevention as well as control of glycemia (Duranti et al., 2008). The observed effects probably derive from the synergistic combination of the activities of many seed components, whose specific role is still to be elucidated.

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Lupinus albus gamma conglutin decreases glucose in healthy subjects and type 2 diabetes mellitus patients.

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State of art and aim: *Lupinus albus* gamma-conglutins were isolated and characterized using size exclusion (SE) and reverse phase (RP) HPLC and electrophoresis before performing their mass spectra analysis (MALDI-TOF). Thereafter, normal individuals before a second oral glucose tolerance test (OGTT) orally took these characterized proteins. Gama-conglutins, also was administered to type 2 diabetes mellitus human volunteers after a two days discharge of their recently indicated metformin prescription in order to precise their magnitude of hypoglycemia induction.

Results and discussion: Normal adult subjects (n=15) received a 75g oral dose of glucose that was drunk within 5 minutes to define their response to an oral glucose tolerance test (OGTT) one day before but also, 10 minutes after they orally received 100 mg/Kg of *Lupinus albus* gamma-conglutin. Most of the selected type 2 suffering diabetes patients (n=16) were overweight and obese and all were recently receiving metformin. All patients were explored for normal liver, kidney, and heart functioning as well as to clarify of not suffering of lactic acidosis. In addition, they were not taking cimetidine neither cephalexin. Clinical history, physical examination and type 2 diabetic patients evolution notes were carefully monitored by a physician and nurses at the health clinic at Jocotepec before and during the period of metformin intake and its withdrawal during a two days period. Interruption of Metformin oral intake was done under medical guidance, without nutritional and exercise habits modifications two days previously to the measurement of basal blood glucose, subsequent oral administration of 100 mg/Kg of *Lupinus albus* gamma-conglutin and glucose measurements every 30 minutes up until 120 min.

Biochemical assays of transaminases, creatinine, urea and CK were within the normal values. Glycemia decrease was statistically significant during 60 min of the oral glucose tolerance test in normal healthy subjects ($p<0.05$) and, although statistically significant in type 2 diabetic patients the glycemia decreased less than observed in healthy subjects and their response seemed to be dependent of the glycemia range group. In conclusion, *Lupinus albus* gamma conglutins from the Atemajac de Brizuela, Jalisco habitat, after being carefully characterized through: SE, RPHPLC, electrophoresis and MALDI-TOF) as the 45kDa gamma-conglutin displayed a short duration hypoglycemic effects in normal volunteers and DM2 patients. A suitable method as well as a greater number of patients are needed to obtain a higher yield of gamma-conglutins to confirm the benefits of the herein reported hypoglycemic effects in normal volunteers and type 2 diabetic patients.

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Tags: gamma-conglutin, type 2 diabetes, normal human volunteers, proteomics

Short Term Effects of Lupin versus Whey Protein on Glucose and Insulin Responses to a Standardized Meal

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State of art and aim: Whey protein is known to reduce postprandial glycaemia in type 2 diabetic subjects (FRID et al. 2005). Lupin protein as a vegetable alternative is currently also under investigation inducing similar effects (DOVE et al. 2011). The present study aims to assess and compare the acute glycemic and insulinotropic effects of whey and lupin protein supplemented to a carbohydrate-rich standardized meal (reference meal) in healthy volunteers. A total of 12 individuals, including healthy men and women aged between 20 and 45, took three different test meals (reference meal; reference meal+whey protein; reference meal+lupin protein) in a balanced randomized order. Volunteers' blood glucose and insulin levels were analyzed for baseline and at eight time points (+10, +20, +30, +40, +60, +120 and +180 minutes) after the ingestion of the test meal. The trial is registered at Clinical trials with the number NCT02413671.

Results and discussion: Supplementation with whey and lupin protein led to a significant lower increase in postprandial blood glucose levels compared to the reference meal itself ($p < 0.001$).
First significant differences compared to the reference meal could already be observed at ten minutes after the ingestion for both, the supplementation with whey ($p=0.013$) and the supplementation with lupin protein ($p < 0.001$). A significant difference between whey and lupin protein meal could only be detected at time point +20 ($p=0.040$), whereat the increase for whey protein was higher than for lupin protein. The expected insulin increase after the different test meals was significantly different compared to the reference meal ($p=0.001$). Supplementation with whey protein led to a higher increase than the reference meal at time points +20 ($p=0.004$) and +30 ($p=0.032$), whereas the addition of lupin protein caused a lower insulin increase than the reference meal being significant at time point +10 ($p=0.047$). The insulin increase after whey protein supplementation was higher than the insulin increase after lupin supplementation at time points +10 ($p=0.037$), +20 ($p < 0.001$) and +30 ($p=0.014$). Our results conclude that lupin and whey protein can lower the increase of postprandial blood glucose levels to nearly the same extent, suggesting that lupin protein can be used as a valuable alternative to whey protein to reduce glycaemia. However, the insulin response of both seems to be different.

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Tags: Lupin protein, whey protein, glucose and insulin response

Lupins: Potential Oral Health supplement for Management of Diabetes

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State of art and aim: In recent years, leguminous seeds have played a primary role in the search for vegetable sources of proteins on account of their high nutritional value and lower production costs as compared to animal proteins. Lupin (*Lupinus albus*) seed protein, specifically gamma-conglutin, is unique amongst legume proteins, because it has shown promise in glycemic control, a key to healthy and productive life. (Scarafoni *et. al.* 2007). Thus, there is an opportunity to add value to the lupin crop if gamma-conglutin can be extracted efficiently. However, a major challenge in their utilization is non availability of extraction process with satisfactory yields and selectivity for extracting gamma-conglutin on commercial scale. This study reports development and optimization of a scalable, efficient extraction process and its antidiabetic activity assessment in specific model. Although some important parameters on protein extraction are well established, systematic studies on overall yield including relevant processing parameters are still scarce. Design of Experiment (DOE) was performed to determine both the optimum operating conditions and the optimum design configurations for the extraction process.

Results and discussion: Based on risk analysis and critical quality attributes, the critical process parameters for extraction were established and optimized using JMP software with the yield of lupin proteins >90%. Extraction of gamma-conglutin and its antidiabetic activity was confirmed by blood sugar lowering effect in animal model. Extracted lupin proteins showed >50% reduction in blood sugar level within 4 hours of oral administration. Lupin protein extract was proven to be 10 times more potent than standard antidiabetic drug. The present work thus forms a basis for developing scalable and selective extraction process for bioactive gamma-conglutin with high yield and purity from lupin as a potential anti-diabetic oral health supplement.

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Tags: Lupin proteins, Extraction, Design of Experiment, Antidiabetic activity

Microbial biosynthesis to enhance vitamin B_{12} contents in Lupin flour

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Abstract
Vitamin B_{12} or cobalamin is an essential water-soluble vitamin. Fermented soy bean products, such as *natto* and *tempe*, are rich in cobalamin, even though soy bean is low in cobalamin. During fermentation, polysaccharides, proteins and lipids are hydrolysed by microbial enzymes into products with desirable sensory properties. Fermentation also facilitates some microorganisms to synthesise cobalamin. Based on this premise, this study aimed to enrich lupin flour with cobalamin through natural fermentation. Lupin flour (50g) was inoculated with commercial *tempe* starter culture or ground *tempe*. After mixing with 100g sterilised RO water, treated flour and un-inoculated flour were incubated for 20 hours at 30°C. Freeze-dried fermented flour was then prepared for cobalamin analysis. Samples were treated with pepsin in the presence of potassium cyanide and extracted using immuno-affinity columns. The column contained specific antibodies that were selectively bound to cyanocobalamin, which was then detected for quantitative analysis. Mean cobalamin determined in fermented lupin flour ranged from 18-30 μg/100g (dry weight basis); the mean differences were not statistically significant ($p>0.05$). The data suggested that cobalamin might have been synthesised by endogenous microorganisms present in the lupin flour. Addition of starter culture into sterilised or non-sterilised flour did not significantly increase the cobalamin levels ($p>0.05$). Further work is currently underway to identify cobalamin vitamers present in fermented flour and to investigate whether intermediates of cobalto-complexes contributed to cobalamin levels measured here.

Keywords: lupin flour, B_{12}, cobalamin, cyanocobalamin, natural fermentation, HPLC.

Introduction
Vitamin B_{12} and cobalamin are the generic terms encompassing the naturally occurring vitamers and cyanocobalamin, which is the synthetic form added as a fortificant and used in dietary supplements. The vitamers found in foods are methylcobalamin, adenosylcobalamin, hydroxycobalamin, cyanocobalamin and aquocobalamin. As co-enzymes, cobalamin plays a significant role in the metabolism of propionate, amino acids and single carbon. The chemical structure of cobalamin is characterised by a corrinoid ring with cobalt in the centre of the molecule. Bio-synthesis of cobalamin is limited almost exclusively to bacteria. Cobalamin is therefore found in foods that have been bacterially fermented and those derived from animal' tissues that have obtained it from their ruminal or intestinal microflora [1]. Soy beans contain low or undetectable cobalamin, however fermented soy products, like *tempe*, contain a significant amount of cobalamin [2]. It has been reported that the levels of cobalamin in *tempe* ranged between 0.04-0.085 μg/100g, dry weight basis [3] and 0.7-8.0 μg/100g, wet weight basis [4].
Lupin is taxonomically part of the legume family (*Fabacea*). Similar to other legumes, lupin is able to fix atmospheric nitrogen through the formation of root nodules, the highly specialised organs that result from the symbiosis between the host plant and the soil rhizobia [5]. Rhizobia require cobalamin and cobalt for their growth and DNA synthesis [6, 7]. The aim of this study was to investigate whether fermentation of lupin flour would enhance the cobalamin levels in lupin flour. It is known that fermentation biologically enriches food substrates with vitamins, protein, essential amino acids and essential fatty acids [8].

Results and discussion

Determination of cobalt

The accuracy of data obtained by ICP-MS (NexION 300D, Perkin Elmer, Billerica, MA, USA) was assessed by analysing the standard reference material (SRM 1573a). The analytical value of SRM 1573a tomato leaves was 62.3±1.9 µg/100g (dry weight basis) and was comparable with the certified value (57±2.0 µg/100g, dwb). Mean cobalt in raw lupin flour was 11.9±0.5 µg/100g (dwb) and in naturally fermented lupin flour was 10.9±0.01 µg/100g. Cobalt is an essential trace mineral required for the growth of root nodule bacteria, that reduce the atmospheric nitrogen in order to make nitrogen available to the plant [9].

Quantitative analysis of cobalamin

Samples of fermented lupin flour were processed according to published methods [10-12] with some modifications. Samples suspended in 50 mM sodium acetate buffer (pH 4) were treated with pepsin in the presence of 1% potassium cyanide. Pepsin treatment at 37 °C for 3 hours released the endogenous cobalamin from its binding protein. In the presence of potassium cyanide, cobalamin vitamers were converted to cyanocobalamin. This was subsequently extracted using Immuno-affinity columns (EASI-EXTRACT® VITAMIN B12, R-Biopharm, Sydney, Australia). The columns contain specific monoclonal antibodies that selectively isolate and concentrate cyanocobalamin that can be chromatographically detected during HPLC analysis. The specificity of the antibodies improved sample clean-up and reduced the matrix interferences, thus improving the peak detection and identification (Figure 1).

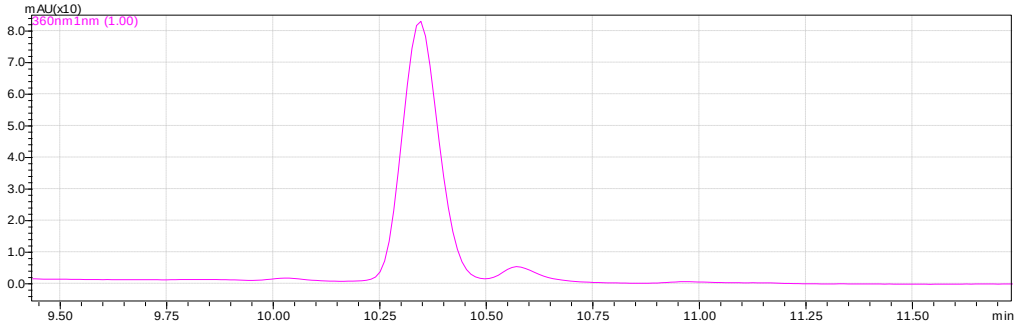

Figure 1 The cyano-cobalamin peak in fermented lupin flour that was detected at 360 nm with the retention time of 10.30 mins.

Standard calibration curves were determined by LINEST analysis and sample concentrations were calculated using linear regression y=ax+b. Calibration curves were linear between 0.15-60.0 µg/mL cyanocobamin (R^2=0.997).

Table 1. Mean cobalamin in samples of fermented lupin flour with varying treatments (µg/100, dry weight basis).

Samples	Mean cyanocobalamin (µg/100g)
Non-sterilised flour-natural	32.7
Non-sterilised flour-starter culture	20.0
Sterilised flour-starter culture	18.4
Non-sterile sample (flour, water,not autoclaved)	20.7
Non-sterilised flour-tempe	18.6

The highest mean cyanocobalamin (32.7 µg/100g, dwb) was found in naturally fermented lupin flour. The mean difference was not significant compared to other treated flour samples (p>0.05). Inoculation of lupin flour with commercial *tempe* starter culture (Indopal brand, Jakarta, Indonesia) did not increase the cyanocobalamin level. There was no significant difference in mean cyanocobalamin when sterilised and non-sterilised flour samples were fermented at 30°C for 20 hours (p>0.05). The typical levels of cobalamin in foods range from 0.3 to 25 µg/100g and B_{12} fortified breakfast cereals contain approximately 30 µg/100g [1]. The data presented here suggested that naturally fermented lupin flour is

likely a good source of cyanocobalamin.
According to Martens *et al* (2002), C-5 skeleton of glutamate is the initial substrate used by the bacteria for cobalamin biosynthesis that gives rise to 5-aminolevulinic acid, the first general precursor of tetrapyrroles. Glutamic acid is found in higher amount in lupin than other amino acids. Insertion of cobalt to precorin-2 intermediate leads to the formation of adenosylcobyric acid, which is the precursor of adenosylcobalamin [13]. Adenosylcobalamin and other derivatives are likely present in lupin flour and measured as cyanocobalamin. Lupin kernels obtain their nutrients through the root system that has rhizobia in the root nodules. It can therefore be suggested that lupin kernels and flour might contain endogenous cobalamin. Natural fermentation would have enhanced the amount of cobalamin measured here. Further work is therefore warranted to identify the presence of adenosylcobalamin and other vitamers in lupin flour. In this study, measured cyanocobalamin might have been derived from intermediates of cobalto-complexes that have likely been bound to the epitopes of the antibodies during extraction using the immuno-affinity columns.

Conclusions

Fermentation is a valuable technique in preserving foods that also enhances food quality by improving the sensory and nutritional properties. Cobalamin levels in fermented lupin flour ranged between 18-32 μg/100g (dwb). The result suggested that cobalamin might have been synthesised by microbes naturally present in lupin flour. Addition of commercial *tempe* starter culture or ground *tempe* itself did not increase cobalamin levels in flour ($p>0.05$). As rhizobia inhabiting the root nodules synthesise cobalamin, it is therefore possible that lupin kernels and flour might contain endogenous cobalamin. Current work is underway to identify cobalamin vitamers in lupin kernels, flour and fermented flour. Future work will investigate any cross-reactivity between antibodies and intermediates of cobalto-complexes during the extraction of cyanocobalamin using the immune-affinity columns.

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Lupin ferritin application for enrichment food in iron

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Keywords: iron, ferritin, iron deficiency, lupin sprouts

State of art and aim:

Iron deficiency is a common malnutritional disorder and affects more than 2 billion people. Due to the scale of this issue, many international organizations, with the World Health Organization (WHO) and United Nations Children's Fund (UNICEF) at the head as well as governments of many countries, have taken many actions to prevent it [*WHO, 2011*].

Food contains two types of iron, i.e. heme iron, present in food obtained from animal organisms, and non-heme iron, typical plant form of iron, but occurring also in meet. These two forms differ significantly in bioavailability: absorption of non-heme iron usually reaches 2-20% compared to 15-35% bioavailability of the heme iron (*USA Government, 2014)*.

Simultaneously, increasing heme iron consumption is very complicated, because of various economic and social factors. Thus, a good plant iron source, with a high bioavailability is sought-after. Plant biofortification is one of the considered solutions to that problem, because even edible plant parts rich in iron, such as legume seeds, contain too little of it, e.g. raw bean seeds contain ~10 mg/kg *(Sczekan & Joshi, 1987)*. One of the proposed strategies to significantly increase iron content in plant was legume is seeds germination in special conditions, i.e. high concentration of Fe^{2+} ions. Obtained sprouts are rich in iron enclosed in the ferritin (*Zielińska-Dawidziak et al., 2012)*. The protein has some unique properties, such as a high possibility of iron concentration (up to 4500 atoms) closed in the peptide shell. Moreover, ferritin displays good resistance to variable pH and digestive enzymes (*Theil et al., 2004*).

Results and discussion:

Lupin seeds have a long tradition of use for human consumption only in some countries, mainly South American and some European ones. In others it is still regarded only as a feed ingredient. Proposed new technologies of lupin processing may increase its application for humans, especially due to the unique protein component. Fortification of lupin in ferritin may be a way to obtain a good source of iron in the diet. Lupin seeds, compared to other plants, have huge tolerance to the presence of iron in their growing environment. While culturing in the solutions of $FeSO_4$, they sprout in the concentration up to 25 mM, forming shorter and bold seedlings, partially dyed red-brown, but still well-formed (Fig.1). Simultaneously, a huge amount of iron, up to ~700 mg/g d.m., is accumulated in the sprouts, depending on the genus (Tab.1), species and varieties (Tab.2).

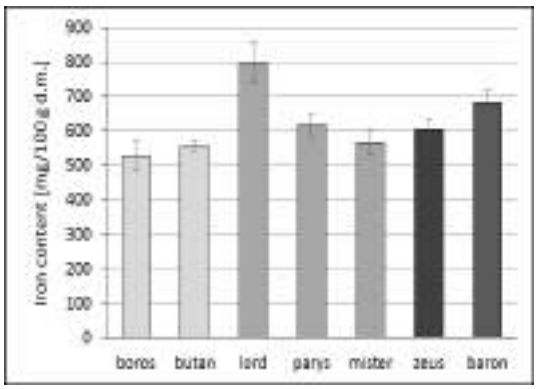
Figure 1. The sprouts of yellow lupin seeds after three-day-cultivation in the solution of 25 mM $FeSO_4$.

Because of the good yields (about 10 times higher than for alfalfa and about 2-3 times higher than for lentil) and quite good tolerance to the climate conditions of Central Europe, the lupin seeds seem to be a very interesting raw material for their biofortification in ferritin iron.

Table 1. Tolerance of legume plants to the $FeSO_4$ concentration in growth medium and accumulation of iron in the obtained sprouts.

Genus	Tolerance [mM $FeSO_4$]	Obtained level of iron binding [mg/100 g d.m.]
Lupin	25	~530-810
Soy bean	25	~600
Alfalfa	25	~1800
Lentil	14	~550

Figure 2. Iron content in sprouts obtained after seven-day-cultivation from: white lupin seeds (Boros and Butan vars.), yellow lupin seeds (Lord, Parys and Mister vars.) and Lupinus angustifolius seeds (Zeus and Baron vars.).



Iron is most efficient accumulated in husk and growing radicle and cotyledon (Tab. 2). However, there is no point in separation of the sprouts fragments. More important is proper formulation of the preparation. The sprouts are a very unstable kind of food, susceptible to microbiological contamination. Therefore, in the experiments drying and milling process were proposed to increase the shelf life of the products constructed with the addition of sprouts enriched in ferritin iron.

Table 2. Iron content in different anatomical parts of lupin sprouts.

Anatomical part	Iron content [mg/100 g d.m.]		
	Boros	Lord	Baron
Seeds	415±8 *a**	460±13 *a*	519±4 *a*
Husk	874±6 *d*	1202±6 *d*	851±15 *c*
Radicle	633±14 *c*	826±53 *c*	1004±3 *d*
Total	567±15 *b*	804±27 *b*	655±4 *b*

**The values expressed as an average ± standard deviation marked with different letters are significantly different (p= 0.05)*

Properly prepared sprouts, delivering an iron in the form promising for supplementation, became a component of many food products. The conducted experiments confirmed the stability of obtained plant ferritin in some food products (pastas, extruded and bakery products), even if these experiments were conducted on soy sprouts. However, it could be expected that for the lupin sprouts the results will be comparable.

Two instant deserts were the first group of the produced product were: 'jellies'* (lemon and orange) and 'puddings'* (chocolate, cream and cherry). Slight losses in the iron content of ferritin were observed in the products prepared to consumption with hot water. They were greater in the case of the analyzed 'pudding' samples, in which the presence of Fe^{3+} ions was also detected, i.e. iron form with strongly limited absorption. The average ferritin iron content was 67% in 'jellies' and 45% in puddings. Products prepared in this way were accepted in the performed sensory tests (the most favorable was chocolate pudding).

Triticale crisp bread was produced with the 5% addition of sprouts. The resulting product was subjected to sensory evaluation and speciation of iron. The product did not obtain consumer acceptance, because of the perceptible grassy flavor. Moreover, 99% of ferritin was degraded in a consequence of the applied pressure and temperature. However, produced corn snacks were positively assessed by consumers (due to the peanut flavor). Decreasing the extrusion temperature prevents ferritin denaturation and the obtained product contained 45% of iron in that form. The use of sprouts to the production of expanded rice wafers was also proposed, even if the application of high temperature is generally needed, but the time of its action is really short (some seconds). Unexpectedly good tolerance of ferritin to the production process conditions was noted – almost 70% of ferritin iron was stabile in these wafers.

While, during bread baking, partial degradation of ferritin was observed. Complexed iron content in the product was 55-68%, highly variable and depended on the obtained product batch.

Raw pastas were prepared with maintaining a huge amount of stabile ferritin (~70%). But after their preparation for consumption (7 minutes of boiling) the iron losses were bigger and reached about 45%.

The most important crucial of ferritin iron used in fortification is its isolation from the surrounding. This protects the food components against the oxidation caused by iron ions, and also reduces the products discoloration *(Hurrel, 2002; Davila-Hicks et al., 2004)*. Nevertheless, the protein stability during digestion processes is more important for the nutritional value of the products which contain ferritin.

Susceptibility of lupin ferritin was also analyzed during *in vitro* experiments conducted in the artificial digestive tract, excluding the mouth stage. Firstly, dried and powdered sprouts were two-hour-digested in the stomach at pH 2.0 with the addition of pepsin. Secondly, changes were introduced to the environmental conditions, in order to simulate digestion in the upper small intestine (pH 7.4, addition of trypsin and bile salts). Partial degradation of ferritin in the stomach was observed (up to ~25%), but at the intestine stage, up to 35% of total iron in the form of ferritin, containing mineral core, was probably released. These experiments support the hypothesis that ferritin may escape the degradation in the stomach and differ from those obtained during *in vitro* experiment conducted on pure ferritin preparation. Ferritin stability during digestion processes has been suggested in many experiments conducted *in vivo*, both performed on humans *(Lönnerdal et al., 2006; Theil et al., 2012)* and in animal models (mainly with rats) *(Beard et al., 1996; Zielińska-Dawidziak et al., 2012)*. The *in vivo* experiment proved many times a good ferritin iron bioavailability, which would not be possible if ferritin is decomposed at the stomach stage of digestion. We may only suggest that ferritin, delivered to the human organism as a food component, is either protected against its denaturation in the stomach by other food ingredients or renaturation of ferritin in the intestine may occur (*Zielińska-Dawidziak, 2015*). Moreover, a small percentage of ferritin available to the absorption by the enterocytes results also from the problems of iron extraction from the digested food.

If stability of ferritin in the stomach is proved, the product enriched in ferritin iron could have a unique nutritional value, because the absorption of ferritin iron takes place via mu2 (AP2)-dependent transporter (*San Martin et al., 2008*). Thus, its preparations may be offered to humans with disturbed iron absorption (e.g. suffering from inflammatory bowel disease).

Simultaneously, a huge amount of iron closed in the apoferritin shell could lead to some doubts connected to the ferritin safety, when it is absorbed in endocytosis process. Uptake of thousands iron atoms with the one absorbed *via* enterocytes molecule, could suggest both an easy overdose the metal in these cells and increased danger inflammatory processes occurrance. However, *in vivo* experiments conducted on rats with ferritin filled with lead ions proved that ferritin absorption is strongly controlled and regulated. This uptake depends on the iron status of individuals, as it is also observed for other iron forms (*Zielińska-Dawidziak, 2014*).

Moreover, it should be remembered that the ferritin preparations used as a supplement in the form of powdered sprouts contain many other constituents with high nutritional value, e.g. antioxidants. These compounds are well-known agents inhibiting inflammation processes. The parameters of the lupin seed germination technology has been altered in such a way that the antioxidant potential (expressed in mM of Trolox/kg) of the obtained lupin sprouts increased almost tenfold, when compared to dried seeds.

Discrepancies among results concluded from experiments concerning the bioavailability of ferritin,

should not undermine the possibilities of its application as a food supplement, because the *in vivo* results are very optimistic, both in rats and humans. It may only suggest that food composition may strongly influence this bioavailability, and properly designed food enriched in lupin ferritin may become a really valuable iron supplement.

* These instant desserts are typical Polish ones, named 'jelly' and 'pudding' for the purposes of article. 'Jelly' – Polish 'kisiel' - is a fruit juice, sweetened and condensed with gelatinized starch; 'pudding' – Polish 'budyń' is a flavored milk condensed with flour (gelatinized starch).

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Molecular investigation of the mechanism of action through which lupin peptides induce hypocholesterolemic effects on HepG2 cells

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Abstract
Previous experiments in suitable animal models and mild hypercholesterolemic individuals have shown that the consumption of lupin proteins may be useful for controlling total and low-density-lipoprotein (LDL) cholesterol levels. With the objective of providing evidences that peptides generated by the digestion of lupin proteins may be responsible of the observed activities and for investigating the mechanism of action, HepG2 cells were treated with lupin peptides obtained either by pepsin (P) or trypsin (T) hydrolysis and molecular and functional investigations were performed on the LDL receptor / SREBP2 pathway. Our findings indicate that peptides obtained from the hydrolysis of lupin proteins are able to interfere with the HMGCoAR activity. Immunoblotting experiments showed that the treatments with lupin peptides induce an up-regulation of the SREBP2 protein level and consequently to the up-regulation of the LDL receptor. From a functional point of view, the increase of LDLR proteins leads to an increase of the HepG2 cells ability to up-take LDL with final hypocholesterolemic effects. The activation of LDLR/SREBP2 pathway is regulated by the activation of PI3K/Akt/GSK3β pathways.

Keywords: cholesterol regulation, plant protein, *Lupinus*, HMGCoAR.

Introduction
Lupin is a protein-rich grain legume, which has been domesticated long time ago and cultivated in different continents, either for animal or human nutrition. This generic term indicates four species: *Lupinus albus* (white lupin), *Lupinus angustifolius* (narrow-leaf lupin), *Lupinus luteus* (yellow lupin), and *Lupinus mutabilis* (Andean lupin). Interestingly, it has been demonstrated that lupin consumption may provide some health benefits, particularly in the area of hypertension and dyslipidemia prevention. In particular, previous experiments in suitable animal models and in mild hypercholesterolemic individuals have shown that the consumption of lupin proteins may be useful for controlling total and low-density-lipoprotein (LDL) cholesterol levels [1-3].
The majority of plasma cholesterol is transported by the LDL fraction and the cellular uptake of LDL is mediated by the LDLR. The circulating level of LDL is largely determined by its rate of uptake through the hepatic LDLR pathway [4]. In general, the LDLR expression is finely tuned by changes in intracellular cholesterol. A transcription factor known as the sterol-responsive element binding protein 2 (SREBP2) plays a critical role in LDLR mRNA expression. Among SREBP2 gene targets, the 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase (HMGCoAR) is particularly important. This enzyme plays a key role in the intracellular cholesterol biosynthesis, since it is the rate-controlling enzyme in the mevalonate pathway. Recent reports [5] identify a crucial signaling pathway, via phosphatidylinositol-3-kinase (PI3K)/Akt, as an important player in the regulation of cellular lipid metabolism. This pathway is the best known for its role in promoting cell growth, proliferation, and survival through increased glucose utilization and prevention of apoptosis. In particular, Akt has recently been implicated in a novel form of lipid metabolism regulation, through the SREBPs [6]. The physiological coordination between Akt and SREBPs pathways is needed to produce the lipids for new membrane synthesis, which in turn is required for growing and proliferating cells [5]. Even though

many reports have been focused on SREBP-1c isoform [6], new efforts are directed to explore and to investigate the link between Akt and SREBP2 [5,6].
The objective of the present study was to characterize in detail the molecular mechanism at the basis of the cholesterol lowering properties of *L. albus* protein observed in experimental and clinical investigations. Starting from the hypothesis that the activity may depend on specific peptides encrypted in the protein sequences, a total protein extract from lupin seed was hydrolyzed either with pepsin (P peptides) or with trypsin (T peptides). The HepG2 cell line was treated with both kinds of peptides and molecular and functional investigations were performed on the LDLR-SREBP2 and PI3K/Akt pathways, using a combination of techniques.

Results and discussion

The main findings of this study are the following: i) T peptides are able to directly interfere with the HMGCoAR activity, whereas P peptides are much less effective; ii) Both P and T peptides modulate the cholesterol metabolism in HepG2 cells, through the up-regulation of the pathway involving the LDLR; iii) Both P and T peptide treatments increase the LDL-uptake; iv) The activation of Akt/GSK3β pathway is involved in the up-regulation of the LDLR-SREBP2 pathway.
HMGCoAR is the rate-controlling enzyme of cellular cholesterol biosynthesis pathway and therefore it constitutes the target of numerous investigations aimed at lowering the rate of cholesterol biosynthesis. Therefore, initially in vitro experiments were performed using the purified catalytic domain of the enzyme with the objective of clarifying whether lupin peptides are able to directly inhibit the activity of HMGCoAR. P peptides inhibit the enzyme with a statistical significance (-17%) only at the maximum tested dose (2.5 mg/ml). On the contrary, T peptides show a statistically significant reduction of the HMGCoAR activity by 37% at 0.25 mg/ml, by 57% at 0.5 mg/ml, and by 61% at 1.0 and 2.5 mg/ml. The LDLR expression and the receptor protein localization at cellular membranes are strictly correlated to the intracellular cholesterol biosynthesis pathway. In facts, the transcription of the LDLR and the genes required for cholesterol and fatty acid synthesis are controlled by membrane-bound transcription factors called SREBPs and the intracellular cholesterol acts with a negative feedback inhibition mechanism. The SREBP2 isoform is responsible for the LDLR and HMGCoAR transcription and the SREBP2 maturation is regulated by the intracellular cholesterol homeostasis. Thus, the up-regulation of LDLR represents a useful strategy to control plasma LDL cholesterol levels. Our findings demonstrate that both lupin peptide mixtures are able to up-regulate the LDLR protein levels through an increase of SREBP2 protein. P peptides up-regulate the SREBP2 protein level by 100%, 148%, and 162% *vs.* the control, while T peptides increase the SREBP2 protein level by 80%, 73%, and 44% *vs.* the control, after 0.5, 1.0, and 2.5 mg/mL treatments, respectively. However, a detailed investigation of the LDLR pathway revealed that lupin peptides produce different effects on the HMGCoAR level. In particular, whereas P peptides are able to increase the enzyme protein level with a dose-dependent manner, T peptides up-regulate the enzyme protein levels in a significant way vs. the control at 0.5 mg/ml, the increase remains statistically significant, but it is smaller at 1.0 mg/ml, and it is finally completely abolished at 2.5 mg/ml (Figure 1).

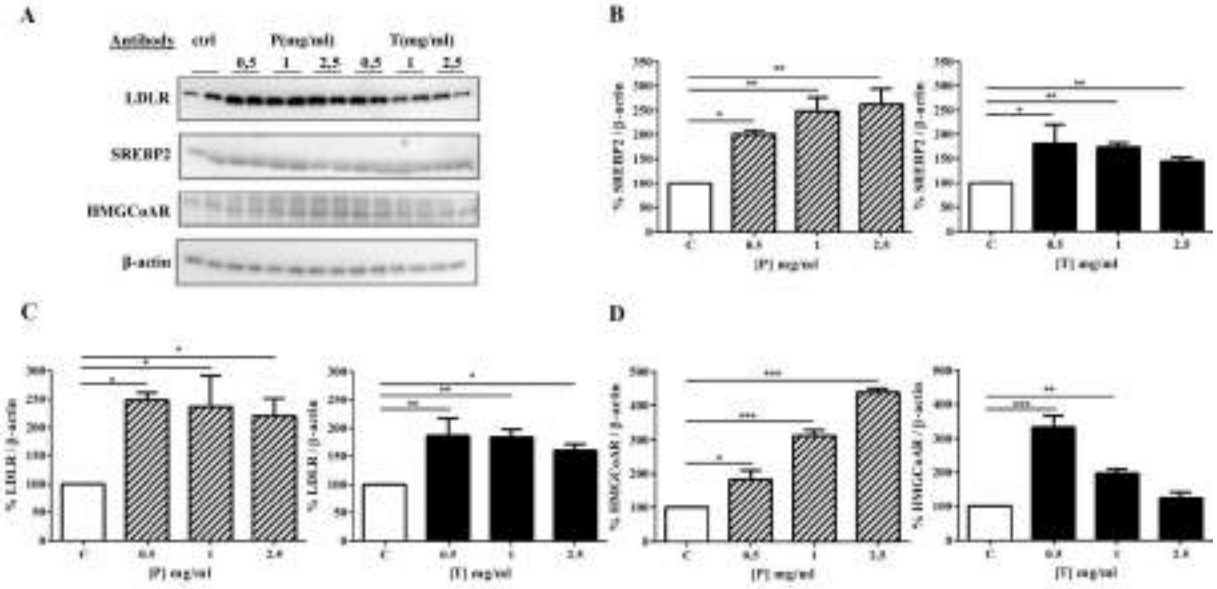

Figure 1. HepG2 cells (1.5 x 10^5) were treated with 0.5, 1.0, and 2.5 mg/ml of P and T peptides for 24 h respectively. The SREBP2, LDLR, HMGCoAR, and β-actin immunoblotting signals were detected using specific anti- SREBP2, anti-LDLR, anti-HMGCoAR, and anti- β-actin primary antibodies respectively (Panel A). The SREBP2 (Panel B), LDLR (Panel C), HMGCoAR (Panel D) signals were quantified by ImageJ Software and normalized with β-actin signals. Bars represent the averages of duplicate samples ± SEM of three independent experiments. * $P < 0.05$, ** $P < 0.001$ *vs* C. P = Pepsin peptides, T= Trypsin peptides, and C = control.

These evidences suggest that both peptide mixtures modulate the cholesterol metabolism pathway, through the induction of LDLR protein levels (at 0.5, 1.0 2.5 mg/ml P peptides mediate a 147%, 136%, and 120% induction of the LDLR protein; whereas T peptides mediate a ~85% up-regulation at 0.5 and 1.0 mg/mL and a 61% up-regulation at 2.5 mg/ml *vs.* the control) due to an increase of SREBP2 protein, although their potency of induction is different.

In agreement with immunoblotting results, the increase of LDLR protein levels leads to an increase of LDL uptake. In particular, the treatment with P peptides at the concentration of 1.0 and 2.5 mg/ml increases the LDL uptake by 42% and 45%, respectively, *vs.* the control, whereas the LDL-uptake increase was not statistically significant at 0.5 mg/ml. On the other hand, at the concentration of 0.25, 0.5, and 1.0 mg/ml T peptides significantly raise the LDL-uptake by 52%, 50%, and 70%, respectively, *vs.* the control. Our findings suggest that both P and T peptides are able to significantly induce the LDL clearance and this result is strictly correlated to an increase of LDLR protein level.

Recently, studies have indicated that there are links between the Akt and the SREBP pathways: in facts, emerging evidences show that Akt is implicated in the regulation of lipid metabolism through the activation of SREBPs. Luu and coworkers showed that insulin-like growth factor-1, an inducer of Akt signalling, acutely increases SREBP2 activation [5]. This study provided evidence that Akt contributes to the regulation of cholesterol metabolism through activating SREBP2.

Even if the precise target(s) of Akt still remains elusive and not clarified, part of our investigation was dedicated to evaluate the effects of lupin peptides on the PI3K/Akt pathway. Our study provides the experimental evidences that either P or T peptides from lupin protein are able to induce increases of Akt and GSK3β phosphorylation levels, which are completely abolished by the treatment with a well-known PI3K inhibitor, such as wortmannin. A constitutively active form of Akt activated the LDLR. Our findings clearly support this study, since both P and T increase the LDLR protein levels and induce an increased fluorescent LDL-uptake at HepG2 cells. Moreover, after treatment with both lupin peptides in the presence of wortmannin, the LDL uptake is blocked vs. the P and T treatments alone, demonstrating that the inhibition of PI3K/Akt has general effects on cellular lipid homeostasis, although the precise Akt target(s) is(are) not definitely assigned yet (Figure 2).

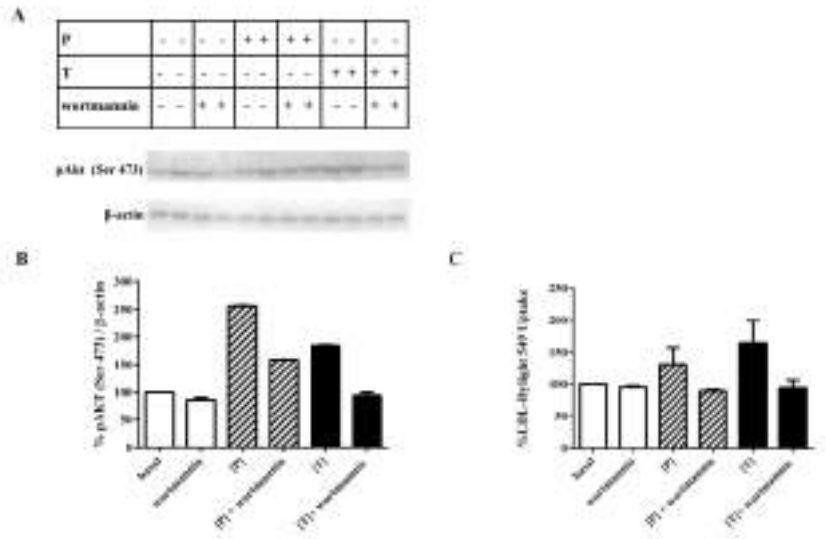

Figure 2. HepG2 cells (1.5 x 105) were treated with 1.0 and 0.5 mg/ml of P and T peptides in presence or absence of wortmannin for 24 h respectively. The phosphorylation level of Akt (Ser473) (Panel A) and β-actin immunoblotting signals were detected using specific anti-posphoAkt (Ser473) and anti-β-actin primary antibodies respectively. Relative intensity of posphoAkt signals (Panel B) were quantified by ImageJ Software and normalized with β-actin signals. Panel C shows the LDL-uptake after treatment with the PI3K inhibitor. Specific fluorescent LDL-uptake analyzed by Synergy H1 (Biotek). Bars represent the averages of duplicate samples ± SEM of three independent experiments. pAkt = posphoAkt, P = Pepsin peptides, T= Trypsin peptides, and C = control.

Conclusions

In conclusion, we have provided evidence that peptide mixtures obtained by the hydrolysis of lupin proteins are able to modulate the LDLR/SREBP2 pathway with the final effect of an increased LDL uptake. Since as indicated above, both P peptides and T peptides are complex mixtures, it appears very difficult to sort out which may be the peptides responsible for the observed activities. It is, however, possible to affirm that the diversity in the behaviors of the two peptide mixtures depends on their different compositions. Further work will be necessary to investigate these aspects in detail in the future.

Acknowledgements

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Sensitization pattern as a tool to prevent clinical events due to cross-reactivity between lupin and peanut in allergic children

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State of art and aim: Clinical events associated with allergy to lupin are increasing due to the wide use of lupin flour in bakery, pasta and other formulations, including gluten-free products. Published case reports describe both primary sensitization and cross-reactivity with other legumes/vegetables. The cross-reactivity between lupin and peanut has been described by different authors, and it is an important point to consider taking into consideration that allergy to peanut is often severe and associated with anaphylactic shock.
The most important lupin allergens are alpha- and beta-conglutin, followed by gamma- and delta-conglutin. The aim of this study was the characterization of the pattern of sensitization to lupin in a group of children allergic to peanut, using both *in vitro* methods (CAP-Test and immunoblotting) and *in vivo* approaches (fresh food skin prick test-FFSPT and oral challenge-DBPCFC). The second objective of the research was the assessment of the role of different allergens in the symptomatic clinical cross-reactivity.

Results and discussion: The study involved 12 children allergic to peanut, as confirmed by oral challenge or by history of anaphylactic shock. Children were included in a DBPCFC (Double Blind Placebo Controlled Food Challenge) study, where their clinical reactivity to a pasta containing lupin flour was assayed. The symptomatic cross-reactivity was observed in 2/12 subjects (17%). In parallel, data on sensitization pattern to peanut and lupin were collected by CAP-test, FFSPT and immunoblotting. Results showed that beta-conglutin was recognized by cutaneous IgEs from 7/12 peanut-allergic children in FFSPT and serum IgEs from 5/12 in immunoblotting. Moreover, 4/12 and 8/12 patients tested positive to gamma-conglutin in FFSPT and immunoblotting, respectively. Less significant immunoreactive responses were observed to alpha- and delta-conglutins.
In this group of allergic children, beta-conglutin has been identified as the major lupin allergen involved both *in vitro* and *in vivo* cross-reactivity with peanut proteins. The role of gamma-conglutin in the cross-reactivity between lupin and peanut proteins was also relevant and clear, despite the observed unspecificity of the immunoblotting responses.

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Tags: allergy, alpha-conglutin, beta-conglutin, cross-reactivity, peanut

Poster contributions

Note:
Abstracts are reported in the order they were received from the Authors

(P1)

Effect of thermal treatment on the chemical composition and minerals of wild lupin seeds

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State of art and aim: Wild lupins consumption has restrictions due to the presence of alkaloids, however these components can be reduced with a suitable thermal treatment. The aim of this research was to determine the thermal effect on chemical composition and minerals of wild lupins.

Results and discussion: *L. Mexicanus,* L. has a reduction in protein and fat contents of 34.76 to 33.11 of sample and 6.10 to 5.41 g/100 g respectively, and an increase in ash and dietary fiber of 3.84 to 4.53 and 20.9 to 28.48 g/100 g respectively. *L. mexicanus* raw seeds revealed the highest Ca content (3,252.6 mg/kg), *L. elegans* was the highest in Mg with 2,656.4. Highest Fe content was *L. rotundiflorus* (82.8 mg/kg), and *L. exaltatus* in Cu (184.4 mg/kg). All species showed similar Zn content of 73.3 (*L. montanus*) to 89.6 mg/kg (*L. exaltatus*). In all species the Cu decreased, mainly in *L. elegans* with a loss of 76. 71 %.

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Tags: Lupinus, legumes, minerals, thermal effect, dietary fiber.

(P2)

Agronomic and protein characterization of *lupinus angustifolius,* L. varieties cultivated in Mexico

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State of art and aim: Whereas in Mexico the agronomic potential of *L. angustifolius* is unknown, carried out research with the objective of evaluating the yield and chemical composition of six varieties of L. angustifolius (Haags Blaue, Boregine, Borlu, Probor, Sonate, and Boruta) in Zapopan, Jalisco Mexico in experimental field with pH 5.04, without rhizobium inoculum and Ca content of 1.365 mg kg-1. In addition an amino acid analysis and characterization of proteins by gel electrophoresis on protein isolates of Boruta and Probor varieties was performed.

Results and discussion: Grain yield ranged from 3.63 t/ha in Boruta to 4.95 t/ha in Probor, without showing significant differences among all the varieties evaluated. The higher protein content (Dry matter) was recorded in the Probor with 36.61% while the lowest was found in Boruta with 28.40%. The composition of amino acids was found that the seed of Probor has higher content of histidine, isoleucine, leucine and threonine as Boruta, same that cover the FAO requirements. The electrophoretic pattern of SDS-PAGE in protein isolates showed bands ranging from 52 to 10 kDa, where those of weightier corresponded to globulins (conglutins). Our results indicate that *L. angustifolius* varieties grown in Zapopan, Jalisco could be an important source of protein in the form of concentrates or isolates for human or animal consumption in local farms.

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Tags: Lupinus, grain yield, proteins, amino acids composition

(P3)

In vitro micropropagation of sweet varieties of *Lupinus albus,* L. from cotyledonary node explants

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State of art and aim: *Lupinus albus,* L (white lupin) is the most cultivated and consumed lupin in the Mediterranean area, mainly in Portugal. Lupins (*Lupinus* spp.) are cultivated as forage plants because of their high protein content. Despite their beneficial properties, lupins are not extensively used in biotechnological programs when compared to soybean or pea. This limitation is a result of their recalcitrant nature, making them difficult to manage in tissue culture manipulation and transformation. However, the establishment of *in vitro* regeneration cultures is the main prerequisite to transform lupin into a viable plant system for expression/production of various proteins of industrial, agricultural, nutritional and medicinal importance. Some established *in vitro* regeneration and transformation protocols have also been successful for some lupin species, such as *L. angustifolius,* L., *L. luteus,* L. and *L. mutabilis*, L. except for white lupin. Therefore is it important to develop an *in vitro* regeneration protocol for white lupin for further development of plant transformation-based techniques. Preliminary results of our work show that we are successfully developing a protocol to obtain *in vitro* regeneration, via direct organogenesis, in two *L. albus* cultivars, cv. Energy and cv. Rumbo.

Results and discussion: In this study, the regeneration potential of cotyledonary meristems from *L. albus* cvs. Energy and Rumbo was studied on modified Murashige and Skoog (MS) and Gamborg basal salt media, supplemented with B5 vitamin, various concentrations of benzylaminopurine (BAP) and 1-naphthalene acetic acid (NAA), alone or in combination. Preliminary results indicated that multiple bud regeneration of *L. albus* explants could be verified when 0.5 to 3 mg/L BAP was applied in conjunction with low NAA concentrations (0.001 to 0.1 mg/L). Elongated *L. albus* shoots were tested for rooting in different media, including modified MS medium containing 1 to 3 mg/L of different auxins (indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and NAA) as well as auxin-free media. Optimization experiments are currently underway. The results obtained indicate a promising *in vitro* regeneration protocol for various *L.albus* cultivars, making them a prospective platform for plant transformation-based techniques and, consequently, for production of high-value products.

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Tags: *Lupinus albus*, micropropagation, phytohormones, shoot regeneration

(P4)

The role of root carboxylates in ecological intensification by lupin/wheat intercropping system

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State of art and aim: A fundamental aspect of intercropping is to avoid unfavorable intra- or inter-specific competition between the neighboring plants improving the growth and survival of both neighbors. Many studies on multi-cropping systems indicate that root interactions are more important than shoot interactions for determining productivity in mixtures and intercropping advantages (Hauggaard-Nielsen H and Jensen E. 2005). Some effects of root carboxylates on both positive and negative plant-plant interactions may also be mediated by indirect effects on soil resources. Organic acids can play the role of metal chelators in the rhizosphere, but are supposed to have more important effects on phosphorus than micronutrients availability. Among species examined for organic acid production in response to phosphorus stress, lupins exhibit the strongest trends. Despite the large number of studies carried out, further efforts to determine rhizosphere concentrations of organic acids in living soil and to examine the effects of those quantities on phosphorus solubility and uptake are needed to confirm the role of organic acids in plant responses to phosphorus stress (Bais H.P. *et al.* 2006). In addition, it has not yet been determined whether the high rates of organic acid secretion by lupins also increases phosphorus availability to neighboring plants. The aim of this study, carried out on living soil, was to compare root carboxylates composition of lupin grown in pure culture and intercropped with wheat at two levels of phosphorus supply. Effects of carboxylates on the phosphorus availability for the neighbor cereal were also evaluated. The experiment was also replicated with peas using the same systems of sole crop and intercropping at two P-levels. Root carboxylates composition and effects on phosphorus availability were also analyzed.

Results and discussion: Two grain legume species white lupin (*Lupinus albus* L.), field pea (*Pisum sativum* L.) and wheat (*Triticum turgidum* spp durum.) were grown, sole (SC) and in intercropping (IC), in the PVC tubes (V=3,85 dm-3) filled with the mixture of clay loam soil and agriperlite (4:1 v/v) in the climate chamber for 45 days. Organic acid (OA) concentration, *i.e.* oxalic (OX), malic (MA), acetic (AC), fumaric (FU), malonic (MO), maleic (ME), citric (CI) and trans-aconitic (TA) acids was determined by HPLC. Phosphorus supply increased the root biomass in wheat (+ 65%) and in the two intercropping systems (25 and 35% in W/P and W/LU respectively). In contrast, as expected, a quite similar decline was observed in pea (-35%) and lupin (-36%) grown in sole crop. The single amounts of AC, MA and OX (42.83÷ 119.31 μmol g-1 root DM) were significantly higher than AC, CI, FU, ME, and TA. ME values was below 1 μmol. The phosphorus supply caused a considerable increase in the amount of carboxylates with the exception of TA (no significant change) and OX that, in contrast, revealed a significant decreasing. The highest values of all carboxylates were found in lupin and pea grown in sole crop. FU showed the largest amount in wheat grown in sole crop. Instead the lowest values, with the exception of FU, were observed in intercropping. The different pattern of OA detected in intercropping system seems to have a positive influence on the availability of phosphorus facilitating wheat uptake.

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Acknowledgements: This work was supported by Italian Ministry of Research (MIUR), Research Program PON01_1145 "ISCOCEM".

Tags: Root carboxylates in living soil, intercropping system, ecosystem service.

(P5)

Development of a NIR calibration to select white lupin genotypes with high gamma-conglutin fractions

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State of art and aim: New interest in lupin seed protein has arisen because of its overall abundance and the beneficial effects on human health of some of its fractions, namely, the gamma-conglutins. White lupin (*Lupinus albus* L.) has 36-40% protein content with a biological value of 91% in comparison to egg protein. Gamma-conglutins have blood glucose lowering capacity, owing to interaction/binding with insulin and to the insulin-mimetic properties. The aim of this work is to explore opportunities to develop a fast Near Infrared Spectroscopic (NIRS) method for screening and selecting white lupin genotypes with high seed protein, and especially gamma-conglutin, content. This tool could be useful for the nutritional improvement of lupin, both as a food for humans and as feed for animals.

Results and discussion: Ideally, use of NIRS technology in plant breeding would involve non-destructive analysis of seeds, to enable the sowing of individual seeds selected from the progeny of a particular genotypes. Seventy-eight seed samples from 23 cultivars or breeding lines harvested in 2012, 2013 and/or 2014 in two climatically-contrasting sites in Italy were used to collect NIRS spectra on whole grains and to perform chemical analyses of gamma-conglutin content. Gamma-conglutins ranged from 1.0 to 2.3% on a seed dry matter basis (i.e., about 4.5 to 6.7% of total protein in the dry seed). NIRS data were averaged across spectra of 10 individual seeds per sample. A partial least squares PLS NIRS calibration was developed that showed modest predicting power (R^2 = 0.28). A second NIRS calibration performed on ground seed of a subset of 46 samples displayed moderate predicting power (R^2=0.63). NIRS calibration based on spectra of these flour samples was also carried out for total protein content in the seed, obtaining high predictability (R^2=0.96) in line with previous findings. Our results suggest that NIRS prediction of gamma-conglutin content is only suitable for seed samples which have been ground, and that there are good prospects for its adoption for selecting segregating genotypes based on responses of bulked seed from progeny.

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Tags: breeding, gamma-conglutin, NIR spectroscopy, protein, white lupin.

(P6)

Uncovering genetic factors associated with leaf and seed alkaloid content in the yellow lupin (*Lupinus luteus* L.)

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State of art and aim: The increasing demand for agricultural food products has forced plant breeders to permanently develop higher yielding commercial varieties to satisfy this human need. In addition, concerns on human health have pointed out the need of producing food products with better nutritional quality and reduced levels of anti-nutritional compounds. Lupin alkaloids are part of these plant compounds, and high content of them, or "bitterness", has a detrimental quality effect on human and animal nutrition given that ingestion of large amounts of them are associated with severe intoxications. The generation of low alkaloid lupin varieties has facilitated the use of these legumes in human and animal nutrition and satisfied food industry standards [1]; however, it has also increased susceptibility to herbivores and transmission of aphid-borne viruses and bacteria [2]. This bitter-pest resistance/sweet-food industry balance has generated a complex challenge for lupin breeders, whom must select for sweet genotypes, but must still have some levels of alkaloids to reduce damages associated to pests and diseases. Thus, generating breeding tools to aid the efficient manipulation of alkaloids could significantly increase the generation of better adapted good nutritional quality lupin varieties. Several research initiatives carried out at the Agri aquaculture Nutritional Genomics Center (CGNA) have allowed the identification of QTLs associated to alkaloids; however, there is still uncertainty of what gene(s) are directly involved in the biosynthesis of these compounds. The main goals of this study are to genetically dissect the natural variation of alkaloid content in leaf tissue and mature seeds on a diverse set of *L. luteus,* narrow down alkaloid associated genomic regions and ultimately, find candidate genes involved on alkaloid biosynthetic pathways.

Results and discussion: Alkaloid content was measured, in leaves and mature seeds, from a diverse yellow lupin core collection during two seasons using HPTLC technology. Lupin accessions were genotyped using a set of ~300 molecular markers (SSRs, EST-SSRs, SNPs and INDELs). Association analyses uncovered several genomic regions associated to lupinine, spartein, and gramine alkaloid content in both lupin tissues. Currently, we are carrying out bioinformatic analyses to target candidate genes, such as lysine decarboxylase, and key enzyme on the quinolizidine pathway [3], as an attempt uncover yellow lupin genes behind the production of these plant compounds.

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Tags: alkaloids, *Lupinus luteus*, associative mapping.

(P7)

Mexican *Lupinus*: potential uses in pest control

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State of art and aim: *Lupinus* belongs to the *Fabacea* family. Plants of this genus contain quinolizidine alkaloids (QA); these compounds are synthesized in wild plants as part of a strategy against herbivores. Several studies have shown that QA provide insecticidal effects. Mexican *Lupinus* synthesizes these secondary metabolites in high amount. The aim of this study is to review results of our group to evaluate the biological effect of QA on two model insects: larvae of *Spodoptera frugiperda* and *Aedes aegypti*.

Results and discussion: Results show that QA have a negative effect on larvae development of *S. frugiperda* and *Ae. aegypti*. On *S. frugiperda*, QA led to a decrease on feeding of larvae, which affects directly the development on the pupa stage - adult turn in adult- causing malformations like folded and twisted wings, preventing adults to fly. Other live activities (dispersion, feeding, reproduction) are also influenced. Oviposition and hatching were affected (74 and 84 %, respectively) with the 5000 ppm extract. In *Ae. aegypti* QA induced a prolonged time of molting from third to fourth instar for five days and one day pupal-adult instar and also caused malformations in parts of the gills, pecten, siphon and abdomen in larvae of the third instar. These malformations prevented the larvae to move into the water and they prevailed in the depth of the container resulting in death. This work evidences that QA are an alternative solution for pest control.

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Acknowledgements: We thank you to SIP projects. Dr. Rodolfo Figueroa Brito and Kalina Bermúdez Torres are in debt for CONACyT , EDI and SIBE. Raúl Simión Flores Michi and Leticia Reyes Izquierdo have been souported by BEIFI. We thank you Engineer Juan Sánchez Arriaga of the Ministry of Public Health of Iguala, for providing technical assistance and egg masses of *Ae . aegypti.*

Tags: Quinolizidine alkaloids, *Spodoptera frugiperda*, *Aedes aegypti*, Malformations

(P8)

Screening of Lupin Varieties for Organic Mixed Cropping in Switzerland

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State of art and aims: From 2010 to 2014, Swiss blue lupin (*Lupinus angustifolius*) acreage was only about 50 hectares annually (www.swissgranum.ch/files/2014-05-20_schaetzung_anbauflaeche_2014_stand_20.5.2014.pdf). White lupin (*Lupinus albus*) has not been grown at all since 2004 and is not recommended for Swiss agriculture due to risk of anthracnose infection causing severe damage to the crop. Recently, demand for domestically grown organic protein crops has been increasing rapidly in Europe. Therefore, efforts have started to encourage farmers to grow grain legumes. In order to promote organic lupin production in Switzerland, the objective of our study was to screen new cultivars of white (*Lupinus albus*), blue (*Lupinus angustifolius*), and yellow lupin (*L. luteus*) for their tolerance to anthracnose and their suitability for mixed cropping systems. Barley and wheat were reported as suboptimal intercrops (Böhm *et al.*, 2008), so we tested intercropping with spring and winter oats in order to suppress weeds (Hauggard-Nielsen *et al.*, 2008).

Results and discussion: In the relatively dry spring and early summer of 2014, both lupins and oats developed fairly well, but in the wet summer after flowering, weed suppression became insufficient and the plants suffered from competition from the taller oat plants and weeds. The total yield of the spring oat-lupin mixture averaged 1.69 t ha^{-1} for white lupin with a lupin yield of 0.66 t ha^{-1}, 2.91 t ha^{-1} for blue lupin with a lupin yield of 0.5 t ha^{-1}, and 3.01 t ha^{-1} in yellow lupin with a lupin yield of only 0.17 t ha^{-1}. Regardless of whether certified seed had been sown, anthracnose was present in all cultivars. In the oat variety screening, the total yield of the oat-lupin mixture averaged 2.61 t ha^{-1} with a lupin yield of 0.80 t ha^{-1}. The highest total yield was obtained with the oat, *Avena sativa*, L., cultivar Symphony (3.12 t ha^{-1} with 0.92 t ha^{-1} of lupins) whereas the highest lupin yield was obtained with the very short oat variety Buggy (2.45 t ha^{-1} with 1.21 t ha^{-1} of lupins). These results suggest that mixed cropping with acceptable total yields per hectare is possible using blue or white lupin, whereas yellow lupin did not match well with the soil and rainfall conditions in the Northern part of Switzerland and will be discontinued.The screening of white lupin lines as a sole crop revealed some variation in anthracnose tolerance but much less than that reported by Jacob (2014). Overall anthracnose tolerance levels were much too low, therefore breeding activities will be initiated to develop a composite cross population within the scope of the European DIVERSIFOOD project.

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Acknowledgements: We want to thank the following breeders/seed producers for sending us seed: Saatzucht Steinach, Südwestdeutsche Saatzucht, Jouffray-Drillaud, Nordsaat Saatzucht, Poznanska Hodowla Roslin, Dr. N. Drienyovszki (Univ. of Debrecen, HU), Edwin Nuijten (Louis Bolk Instituut, NL), Paolo Annichiarico (CRA-FLC, Lodi, IT), Erik von Baer (Semillas Baer, Chile), Boguslav S. Kurlovich.

Tags: Lupin varieties, mixed cropping, lupin-oat intercropping, anthracnose, organic farming.

(P9)

Endogenous 5-Methyltetrahydrofolate in Lupin flour

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State of art and aim: Folate is an essential water-soluble vitamin and present in many different vitamers. The predominant vitamer found in foods is 5-methyltetrahydrofolate. Foods rich in folate can be sourced from dried peas and beans, leafy green vegetables and citrus fruits and juice. This study aimed to optimise a validated ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method, in order to determine the levels of folate vitamers in lupin flour. Folate extraction from lupin samples involved sequential treatments with α-amylase, protease and rat serum, also known as tri-enzyme. Samples were further processed using solid phase extraction, concentrated in a speed vacuum and filtered using the Amicon membrane filter prior to analysis**.**

Results and discussion: The result showed that the level of 5-methylterahydrofolate in lupin flour was 94 μg/100g (dry weight basis), which is higher than in fava beans (36 μg/100g) and lower than in chickpeas (195 μg/100g). Other folate vitamers, such as tetrahydrofolate, 5-formyltetrahydrofolate and 10-formylpteroylglutamate were also detected in lupin flour and quantitation is currently in progress. The results of this study reveal that lupin flour is a rich source of naturally occurring folate. As the use of plant based food ingredients gains popularity, lupin flour may contribute to dietary folate intake.

Tags: vitamin, folate, chromatography, mass-spectrometry, food analysis.

(P10)

'Old World' lupin species evolution: comparative cytogenetic analysis of chromosomal rearrangements

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State of the art and aim: Plant evolution is a complex process leading to many chromosomal rearrangements. 'Old World' lupin species provide good examples of diversification in total and basic chromosome number, as well as in genome size. To explain such differences, researchers suggest at least one round of polyploidisation may have taken place during early stages of genome evolution (Kroc *et al.* 2014), but the exact structural variation and changes of lupin genomes which emerged from the genome duplication process are still unknown.
Fluorescence *in situ* hybridisation (FISH) is the most useful method available for comparative mapping and physical visualisation of chromosomal rearrangements (Lesniewska *et al.* 2011). Use of large DNA fragments (BAC clones) as FISH probes can reveal differences between chromosomes not only within a species, but also between different species within a genus. We used a set of BAC-derived chromosome markers from *Lupinus angustifolius,* L. (2n=40) as probes for FISH performed on four wild relatives: *L. cosentinii,* L. (2n=32), *L. cryptanthus,* L. (2n=40), *L. pilosus,* L. (2n=42) and *L. micranthus,* L. (2n=52). FISH reaction parameters (i.e. temperature and stringency) were adjusted to compensate for possible sequence mismatches in heterologous species, to ensure appropriate accuracy of synteny analysis.
The main goal of this research was to identify chromosomal rearrangements within genus *Lupinus*, and to reveal mechanism(s) behind those changes. Moreover, combining that knowledge with additional bioinformatic sequence analyses will enable us to answer questions about relationships between lupin species and describe the model of their genome evolution.

Results and discussion: By comparing BAC-FISH results, it is possible to characterise individual chromosomes unambiguously across a range of species. These studies reveal rearrangements between particular chromosomes of *L. angustifolius* and its wild relatives. Examples are provided by BAC clones 136C16, 111G03 and 3B18. All of them mapped to the same single chromosome in *L. angustifolius* and *L. cosentinii*. In *L. pilosus*, however, the signal for clone 3B18 was localised to a separate chromosome from that of the other two clones. To better understand the mechanism of lupin chromosome changes, we plan to extend the mapping to further 'Old World' species. Other analyses have shown that BAC 142D13 behaves as a single locus in *L. angustifolius* but hybridises to several chromosomes in the two wild lupin species *L. cosentinii* and *L. pilosus*. This pattern could reflect ancestral polyploidisation or extensive recombination which reorganised the current 'Old World' branch of genus *Lupinus*. There are few studies of wild lupin species, despite their great diversity and ability to adapt to a wide range of biotic and abiotic stresses. Expanded research can highlight them as an excellent source of valuable traits for current and future breeding programs.

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Tags: 'Old World' lupin, FISH, BAC probes, comparative mapping.

(P11)

Effects of the inoculation of lupin seeds (*L. angustifolius,* L.) with rhizobium "HiCoat" on yield parameters and protein content

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State of art and aim: The plant family of legumes has the ability to fix N_2 from the air with the help of nodule bacteria resulting in the fact that these species are not affected by nitrogen deficiency in the soil, but supply even following crops with nitrogen.
Nevertheless, in particular lupins should be inoculated with specific nodule bacteria, because lupin-specific rhizobia are often not present in the soil at the first time of cultivation or after longer breaks in lupin cultivation (8 years). This may be achieved by treating seeds of lupins with "HiCoat", "HiStick" or "Radicin Nr. 6" (Schmiechen et al., 2011).
Rhizobium inoculation increases the yield of *L. angustifolius* when plants were not fertilized with mineral N (Merbach et al., 2008). The aim of this work was to investigate the influence of the inoculation with the rhizobium "HiCoat" on the yield and protein content of *L. angustifolius* seeds.

Results and discussion:
In 2014 the commercial liquid inoculant "HiCoat" was tested on 5 lupin varieties (Boregine, Boruta, Haags Blaue, Mirabor and Probor) at two locations in Germany. At Groß Lüsewitz lupins were grown for the first time and at Bornhof, lupins are continually grown. In Groß Lüsewitz no yield or yield parameters could by determined due to animal damage, but nodules were detected in the roots of HiCoat treated and untreated variants. In Bornhof no significant effects of "HiCoat" on grain yield, thousand kernel weight and protein content, only significant variety differences, were detected.
In contrast to these results, inoculation of lupins are regarded as necessary in Western Canada (Lopetinsky et al., 2014) as it turned out that different peat and granular lupin inoculants are essential for ensure a high biological N_2 – fixation. In these trials only one cultivar was tested. Therefore, additional trials with commercial rhizobial inoculants in different formulations and especially at a location with breaks in lupin cultivation over a long period are needed to come to conclusive results about the need for rhizobium inoculation.

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Acknowledgements: We thank M. Jugert, C. Leesch and B. Lemke for their excellent technical assistance.

Tags: inoculation, lupin, rhizobia, yield, protein

(P12)

Antigenic properties of technologically processed lupin seeds after *in vitro* digestion

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State of art and aim: Despite many nutritional benefits, lupin seeds can be harmful to humans, being a source of allergens. Lupin proteins may induce allergy either by primary sensitization or cross-reaction with antibodies directed against other legumes, especially peanuts. According to the current legislation (*Directives EC 1169/2011*) lupin belongs to the allergenic ingredients which must declared on labels. The allergenic reaction can occur not only after lupin oral ingestion, but also as a consequence of inhalation or skin contact. Although the allergens are usually resistant to food processing and digestion, some of them can be destroyed during food processing (*Sathe et al. 2005*). Nevertheless, it has been confirmed by several researchers that even though the allergenic properties are reduced, the product can still be dangerous to sensitive individuals. There are suggestions (*Crespo et al. 2001)* that people diagnosed as sensitive to lupin during inhalation or skin contact may also suffer upon ingestion of food containing the same allergens. The aim of the study was to determine antigenicity of processed lupin seeds globulins to humans with confirmed allergenic syndrome after inhalation or skin contact.

Results and discussion:

Raw and processed seeds, i.e lactic acid fermented, extruded prior or afterlactic acid fermentation, were *in vitro* digested. Afterwards, the globulins were extracted both from digested and non-digested materials and the antigenicity of these extracts was checked with *in vivo* (skin-prick) and *in vitro* (ELISA) tests. Individuals with allergy syndrome recognized in skinprick test after inhalation exposure to lupin were the donors of sera for the presented experiments. These people had never consumed products containing lupin before the experiments.

Antigenic properties of non-digested, fermented lupin seeds increased by 30%, and by 140% after double modifications. However, extruded preparations displayed decreased antigenicity by 37%. The *in vivo* tests results confirmed those obtained *in vitro*.

Peptides extracted from digested raw and processed lupin seeds were not recognized as immunogenic with applied sera. The antigenic properties reduction of all digested materials was noted as almost 100% after *in vitro* analyses, and it was also significant in skin-prick tests. It can be concluded that the induction of hypersensitivity reactions of the immune system after lupin ingestion may be carried out within the mucosa of the mouth, esophagus or the stomach.

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Tags: antigenicity, food processing, *in vitro* digestion

(P13)

Antioxidants formation in lupin sprouts germinated in stress condition

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State of art and aim: Germination is a well-known method to enhance the nutritional value of seeds and grains. Moreover, the value depends on the process conditions and it may be easily modified. During germination in high concentration of iron (II) ions a strong accumulation of that microelement is observed, especially in lupin sprouts. Iron is bound by different molecules and this accumulation is a part of the defence system against toxic concentration of iron in plant cells. Simultaneously, the huge change in the formation of other antioxidants molecules starts, such as phenolic and flavonoids compounds. These compounds are desired component in the human diet. Thus, their increased content would be an additional benefit, apart from the high iron concentration, of those lupin sprouts. However, not only the content, but also the profile of the compounds differs depending on the following factors: lupin genus and variety, germination time and conditions.

Results and discussion: It has been presented previously that in the lupin seeds, regardless of the genus or variety (lupin yellow, white or *angustifolius*), there are two dominant phenolic compounds from the group of flavone, i.e. apigenin derivatives, but the concentration of phenolic acids is small.Methanol extracts of seeds sprouted in high iron concentration were free of gallic acid and the total concentration of other phenolic acids significantly decreased, both in sprouts obtained from the *L. luteus* (´Lord` var.) and *L. angustifolius* (´Baron` var.). Simultaneously, a whole range of phenolic compounds and unidentified derivatives of flavone and isoflavones was synthesized. RP-HPLC/DAD analysis showed two dominating peaks, observed previously in the seeds: apigenin derivatives (apigenin-6,8-di-*C*-β-glucopyranoside and apigenin-7-*O*-β-apiofuranosyl-6,8-di-*C*-β-glucopyranoside) (Siger *et al.* 2012). However, many isoflavones, unidentified and unobserved earlier in lupin sprouts, were also present. The conducted analysis of UV-Vis spectra suggests that these compounds belong to genistein derivatives. Obtained enrich in iron lupin sprouts contain significantly more different derivatives of genistein than apigenin, which are characteristic of the raw seeds. HPLC/ESI/MS^n analysis made identification of the following compounds possible: apigenin, genistein, 2' - hydroxy genistein-4,7'-O-diglucoside, genistein-4,7'-O-diglucoside, 2'-hydroxy genistein-glucoside, malonylated apigenin-O-glucoside, malonylated genistein O-rhamnosyl-O-diglucoside. Three from these compounds could not be recognized at this stage of the experiments. The work on their identification requires additional analyzes using other analytical techniques, such as NMR.

References:
Siger A *et al.* (2012) *J Food Comp Anal*, 25, 190
Acknowledgements: The presented experiments were a part of the project "New bioactive food with designed functional properties" (POIG 01.01.02 -00-061/09), carried out by the Poznań University of Life Sciences.

Tags: antioxidants, iron, lupin sprouts

(P14)

Generation of reactive oxygen species in lupin sprouts germinated in abiotic stress condition

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State of art and aim: In growing plants abiotic stress influences the synthesis of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (*Ajiboye et al, 2011*). These compounds may cause undesired damage of cellular molecules, especially lipids, nucleic acids and proteins. In order to increase the content of ferritin in sprouts, lupin seeds were placed in the medium with high iron concentration. An analysis of ROS/RNS level changes during germination and the sprouts total antioxidant potential were conducted.

Results and discussion: As an experimental material *L. angustifolius,* L. (var. Baron)., and *L. luteus,* L. (var. Lord) seeds were cultured in Fe(II) ions solution. The conditions of the experiment were chosen to maximize ferritin expression. To observe changes of ROS and RNS generation, a 2',7'-dichlorodihydrofluorescein (DCFH) fluorescence probe was used. The applied conditions induced a strong abiotic stress in plant, leading to ROS and RNS formation. Two days after application of culture medium rich in Fe(II) ions, i.e. on the fifth day of lupin seeds germination, maximum overproduction of these compounds was observed. In extracts obtained from sprouting seeds on that day relative fluorescence intensity of the probe was ~118 [a.u]. Whereas, in raw, water-swollen seeds it was only ~13 [a.u] for Baron variety. On the following days of sprouting, the ROS and RNS activity decreased to ~66 [a.u]. Similar results were obtained for the other lupin variety, i.e. Lord. The ROS and RNS activity increased from ~12 [a.u] on the first sprouting day to ~115 [a.u] on fifth day, and decreased to ~70 [a.u] on the seventh cultivation day. Simultaneously, the total antioxidant potential steadily increased in obtained sprouts, changing in the range of 3.9 to 11.9 [µM Trolx/g d.m.] for var. Lord, and 3.1 to 10.7 [µM Trolox/g d.m.] for var. Baron. The obtained results confirmed high tolerance of lupin seeds to the iron presence in the culturing medium. The adaption to inconvenient conditions has already been observed two days after the stress application and consequently, the overproduction of nutritionally desirable compounds, i.e. antioxidants was observed.

References:

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Acknowledgements: The presented experiments were a part of the project "New bioactive food with designed functional properties" (POIG 01.01.02 -00-061/09), carried out by the Poznań University of Life Sciences.

Tags: antioxidants, iron, lupin sprouts

(P15)

The effect of ciprofloxacin on yellow lupin plants

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State of art and aim: Extensive use of pharmaceuticals results in their presence in the soil and the aquatic environment. A significant portion of antibiotics used in veterinary medicine gets into the soil together with the urine and feces of animals and manure. Most antibiotics are not completely metabolized in the body; 30-85 % of the dose is excreted unchanged. Ciprofloxacin is one of the most used fluoroquinolones, a group of synthetic antibiotics with a broad spectrum of action, which is often applied in veterinary and human medicine. The aim of this study was to investigate the effect of ciprofloxacin contained in the substrate on yellow lupin plants (Teixidó *et al.* 2014).
Results and discussion: The content of the dry weight of the lupin growing in medium containing different amount of ciprofloxacin (3; 9; 15; 30; 90 mg/kg dry soil) was examined. Untreated control plants were watered with distilled water. The dry weight of the plants increases together with the concentration of the antibiotic. The lowest content of dry matter was observed in the control sample (approximately 6 %), while the highest percentage of dry matter was at the highest concentration of the drug (7.45 %). This trend repeated itself on consecutive days of measurement. Moreover, the dry matter content at the same concentrations of ciprofloxacin increased with exposure time of the plant to the antibiotic. The highest content (18.92 %) of dry matter was observed on the tenth day of lupin drug treatment at the highest used concentrations. The dry weight of lupin seedlings treated with enrofloxacin showed a smaller increase with increasing drug concentrations, reaching 15 % of the fresh weight at the highest concentration (50 mM) (Adomas *et al.* 2013). The parameter defining the general state of the plant can also be the chlorophyll content. The relative leaf chlorophyll level or so-called greenness index was determined by using a Minolta SPAD-502 chlorophyll meter. Measurement of chlorophyll content showed a decrease with increasing concentration of ciprofloxacin in the medium. Decline occurred gradually during the period of antibiotic treatment of the plants. The lowest chlorophyll content compared to the control sample was observed on the tenth day at the highest concentration of ciprofloxacin (approximately 39 and 18.3 SPAD respectively). Feito *et al.* (2012) showed that changes in chlorophyll autofluorescence are dose-dependent. Catalase activity was also studied, an increase of catalase activity occurred with increasing amount of antibiotic.

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Acknowledgements: The present study was financially supported by project NCN Poland, UMO-2011/01/B/NZ9/02646

Tags: ciprofloxacin, lupin, antioxidant enzymes, catalase, dry weight

(P16)

Tracking of biotin carboxylase gene in narrow-leafed lupin against other legumes

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State of the art and aim: The issue of quantity and quality of lupin oil recently has received particular attention in the research and breeding communities. The complete lipid synthesis pathway contains many enzymes acting in different cellular compartments. Acetyl-coenzyme A carboxylase (ACCase) occupies the start point of the lipid synthesis pathway (Podkowiński et al. 2011). Therefore, the aim of the study was to analyze the narrow-leafed lupin (*Lupinus angustifolius,* L.*)* gene encoding enzyme involved in the first step of fatty acid synthesis: biotin carboxylase (BC) of plastidic acetyl-coenzyme A carboxylase.

Results and discussion: The *L. angustifolius* bacterial artificial chromosome (BAC) genomic library was screened with probes, representing a fragment of the BC subunit sequence of the plastidic acetyl-coenzyme A carboxylase. From among 13 BAC clones selected by hybridization, 7 showed single-locus signals on mitotic chromosomes after fluorescence *in situ* hybridization (BAC-FISH), and provided a set of cytogenetic chromosome landmarks. Five BAC-derived markers were generated and introduced into the genetic map of the species (Książkiewicz et al. 2014). Based on the results of cytogenetic and genetic mapping, followed by annotation of 26 BAC-end sequences, one clone containing BC partial sequence at the 3`end was chosen for sequencing. Sequences of BAC and BAC-ends were aligned to the draft genome sequence of the narrow-leafed lupin (Yang et al. 2014). The alignment showed that the BC gene has one copy in the *L. angustifolius* genome and is localized in the gene-rich region. Copy number of the analyzed gene was verified using Droplet Digital PCR. To reconstruct the evolution of the biotin carboxylase gene, the sequence revealed in this study, together with those extracted from sequenced legume genomes (*Arachis duranensis, Arachis ipaensis, Cajanus cajan, Cicer arietinum, Glycine max, Lotus japonicus*, *Medicago truncatula, Phaseolus vulgaris, Vigna radiata,* all L.) were analysed phylogenetically. Comparative mapping of the sequenced BAC to these genomes provided new evidence of shared synteny of BC gene-rich region among legumes and sheds light on the structure, stability and evolution of legume genomes. This study provides the framework for further research of fatty acid synthesis pathway genes in the *L. angustifolius* genome.

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Tags: fatty acid synthesis, acetyl-CoA carboxylase, biotin carboxylase, legumes, narrow-leafed lupin, genetic and cytogenetic mapping, synteny, phylogenesis

(P17)

Effect of sowing rate of *Lupinus albus* early cultivar Deter 1 on growth parameters and seed yield

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State of the art and aim: Only early spring sowing types of *Lupinus albus* mature in Central region of the European part of Russia. We succeeded in breeding early cultivars - Start, Gamma, Delta, Dega with pod set located only on the main stem and first branch level [1]. The plants of determinate cultivar Deter 1 are without lateral branches. This cultivar matures 7-10 days earlier than Start and Gamma, and may be grown in Moscow region. The plants with the reduced branches are less productive because of lower leaf area index (LAI) and photosynthetic potential (Ph.P). The value of these characteristics can be regulated by modifying sowing rate and plant density. A 2-year field experiment was conducted in Moscow region to study the effect of sowing rate on photosynthetic activity and seed yield of determinate early maturing cultivar Deter 1. The sowing rate was: 1) 50; 2) 80; 3) 100; 4) 120; 5) 140 seed m^{-2} (row width was 15 cm). Leaf Area Index (LAI), Photosynthetic Potential (Ph.P), Wet and Dry Matter Accumulation (WMA, DMA), Net Assimilation Rate (NAR), and seed yield components were determined.

Results and discussion: Seedlings density was usually close to the sowing rate. Density changed during vegetation, especially in stress conditions such as a strong rain, wind or drought. In this case plots with high density suffered the most. In the stress year of 2013 density before harvesting in comparison with seedlings decreased, %-wise: 1) 30; 2) 35; 3) 42; 4) 50; 5) 65. The plant height was 72-78 cm in 2012 and 53-59 cm in 2013. The plants were higher when the density increases. The period from seedlings to full maturity has been 101-107 days depending on the weather. Maximum seed yield was obtained at a sowing rate 100-120 seed m^{-2} and was 3.9 t ha^{-1}. In this case leaf area increased rapidly. Ph.P., DMA and seed yield were 1.3-1.4 times less if sowing rate was 50 seed m^{-2}, though NAR was 15-20 % higher. The yield of seeds per m^2 of ground was 1200 if sowing rate was 100 seed m^{-2}, which is 40 % more than when the rate was 50 seed m^{-2}. The weight of 1000 seeds of Deter 1 was 300-330 g in 2012 and 420-450 in 2013. It is less at plots with high density. Sowing rate of 100-120 seed m^{-2} provides higher and more stable seed yield for cultivar with reduced branches.

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Tags: *Lupinus albus*, determinate cultivar Deter 1, sowing rate, seed yield

(P18)

Wild Mexican lupins: proteomic analysis of the seeds of four species

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State of art and aim: *Lupinus* is a wide genus, comprising between 200 and 500 species, most of them represented in America. Mexico is a secondary distribution center with more than 100 species growing along the highlands (Bermudez Torres *et al.*, 2009). Due to morphological similarities, the taxonomy of wild *Lupinus* species is still incomplete. It is therefore useful to collect morphological, chemical, and molecular data for a correct differentiation of these plants. The aim of this work is the characterization of seed proteins of four wild Mexican *Lupinus* species.

Results and discussion: The seeds of four species were collected: *Lupinus aschenbornii* Schauer, *Lupinus campestris* Cham and Schlecht, *Lupinus hintonii* C.P. Smith, and *Lupinus montanus* Kunth. Plants grown in the Mexican National Park Iztaccihuatl-Popocatepetl at different altitudes.
Total protein extracts (TPEs) were obtained from seeds and single proteins were purified from TPEs by chromatographic procedures (Brambilla *et al.*, 2009). Both TPEs and single proteins were analyzed by electrophoretic techniques, either SDS-PAGE or 2D-electrophoresis.
With these techniques, a detailed characterization of the major seed proteins of *L. aschenbornii, L. campestris*, *L. hintonii*, and *L. montanus*, were obtained. Experimental data were compared with those of domesticated species (*L. albus* and *L. angustifolius*) whose proteome has been already described in literature (Wait *et al.*, 2005). SDS-PAGE analyses revealed that mature proteins, i.e. α-, β-, γ- and δ-conglutins, are composed of numerous polypeptides showing a large heterogeneity both in terms of MW and pI values. Furthermore, these proteins undergo a similar fate, in terms of proteolytic processing and post-translational modifications such as phosphorylation and glycosylation.

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Brambilla F *et al.* (2009) *Proteomics,* 9, 272
Wait R *et al.* (2005) *J Agric Food Chem,* 53, 4599

Tags: 2D-electrophoresis, seed protein purification, wild Mexican *Lupinus*

(P19)

Evaluation of ACE-inhibitory activity of enzymatic hydrolysates of proteins of different lupin species

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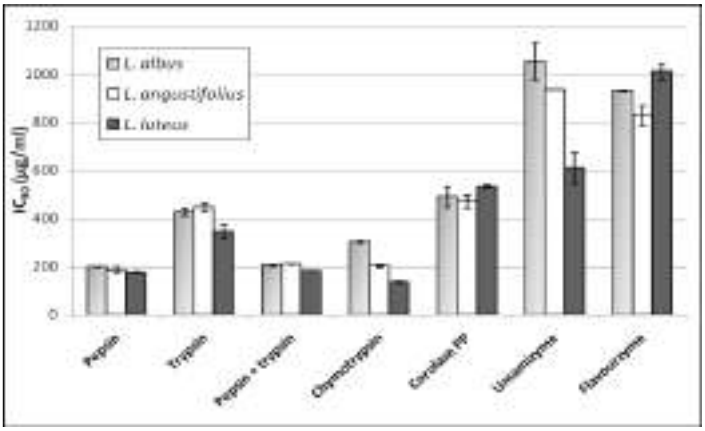
State of art and aim: Lupin consumption may reduce systemic hypertension in animals and humans (Arnoldi *et al.*, 2015). Hypertension is usually treated with drugs, such as angiotensin I converting enzyme (ACE; EC 3.4.15.1) inhibitors. They act towards ACE, an enzyme that plays an important role in regulating blood pressure in the renin-angiotensin system, because it catalyzes the conversion of the biologically inactive angiotensin I to the potent vasoconstrictor angiotensin II and inactivates the potent vasodilator bradykinin (Skeggs *et al.*, 1956). The use of dietary supplements and functional foods are encouraged in subjects with mild hypertension to delay the use of synthetic drugs. Specific dietary ingredients are ACE-inhibitory peptides produced by hydrolyzing proteins from different animal or plant foods such as milk, soy, and pea. The objective of the present investigation is the comparison of the activity of peptides mixture obtained hydrolysing with different enzymes the proteins of three lupin species, *L. albus*, *L. angustifolius*, and *L. luteus*.

Results and discussion: Pepsin gave peptides with the best ACE-inhibitory activity, followed by pepsin+trypsin, chymotrypsin, trypsin, corolase PP, umamizyme, and flavourzyme (see Figure). The three species showed similar activity scales, but after pepsin+trypsin and chymotrypsin treatments, *L. luteus* peptide mixtures resulted to be significantly the most active.
In conclusion, this investigation indicates that lupin proteins may be a valuable source of ACE-inhibitory peptides, which may explain the activity observed in experimental and clinical studies and foresee the application of lupin proteins into functional foods or dietary supplements.



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Tags: ACE-inhibitory activity, enzymatic hydrolysis, hypotensive activity

(P20)

Chemical and genetic data for a multivariate characterization of four Mexican *Lupinus* species

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State of art and aim: Mexico is a secondary dispersion centre of *Lupinus* with 60-100 species growing on highlands at 2000-4000 m above sea level (Bermudez Torres *et al.*, 2009). The Trans Mexican Volcanic Belt, centred around 19°05' N, crossing Mexico from East to West, is a confluence zone of two biogeographically regions, the Neotropical and the Nearctic region, in which *Lupinus* biodiversity is high, since more than 30% of the Mexican species are represented here. In this geographical area, almost 20-25 *Lupinus* species were mentioned, but their taxonomy is often chaotic, because of duplicity of names and high number of synonyms. For this reason, strict identification of wild Mexican *Lupinus* taxa is still uncertain.
The object of this investigation was to develop a multivariate reference pattern useful to help assigning any undefined species to a distinctive lineage.

Results and discussion: Four species, with visible morphological differences, were selected: *L. campestris*, *L. hintonii*, *L. montanus*, and *L. aschenbornii*. Our strategy was to characterize these species by a multivariate approach: morphological, chemical, and multilocus genetical analysis. Chemical data are represented by quinolizidine alkaloids content, which are important secondary metabolites of *Lupinus* usually identified and quantified by GC-MS (Boschin *et al.*, 2008).
The genetic analyses was a combination of ISSR and barcoding analysis. ISSR markers have been widely used to detect the genetic diversity of plants and has some advantages, such as high polymorphism and high reproducibility (Ferval *et al.*, 2013). Metabarcoding consists in sequencing various recognized barcode genes from nucleus and organelles, used to build species trees. We use two nuclear genes and three chloroplastic ones.
The four species are well separated considering either the chemical data or the 5 genes of the genetic analysis; *L. montanus* possesses the highest divergence between individuals of each species. Differently, ISSR data grouped the two species growing at the highest elevations, i.e. *L. montanus* and *L. aschenbornii* from the others. Whatever the data considered, all the taxa are well distinguished and when several specimens from the same species were available, they always fell in a particular specific group. In conclusion, this multivariate approach can help to clear the taxonomy of wild Mexican *Lupinus* as far as other species.

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Boschin G *et al.* (2008) *J Agric Food Chem,* 56, 3657
Ferval M *et al.* (2013) *South Afr J Bot,* 89, 106

Tags: barcoding, ISSR, Mexican *Lupinus*, morphology, quinolizidine alkaloids, taxonomy

(P21)

Extensive proteome characterization of *L. mutabilis* using a combined approach based on 2D-electrophoresis and mass spectrometry

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State of art and aim. In legumes, seed storage proteins are important for developing seedling and are a relevant source of nutrients for humans and animals. Being a source of low-cost proteins with high nutritional value, lupin is considered more attractive than other protein-rich plants (Holden *et al.*, 2005). Current breeding efforts focus mostly on *Lupinus albus,* L. (white lupin), *L. angustifolius,* L. (narrow-leafed lupin) and *L. luteus,* L. (yellow lupin), but also *L. mutabilis,* L. (pearl lupin) was recently included. Cultivated since more than 2000 years in the South American Andes region, Andean lupin (*L. mutabilis* Sweet) is now regarded as a potential crop for central European and Mediterranean climates. Its seeds possess the highest protein levels among all domesticated lupin species. Indeed, the value of this species is mainly due to the high protein content (40-50%) of its seeds (Santos *et al.*, 1997). The seed protein of *L. mutabilis* is also richer in lysine (Lys) and cysteine (Cys) in comparison to that of the Old World *Lupinus* species (Salmanowicz, 1999). Nevertheless, very little is known concerning the protein structure and proteins composition of *L. mutabilis* seeds, when compared with the seeds of *L. albus*, and *L. angustifolius*. In order to obtain extensive and novel information, a proteome analysis has been conducted in our laboratory using a combined approach based on 2D-electrophoresis and mass spectrometry.

Results and discussion: A species-specific proteome profile of *L. mutabilis* is reported, after a comparative study of the seed protein composition of *L. mutabilis* and *L. angustifolius* through bidimensional electrophoresis. In details, two-dimensional mapping has revealed substantial difference in proteome expression and has shown extensive microheterogeneity of *L. mutabilis*. The proteomic analysis, performed using HPLC-Chip-MS/MS, has shown considerable differences in the structure and composition of α and β conglutins from *L. mutabilis*, when compared with *L. angustifolius* conglutins. Moreover, an overexpression of small subunit of γ-conglutin seems to characterize *L. mutabilis* seeds. From this preliminary screening, new and interesting results has emerged and have helped to improve understanding on the complexity of the protein families present in lupin seeds.

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Santos CN *et al.* (1997) *J Agric Food Chem,* 45, 3821
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Tags: *L. mutabilis*, mass spectrometry, 2D-electrophoresis

(P22)

Improved grain yield and forage nutritional quality obtained by intercropping white lupin (*Lupinus albus* L.) with other annual legumes

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State of art and aim: The available literature on intercropping legumes is rather scarce and usually without a clearly defined methodology (1). The basic principles for intercropping of two annual legumes (2) are: 1)both crop should be sown at the same time; 2) they need to have similar growing habit; 3) they need to have similar maturing/harvesting time; 4) one legume has to be a good standing ability (supporting crop) and the other susceptible to lodging (supported crop). The aim of this research was to assess the potential grain productivity and nutritional quality of forage by intercropping white lupin (*Lupinus albus* L.) with grass pea (*Lathyrus sativus* L.), grain pea (*Pisum sativum* L.) and common vetch (*Vicia sativa* L.).

Results and discussion: The best intercropping combination was white lupin and grass pea, (Table 1) confirming the previous reports in the same agroecological condition (3), followed by white lupin and grain pea. On the other hand, white lupin and common vetch showed the lower values of grain yield (GY) and nutritional qualities in relation with other intercropping combinations and even with the sole crops of each legume. All three intercrops proved to be economically profitable, with the average values of land equivalent ratio for grain yield (LER_{GY}), crude protein yield (LER_{CPY}) and crude fat yield (LER_{CFY}) in all cases higher than 1.0. It is noteworthy that all three intercropping had significantly lower weed infestation than the sole crops, thus implicating rather decreased or totally avoids the use of herbicides.

Table 1. Results of yields of legumes crops and intercropping

Sole crop / Intercrop	Grain Yield kg ha^{-1})	LER_{GY}	Crude protein yield (kg ha^{-1})	LER_{CPY}	Crude fat yield (kg ha^{-1})	LER_{CFY}
White lupin	4789	-	1820	-	182	-
Grass pea	4648	-	1441	-	43	-
Pea	3879	-	970	-	15	-
Common vetch	2234	-	648	-	9	-
White lupin + grass pea	5341	1.13	1916	1.19	162	1.46
White lupin + pea	5022	1.15	1680	1.19	133	1.37
White lupin + common vetch	3945	1.06	1390	1.06	136	1.24
$LSD_{0.05}$	875	0.05	267	0.07	35	0.09

References:
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(2) Mikić *et al* (2012) *Sustain Agric Rev*, 11, 161
(3) Mikić *et al* (2014). *Adv Agron*, 130: 337

Acknowledgements: The projects TR-31022, TR-31024 and TR-31025 of the Ministry of Education, Science and Technological Development of the Republic of Serbia.

Tags: Annual legumes, crude fat yield, crude protein yield, intercropping, white lupin.

(P23)

Forage yield potential of Andean lupin (*Lupinus mutabilis* Sweet)

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State of art and aim: Currently, the breeding programme in Serbia on white (*Lupinus albus* L.), Andean (*L. mutabilis* Sweet) as well as other species of lupins is carried out in the Institute of Field and Vegetable Crops in Novi Sad and is based upon using a collection of nearly 200 accessions of diverse origin and status. Among the institutional achievements are the first Serbian white lupin cultivars, "Panorama" and "Vesna", registered in 2008, and several Andean lupin lines tolerant to the prevailing alkaline chernozem (castanosol) soils in the north of the country (1). The goal of this research was to assess the potential of bitter Andean lupin suitable for ruminant feeding after degradation of alkaloids content by forage production.

Results and discussion: There were significant differences among the tested ten Andean lupin accession in the three-year average values of fresh forage yield (FFY), forage dry matter yield (FDMY) and forage dry matter crude protein yield (FFCPY). The line LM-14-01 had the highest values of all three monitored parameters, 43.2 t ha^{-1}, 10.8 t ha^{-1} and 435 kg ha^{-1}, respectively (Table 1). This results may be explained by the fact that this line had been developed from a hybrid population descending from several local landraces that had been proved tolerant to alkaline soil, in consequence could be considered adapted to the prevailing conditions of the northern Balkans. It may be concluded, apart from its globally main use as a grain legume, that Andean lupin has a considerable potential for forage production, quite comparable to grain pea (*Pisum sativum* L.) or vetches (*Vicia* spp.)(2).

Table 1. Forage, dry matter and protein yields for 10 Andean lupin accessions

Accession	FFY (t ha^{-1})	FDMY (t ha^{-1})	FFCPY(kg ha^{-1})
LUP 15/83	28.9	6.1	247
LUP 17/83	26.7	5.3	215
LUP 21/75	29.0	5.5	220
LUP 64/74	33.4	8.0	319
LUP 97/81	27.8	6.1	246
LUP 501/82	34.9	7.3	294
LUP 509/83	30.7	6.4	257
LUP K'AYRA	35.8	9.0	349
LM-11-03	39.7	10.3	418
LM-14-01	43.2	10.8	435
Average	33.0	6.9	300
$LSD_{0.05}$	8.7	2.1	59

References:
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Acknowledgements: The projects TR-31016 and TR-31024 of the Ministry of Education, Science and Technological Development of the Republic of Serbia.

Tags: Andean lupin, forage dry matter, crude protein, fresh forage

(P24)

Field assessment of narrow leave lupin (*Lupinus angustifolius* l.) in Córdoba, Argentina

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State of art and aim: Field research on lupin crop was carried out over the past fifteen years in the Argentine Pampas. It had the objective of introducing sweet white lupin (*Lupinus albus* L.**)** varieties considering agricultural and climatic conditions of the region. Some of the achievements were a phenology model (Lupifen) which was calibrated to estimate dates of germination, flowering, pod setting and maturity for winter and spring varieties (1) and an agro-ecological zoning map for early, average and late maturity varieties (2). Based on these results a technological package was prepared and transferred to the farmer community. However, anthracnose was a significant limiting factor for the crop expansion. Therefore, in order to overcome it new experimental plots of sweet narrow leaved lupin (*Lupinus angustifolius* L.) cultivars were carried out last year in the same environmental conditions as for the sweet white lupins. The main objective of the study was to evaluate the field performance in terms of crop phenology and productivity in the semiarid region of Córdoba, Argentina.

Results and discussion: Cultivar Caro shows different plant architecture (Fig. 2) and phenological responses (Table 1). Also, significant difference ($p<0.05$) was recorded in crop yield between the first and second planting date. First planting (24/04/14) produced 2996 kg/ha while the second planting (24/5/2014) produced 1831 kg/ha**.** Early planting yield was almost double the yield for the May planting. New field tests for different sowing density are under way for 2015 growing season.


Figure 1

Table 1. Crop stages for 1st. and 2nd. sowing dates in 2015

Crop stages	1st. Sowing date	2nd. Sowing date
Sowing	April 24	May 24
Emergency	May 3	June 5
Flowering	July 4	August 28
Pod setting	July 11	September
Harvest	October 9	November 8

References:

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Tags: Lupin field research; plant architecture; Crop stages; crop yield.

(P25)

Presentation of a recipe book using beans and lupins flour

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State of art and aim: The eating habits of the Argentine population reflect a high level of consumption of meat, saturated fats, refined flours and low intake of complex carbohydrates and fiber (1). New research shows that this consumption pattern has been associated with growing index of obesity and other chronic non-transmitted diseases (2, 3). The incorporation of white lupin beans (*Lupinus albus* L) and its flours can improve the nutritional quality of diets due to its high content of proteins, dietary fiber and a good balance of polyunsaturated/saturated fatty acids fats. In Argentina, lupins beans are sold in health food shops, in pharmacies as medicinal grain and also in grocery stores as pickles (canned and bulk). Despite its important nutritional and culinary qualities, the lupins are barely consumed in the country. With the aim of promoting the consumption of lupins in Argentina several recipes with beans and lupin flour were designed, evaluated and presented in a culinary book that is in the edition process by the University of Córdoba, Argentina.
Materials and Results: The pickled lupins received a high score from the panel and represent to be a good option for low sodium diets. This preserve, comparable to the soybean, has better organoleptic qualities as well as the improvement in nutritional value given by the top composition of lupins amino acids and oils. The stews and salads were innovative, attractive and very good nutritional quality dishes with also high scores. The consistency and the characteristic taste of the lupins, introduces a texture in contrast with the usual ingredients of the prepared dishes. For the baked preparations sensory analyses showed that the incorporation of lupin flour in cakes provided a good leavening capacity and an appropriate texture. Experimental evidence also showed that cakes and cookies were very colourful given by the intense yellow colour of the lupin flour, making it convenient for the use in cakes without egg yolks in cholesterol-free diet. A recipe book is under preparation which includes: description of the nutritional values of lupin beans and flour and recipes of Appetizers, Main dishes, Salads and Desserts. Each recipe has: ingredients, directions, nutritional values and calories.
Conclusion: The recipe book (Fig. 1) designed by the authors, provide useful information for families, restaurants and institutional food managers, to incorporate lupins as a high nutritional ingredient in selected menus.

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Acknowledgements: Alejandro Barbeito for the art work

Tags: Nutritional recipes; nutritional diets, culinary book, lupins beans, bakery recipes

(P26)

The involvement of abscisic acid and ROS in programmed cell death – mediated processes in *Lupinus luteus* flower abscission

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State of art and aim: In the study of abscission the models that have been mainly used are *Arabidopsis thaliana,* L. and *Solanum lycopersicum,* L. However, the knowledge concerning physiological and biochemical aspects underlying that process, particularly in the crop species, still remains poorly understood. Excessive flower abscission in *Lupinus* species is a major cause of yield decrease, as a consequence, there is a reduced interest in the crop by farmers. Therefore understanding of this process in agronomical interest plant would help to regulate crop production.
Flower abscission is a developmental process involving multiple changes in the cell structure, metabolism and gene expression in the abscission zone (AZ), place of flower detachment. Additionally, it is well known that large number of genes regulating abscission are part of the hormone biosynthesis and signaling pathways. Moreover, the latest research provides considerable evidence for the importance of reactive oxygen species (ROS) during abscission. An increase in ROS production can induce lipid peroxidation and alter the membrane bilayers integrity, which consequently leads to programmed cell death (PCD). ROS may be detoxified by antioxidant enzymes such as peroxidase (POX). The main purpose of our research was to provide a comprehensive analysis of the *Lupinus luteus,* L. flower abscission zone. We investigated the ultrastructural changes and *in situ* detection of PCD within floral AZ cells. The role of ROS, POX and ABA in flower abscission was assessed by measurement the level and localization in flower pedicels.
Results and discussion: Progressive abscission is correlated with color changes of the pedicel from green to deep yellow or even brownish, and that leads to flower shedding. In the cells of activated abscission zone several ultrastructural changes, such as chromatin disorganization and condensation, electron dense, ameboid–shaped nuclei and branched plasmodesmata, were detected. These changes were accompanied by elevated ROS and ABA concentration, initially accumulated between vascular bundles. Additionally significant increase in POX level and activity during abscission was detected, which suggest the occurrence of detoxification mechanism in the cells. Presented data show that following abscission, detached organs undergo similar process to the plant PCD. These results provide information about biomolecules that regulate abscission in *Lupinus* which further can be used to design genetic strategies helping to elucidate that process.

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Tags: abscission, abscisic acid, peroxidase, reactive oxygen species, programmed cell death

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(P27)

Photosynthetic parameters and seed yield of narrow-leafed lupin and faba bean under condition of Moscow region of Russia

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State of art and aim: Photosynthetic parameters of crops are directly connected with productivity of plants and seed yield [1-2]. The aim of field experiments was to study and compare the effect of weather conditions on plant development, photosynthetic characteristics and seed yield stability of the productive cultivars of *Lupinus angustifolius* (cv. Crystal) and *Vicia faba* (cv. Maria) under growing conditions of Moscow region. Parameters such as Leaf Area Index (LAI), Photosynthetic Potential (Ph.P), Wet and Dry Matter Accumulation (WMA, DMA), Net Assimilation Rate (NAR), Crop Growth Rate (CGR), seed and protein yield were determined. The average normal precipitation during the vegetation period is 60-70 mm per month. Weather conditions differed throughout the years. Periods with low water supply occurred in time of vegetative growth in 2007 and in time of pod set in 2011. During 2 months in 2010 there was a severe drought with high temperatures. There were favorable weather conditions in 2008, 2009.

Results and discussion: Weather conditions had a great impact on studied parameters. The vegetation length of lupin and faba bean was 100-118 days in years with sufficient precipitation (2008, 2009). It has been a month shorter in dry conditions (2007, 2010, and 2011). Ph.P. of narrow-leafed lupin was 2-3 times lower in years with dry conditions. This especially is a concern for faba bean. Wet and dry matter accumulation also decreased though to a lesser extent – 1.5-2 times. The NAR was 20 % higher for lupin than for faba bean. On average for the 5 years WMA at the stage of pod filling has been 33.3 t ha^{-1} for lupin and 22.8 for faba beans. WMA and DMA were the most variable parameter (V(coefficient of variation)-83 %) for faba bean. Crop growth rate (CGR) of lupin is higher (220 kg ha^{-1} day^{-1}, V-25%) than for faba bean (140 kg ha^{-1} day^{-1}, V-54%). The seed and protein yield of faba bean greatly depends on weather conditions; it was high (5.1 t ha^{-1} and 1525 kg ha^{-1} respectively) only in exceptionally humid vegetation period of 2008. In drought conditions (2007, 2010, 2011) the lowest yield was obtained – 0.80 t ha^{-1} and 280 kg ha^{-1}, V was quite high - 77 %. Lupin cv. Crystal provided rather stable productivity in years with different weather conditions (V - 35 %).

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Tags: *Lupinus angustifolius*, *Vicia faba*, photosynthetic parameters, seed yield.

(P28)

Lupin peptides induce hypocholesterolemic effects through the regulation of the post-translation HMGCoAR activity on HepG2 cells

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State of art and aim: Lupin is a protein-rich grain legume, which has been domesticated long time ago and cultivated in different continents, either for animal or human nutrition. The seeds of these plants have some favorable features, in particular the protein percentage is comparable to that of soybean [1] and the content of essential amino acids is only slightly lower. Besides these important nutritional features, lupin seed may also provide some health benefits, particularly in the area of dislipidemia prevention [2-4]. Our previous findings indicate that peptides obtained from the hydrolysis of lupin proteins are able to interfere with the HMGCoAR activity. Moreover, immunoblotting experiments show that lupin peptides are able to modulate the cholesterol metabolism inducing the SREBP2 activity, which in turn leads to an increase of LDLR and HMGCoAR protein levels. From a functional point of view, HepG2 cells treated with lupin peptides are able to increase their ability to uptake extracellular LDL with a final hypocholesterolemic effects [5]. With the objective of providing further evidences that peptides generated by the digestion of lupin proteins may be responsible of the hypocholesterolemic effects through the regulation of LDLR/SREBP2 pathway, HepG2 cells were treated with lupin peptides obtained either by pepsin (P) or trypsin (T) hydrolysis and molecular investigations of the AMPK pathway activation were performed.

Results and discussion: HMGCoAR is among the most highly regulated known enzymes. In particular, it can be long-term regulated by the control of its synthesis and its degradation or short-term regulated through phosphorylation or dephosphorylation. In particular, HMGCoAR is the direct AMPK substrate. Our findings provide some evidence according to which lupin peptides are able to increase the phosphorylation level of AMPK at the Thr172 residue of the catalytic α subunit, indicating the activation of AMPK, which in turn produces an inhibitory effect on HMGCoAR activity. In fact, the AMPK activation mediated by P and T lupin peptides leads to a significant increase of the phosphorylation levels of the HMGCoAR at Ser872 residue, which is the phosphorylation site of AMPK. For this reason, lupin peptides are able not only to act as competitive inhibitors of the HMGCoAR, but also to inhibit HMGCoAR activity by enhancing AMPK activation.

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(P29)

Increasing incidence of virus-like symptoms in lupins in Chile

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State of art and aim: During the last decade, the occurrence of lupin plants with symptoms associated with virus attack has increased in southern Chile. Affected plants of sweet and bitter *Lupinus albus* show a conspicuous change in leaf morphology, turning the leaflets wider and curled at the edges, and pale foliage color. Infection before flowering prevents all or most seed production, but late infections do not. *Lupinus luteus* plants show similar symptoms, although at lower frequency. *Lupinus angustifolius*, in contrast, shows brown streaking and bending of the upper stem, followed by severe necrosis and quick plant death without seed production, in agreement with symptoms described for the necrotic strain of the *Bean Yellow Mosaic Virus* (BYMV) in Australia. Less often, the pods turn black while the plant maintains green or yellowish foliage, suggesting the presence of the black pod syndrome induced by late infection with BYMV (Kehoe et al. 2014). Lupin crops with plants showing the symptoms described above are usually concomitant with nearby red clover or common bean crops, where aphid populations have boosted in recent years favored by dry springs. During 2014, the presence of BYMV was assessed in symptomatic and asymptomatic plants of *L. albus*, *L. luteus*, and *L. angustifolius* collected from nurseries at INIA Carillanca, near Temuco.

Results and discussion: DAS-ELISA detected the presence of BYMV in the three species (*L. albus*, *L. luteus*, and *L. angustifolius*) in symptomatic plants only. These results were corroborated by RT-PCR with BYMV5'/BYMV3' specific primers for BYMV (Bariana et al. 1994) as symptomatic plants of the three species showed an expected band of 240 bp. In addition, thin sections of symptomatic tissue showed typical lamellar inclusions of subdivision-II type, which are characteristic for BYMV, under a transmission electron microscope (TEM). Finally, a RT-PCR performed on ten symptomatic and two asymptomatic plants of *L. albus* using specific BYMV primers (Duraisamy et al. 2011), capable of amplifying a 1134 bp fragment involved in the transcription of the virus coat protein, generated the expected band only for samples from plants with symptoms. This amplicon is being sequenced to establish the percentage of homology with other BYMV isolates.

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Tags: BYMV, black pod syndrome, virus, Chile.

(P30)

Micellar lupin protein isolate – digestibility, solubility and sensory evaluation as a function of physical treatment of defatted lupin flakes

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State of art and aim: As is the case with other plant, especially legume proteins, lupin proteins become increasingly important for human nutrition. Advantages regarding techno-functional or nutritional properties are well known. Recent studies report on a new quality aspect of a special lupin protein isolate, namely its potential use as fat replacer (Sussmann et al., 2013). In a cooperation project with the Fraunhofer Institute for Process Engineering and Packaging IVV (Freising, Germany) methods of microbial stabilization were defined, because raw materials for the protein isolation procedure, defatted (hexane or carbon dioxide) flakes of lupin (*Lupinus angustifolius* cv. Boregine*)*, exhibit a microbial load of approximately 10^4 cfu/g that mainly consists of spore-forming microorganisms. Consequently different methods of lupin flakes decontamination were examined: dry heat treatment at 130 °C for 60 min and 140 °C for 55 min as well as UVC radiation for 120 min. Following the physical treatment the fat-like micellar protein isolate was extracted.
These decontamination methods and the fact that lupin proteins undergo denaturation from 71,49 °C on (Sirtori et al., 2010) suggest that sensory, functional or nutritional properties of the protein isolates differ depending on the treatment modality.
In the following, the results of the sensory evaluation (according to DIN EN ISO 4120 triangle test, DIN ISO 8587 ranking test), the *in-vitro* protein digestibility as well as the nitrogen solubility as a function of pH-value (2, 4, 6, 8, 10) are described.

Results and discussion: Differences in the taste and mouthfeel of protein isolates were obtained for hexane and carbon dioxide defatted lupin flakes. In ranking tests, we observed the maximum creaminess for the isolates resulting from untreated and UVC-radiated flakes, and a minimum for the 140 °C sample. Furthermore, the popularity of protein isolates increased from the protein isolates of the dry heated to those of the untreated or UVC radiated flakes.
The nitrogen solubility was lowest at pH 4 for all protein isolates tested, with increasing solubility in acidic and alkaline regions. This was due to the isoelectric point of lupin protein, which is between 4.3 and 4.9 (El-Adawy et al., 2001). There was no variation in solubility of protein isolates from both defatted varieties of lupin flakes, but with regard to the decontamination treatments, the solubility decreased with stronger heat treatment of raw materials.
No differences in *in-vitro* protein digestibility could be detected.
In conclusion, the assumption of alteration in protein characteristics has been confirmed. There were changes in sensory profiles and protein solubility, but not in protein digestibility.

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Tags: seed decontamination, lupin protein, solubility, digestibility, sensory properties

(P31)

Protein quality of *Lupinus angustifolius* – prediction of amino acids and crude protein with Near Infrared Spectroscopy

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State of art and aim: The quality evaluation of organic protein feedstuffs, especially the quick and easy determination of the protein quality including the amino acid (AA) pattern in locally grown legumes like lupins, is very important to fulfil the requirements regarding the protein and amino acid supply of organically fed animals, especially monogastrics. The analytical data of organically cultivated feeds, as compared with conventional table values, shows a clear deviation of protein and amino acids between conventional and organic feeds. The standard tabular values (DLG 2014) are not sufficient for the calculation of feed rations. Therefore the ability of NIRS to predict the chemical composition and the essential AA's of organically grown lupins was proofed.

Results and discussion: The statistical parameters of NIRS calibration (see table) showed that the predictions were successful for crude protein, lysine and cysteine. The prediction accuracy for the sulfur-containing AA's, methionine and cysteine and for threonine was satisfactory. The main reason that no more robust calibration equations were developed, was the fact, that especially the methionine contents were very low and the range was very small. Therefore lupin samples from future seasons should be used to improve the quality and to check the robustness of the developed calibrations. Nevertheless, NIRS can be used for the prediction of the protein quality in lupins as well as in other feed components (Aulrich & Böhm 2012) and for the direct, quick and easy calculation of feed rations directly after harvest.

Table: NIRS data and calibration statistics for prediction of crude protein and some amino acids in lupins

Ingredient (n)	Range (g/kg DM)	Mean (g/kg DM)	R_K	SEP
Protein (525)	235 - 420	333.5	0.96	0.88
Lysine (196)	12.1 – 22.4	16.7	0.93	0.65
Methionine (204)	1.71 – 2.17	2.13	0.88	0.12
Cysteine (211)	3.39 – 7.55	4.54	0.91	0.31
Threonine (210)	8.84 – 14.8	11.61	0.85	0.65

R_K: Regression coefficient of the calibration, SEP: Standard error of prediction

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Tags: *L. angustifolius*, protein quality, amino acids, NIRS.

(P32)

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

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State of art and aim: Primary goals in narrow-leafed lupin breeding are high grain yield, yield stability and good seed quality. For that reason 50 varieties and breeding lines (eight with restricted branching and 42 with normal branching) were tested in three different locations in Mecklenburg-Pomerania over three years. Field experiments were conducted in 2010, 2011 and 2012 at Groß Lüsewitz, Groß Dratow and Bocksee and laid out in a randomized complete block design.
The main goal of the study was to assess the genetic and environmental variation in current breeding material, but also to identify breeding lines with low environmental variability.

Results and discussion: The trials demonstrated the effect of location, years, genotypes and genotype- environment interaction on grain yield, protein content and thousand kernel weight (TKW) in lupins with normal and restricted branching.
Yield variability between years and locations was high. Location had a greater influence on grain yield than year or genotype. A significant location-by-year interaction could also be observed.
The variety showing the highest crude protein content over years was Probor. Strong variation was observed between years and in the location-by-year interaction. Yield was negatively correlated with crude protein content. Crude protein yield has been mainly determined by grain yield.
TKW ranged from 108.5g to 166.9g, with normal branching lines having a major range of variation than lines with restricted branching. Thus, TKW variation is more closely correlated to grain yield and crude protein content. This character is greatly affected by genotype.

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Tags: grain yield, crude protein content, environment

(P33)

Replacement of soybean meal with white lupin in the nutrition of rabbit does: effect on milk yield, milk composition and growth performance of their litters over two lactation periods

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State of art and aim: A short-term lactation experiment (one lactation period) revealed that white lupin seeds (WLS) may be a perspective dietary crude protein (CP) source for lactating rabbit does, and can fully replace commonly used soybean meal (SBM). To confirm this finding, however, a longer-term experiment was necessary. Therefore, the aim of this study was to evaluate the effect of lactation (WLSL) and weaning diets (WLSW) based on WLS, in comparison with the lactation (SBML) and the weaning diets (SBMW) containing SBM, on milk yield and milk composition of rabbit does, as well as on the growth of their litters through the longer-term experiment, i. e. over two lactation periods. Reproductive performance of rabbit does was also recorded.

Results and discussion: Does body weights were not affected by dietary treatments. Significant differences were observed in terms of the daily milk production. In the 1st lactation period, average milk yield was higher between d 22 and d 32 of lactation in does fed the WLSL diet (P=0.016), whereas in the 2nd lactation period, milk yield was higher (P=0.019) over the whole lactation (35 days) in these does. Milk composition was determined at d 21 of the 1st lactation period. Milk dry matter (on avg. 27.1 g/100 g), protein (on avg. 9.6 g/100 g), fat (on avg. 15.9 g/100 g) or ash (on avg. 2.0 g/100 g) contents were not affected by dietary treatments. When expressed per kg of metabolic weight, milk output (P=0.009) and fat output (P=0.004) were higher in does fed the WLSL diet. These findings are related to the higher dietary ether extract (EE) content in the WLSL diet, due to higher EE content in WLS than in SBM, and is consistent with our previous results. The milk of does fed the WLSL diet contained less caprylic acid (P=0.001), capric acid (P=0.001), lauric acid (P=0.008) and linoleic acid (P=0.002) and more oleic acid (P=0.001), α-linoleic acid (P=0.001) and eicosapentaenic acid (P=0.005). Different milk fatty acid profile of does fed the WLSL diet corresponds with fatty acid profile of WLS, and confirmed our previous findings that WLS may change milk fatty acid profile in a beneficial way. Reproductive performance of does showed common figures without significant differences between treatments. Growth of litters was not affected by dietary treatments in terms of the 1st or 2nd lactation period. A significantly higher daily solid feed intake of litters (weaning diet offered from d 17 of age) before weaning was observed in does fed the diet based on SBM during both lactation periods; a finding which was probably related to the lower milk yield of their mothers. The longer-term experiment confirmed that the WLS is a suitable CP source for the lactation diet of rabbits in terms of milk yield and composition, feed efficiency, growth of litters, and reproductive performance.

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Acknowledgements: This study was supported by project No. MZERO0714

Tags: rabbit, diet, white lupin, protein source, milk yield, reproduction, growth, litters

(P34)

Alkaloids in seeds of the Polish *Lupinus angustifolius,* L. collection.

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In the presented studies a total content and qualitative composition of maior alkaloids (lupanine, 13-hydroxylupanine, angustifoline and isolupanine) in narrow-leafed lupin (*L. angustifolius* L.) were analyzed. The Polish collection (335 accessions) divided into three classes of origin: (1) wild lines, (2) cultivars and (3) other accessions created by man, were assessed.

A very broad differentiation was stated considering total alkaloid content – from 0.0004% in seed dry weight (line Wt 96128) to 2.8750% in seed dry weight (Wt 95708). In most cases cultivars were characterized by strongly decreased content of alkaloids. The distribution of individual alkaloids was very differentiated also, lupanine – 0.99% - 73.1% of total alkaloid content (mean 46.4%), 13-hydroxylupanine – 15.6% - 71.1% (mean 35.7%), angustifoline – 0% - 49.8% (mean 15.5%) and isolupanine – 0% - 34.2% (mean 2.5%). It can be assumed that typical for the species, particularly its wild lines are above mean values but quite frequent in that 13-hydroxylupanine dominates over lupanine. In some accessions lupanine and 13-hydroxylupanine set 90%-100% of all major alkaloids. The average content of isolupanie (2.5% of all alkaloids) allows to consider it as a major alkaloid of *L. angustifolius* but quite frequently occurred below 1% or even its lack was observed.

Three classes of origin were divided into three homogenous groups considering total alkaloid content as well as individual alkaloids content. Among wild lines the most numerous were high alkaloid but among cultivars low alkaloid accessions. Possibilities of substantial decreasing of total alkaloid content in seeds of *L. angustifolius* were stated, even below 0.01% in seed dry weight.

Also investigated was an influence of a content of individual alkaloids on total alkaloid content in the wild lines class and cultivars. In wild lines 13-hydroxylupanine and angustifoline (additionaly lupanine in the homogenous group with the lowest content) had the strongest influence on total alkaloid content. Among cultivars the biggest, positive effect on total alkaloid content had lupanine and additionally 13-hydroxylupanine and angustifoline in the homogenous group with the lowest total content of alkaloids.

(P35)

LupiBreed – Increasing yield potential, yield stability and seed quality of lupin as protein plant

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LupiBreed is a national collaborative research project starting in 2015 for three years. Its goal is to improve narrow-leafed lupin and yellow lupin as crop plants with regard to kernel yield, yield stability, and seed composition. This shall be accomplished both at the genetic and cultivation levels. To these ends, genebank material shall be introduced to broaden the genetic base of elite breeding materials and improved cultivation systems be devised. Project partners are the Julius Kühn-Institut (JKI), the Thünen-Institut, the Leibniz Institute of Plant Genetics and Crop Plant Research Gatersleben, as well as the private lupin breeder Saatzucht Steinach GmbH&Co. KG. Knowledge transfer and communication occurs by the Gemeinschaft zur Förderung der privaten deutschen Pflanzenzüchtung e.V. (GFP), a consortium of German plant breeding companies.
JKI work packages include an evaluation of a set of mutant (M) lines for agronomically relevant traits (yield parameters, pod shattering, anthracnose resistance, alkaloid and protein content). Promising M lines shall be crossed to pre-breeding lines carrying the anthracnose-resistance genes *LanrBo* or *Lanr1* and combination of trait genes will be controlled by use of molecular selection markers. In yellow lupin, a novel gene for anthracnose resistance shall be characterized genetically and selection markers be developed.
LupiBreed will be one of several projects connected to the national Model Network "Lupin", which is supposed to promote the use of lupins as homegrown grain legumes and is funded by the Federal Ministry of Food and Agriculture.

Tags: narrow-leafed lupin, yellow lupin, kernel yield, yield stability, mutant lines, anthracnose, resistance, molecular marker

(P36)

Genetic background of the quinolizidine alkaloids biosynthesis in the narrow-leafed lupin (*Lupinus angustifolius* L.)

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State of art and aim: Quinolizidine alkaloids (QA) are the main antinutrional components in lupins. Due to their toxicity and bitter taste, reduction of the alkaloids content in seeds is especially important for the propagation of lupins as a valuable protein source in animal feeding and human consumption. The biochemical synthesis pathway of lupin alkaloids is partially known, however genetic background of QA biosynthesis still remains poorly investigated. Up until now, only one recessive gene *iucundus* controlling total alkaloid content has been used in the narrow-leafed lupin breeding programs. Unfortunately, its molecular function has not been yet determined. The main goal of the presented study was to identify the genes underlying QA biosynthesis on the basis of the results of the transcriptome sequencing experiment (RNA-Seq) as well as their genetic mapping in the narrow-leafed lupin genome.

Results and discussion: On the basis of the results of RNA-Seq experiment conducted for the narrow-leafed lupin, and the analysis of differentially expressed genes in the group of bitter versus sweet genotypes, thirteen genes were found to be especially interesting on the basis of their molecular function suggesting their involvement in the QA biosynthesis pathway. This group comprise two previously reported genes: *LDC* (Bunsupa et al. 2012b) and *LaAT* (Bunsupa et al. 2011), but also cinnamoyl-CoA reductase, three different genes of copper amine oxidase and five transcription factors genes. We have not identified significant differences in the expression level of *HMT/HLT* gene in the bitter and sweet genotypes, what is in accordance with the results obtained by Suzuki et al. (1994), who suggested that the alkaloid level in plants is not dependent on the activity of the HMT/HLT enzyme. Identified QA biosynthesis genes were mapped in the narrow-leafed lupin genome but only one, ethylene-responsive transcription factor RAP2-7 was found to be closely linked to the total alkaloid content gene *iucundus* . RAP2-7 belongs to apetala2/ethylene response factor (AP2/ERF) family, that regulates plant secondary metabolism (Yu et al. 2012). Our results suggest that, RAP2-7 marker may have practical application in the narrow-leafed lupin marker assisted selection (MAS). In our further studies the correlation between this marker genotype and plants phenotypes of the narrow-leafed lupin Polish cultivars and core collection accessions will be tested. Although the results obtained need further confirmation, they are an important step towards better recognition of genetic background of QA biosynthesis pathway.

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Tags: gene identification, alkaloid biosynthesis, RNA-Seq, narrow-leafed lupin

(P37)

The effect of tetracycline on lupin seedlings (*Lupinus luteus* L.) physiology

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State of art and aim: Pharmaceutical drugs have been repeatedly detected in the environment. Residues of tetracycline antibiotics used in human and veterinary medicine for treatment purposes and as growth promoters can be found in manure, soil and water (Daghrir, 2013). These drugs, when taken up by plants from contaminated soil, can have a toxic effect on these organisms (Adomas et al. 2013). The present study aims to investigate the impact of tetracycline (1, 5, 20, 50, 100 mg per pot) on guaiacol peroxidase activity, chlorophyll content and osmotic pressure of yellow lupin seedlings (*Lupinus luteus* L.). EC_{50} for osmotic potential was determined. The research was carried out on the third day after watering lupin seedlings with the tetracycline solutions.

Results and discussion: The current research demonstrated a decrease of chlorophyll content and osmotic pressure in the seedlings with increasing tetracycline concentration. EC_{50} for the osmotic potential was 42 mg. Chlorophyll content in leaves decreased by 26, 26.5, 27, 38.5 and 49% for 1, 5, 20, 50, 100 mg tetracycline/flowerpot respectively. Moreover, an increase in guaiacol peroxidase activity was observed with increasing concentration of tetracycline. The maximum activity, about 44% higher than the control was noticed at the concentration of 50 mg. Our results demonstrate the ability of yellow lupin seedlings to absorb tetracycline . In previous studies the phytotoxicity of pharmaceutical drugs such as diclofenac and enrofloxacin to lupin plants has been shown (Adomas et al. 2013, Ziółkowska et al. 2014). The current studies demonstrated the toxic effects of tetracycline on lupin on third day after the application of the antibiotic. Moreover, tetracycline caused an oxidative stress reaction in seedlings.

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Tags: tetracycline, guaiacol peroxidase, lupin

(P38)

Project "Lupi-Breed": Improving yield potential, yield stability and seed quality of lupins as protein plants
Work package: Weed suppression and intercropping

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State of art and aim: The overall goal of the project, which has been started in spring 2015, is to improve productivity of narrow-leafed sweet lupin (*Lupinus angustifolius*) and yellow sweet lupin (*L. luteus*) with regard to kernel yield, yield stability, and seed composition. In addition, the question shall be addressed whether the narrow genetic basis of current breeding materials may be broadened by including genebank accessions as plant genetic resources. Another task is to optimize lupin cultivation systems for non-chemical weed control by optimizing intercropping cultivation and the establishing of a system for testing the weed suppression of new varieties resp. pre-breeding lines.
These aims should be achieved in the joint project, carried out at the Julius Kühn Institute with the Institute for Breeding Research on Agricultural Crops (coordination) and the Institute of Resistance Research and Stress Tolerance, the Thünen-Institute of Organic Farming, the Leibniz Institute of Plant Genetics and Crop Plant Reseach and Saatzucht Steinach & Co. KG.
The project is expected to provide fillips to lupin breeding, to improving agricultural practice and to enhancing acceptance of lupins as protein sources for feed and food applications.

Results and discussion: A field experiment was carried out in the years 2006 – 2008 with two varieties (*cv.* Boruta, determinated type and *cv.* Bora, branched type) and different row distances (12.5, 25.0 and 37.5 cm). The results showed a better weed suppression of the branched variety Bora (Böhm & Aulrich 2011). An experiment carried out in the years 2005-2007 at two sites and different seed ratios of the two blue lupin varieties Boruta and Bora intercropped with wheat or barley showed that the yield percentage of blue lupins was very low and did not reach more than 25 % (Böhm et al. 2008). On basis of these results we would like to integrate the question of fitness of pre-breeding lines with respect to their weed suppression and their suitability for intercropping. At the Thünen Institute of Organic Farming (i) a test system will be developed for the detection and evaluation of the weed suppressive effect of blue lupins, (ii) this test system will be tested with pre-breeding lines of blue lupin and (iii) the intercropping of blue lupin will be optimized with regard to weed suppressive effects and the highest possible yield of lupin. For this purpose, (i) in the first project year 2015 two phenological different varieties will be cultivated combined with selected "artificial weeds" and different seed rates, (ii) in the second and third project year this test system will be tested with eight selected pre-breeding lines in field trials and (iii) different seed densities of the mixing partners will be cultivated in combination with the selected pre-breeding lines. The realized lupin yield, as well as the accumulated weed biomass and the PAR measurements, will be evaluated. First results will be presented in a poster at the conference.

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Tags: *Lupinus angustifolius*, intercropping, weed suppression, breeding

(P39)

Influence of variety and sulfur fertilization on the contents of crude protein, and selected amino acids in blue lupins (*Lupinus angustifolius* L.)

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State of art and aim: Legumes are the most important nitrogen suppliers in organic farming, have positive effects in crop rotation and are important protein feedstuffs. In 100 % organic feeding, the amino acid (AA) supply of feedstuffs is of increasing interest. The first and second limiting AAs for swine and poultry are lysine (Lys) and the sulfur-containing AAs (SAAs), methionine (Met) and cysteine (Cys), respectively. Since SAAs are limiting in the diet of monogastrics and, besides tryptophan, also in grain legumes, it was meant to examine, if sulfur (S) fertilization could increase the SAA contents in blue lupins (*L. angustifolius* L.) and if it affects the Lys content in different varieties.

Results and discussion: Two branched (´Boregine´, ´Probor´) and two determinated (´Boruta´, ´Sonet´) blue lupin varieties were cultivated at the experimental station in the years 2012 and 2013. In each year, the varieties were grown either with or without S fertilization (40 kg S ha^{-1}; $MgSO_4$). Laboratory analyzes were done with NIRS, statistical analyzes with SAS 9.4 (proc glm). In 2013, the crop yield (3.65 ± 0.38 t ha^{-1}, 86% DM) and the contents of the measured ingredients (CP: 33.80 ± 1.77 g 100 g^{-1} DM and Lys: 4.94 ± 0.14, Met: 0.68 ± 0.03, Cys: 1.39 ± 0.03 g 100 g^{-1} CP) were higher than in 2012 (Table). There was an effect of the variety on all parameters. In 2013, ´Sonet´ had a significantly lower crop yield and CP content than the other varieties. However, ´Boruta´ showed a high yield difference in both years. Additionally, the variation of the Met contents between the years was higher for ´Probor´ and ´Boregine´ than for the other varieties. ´Boregine´ had the highest and ´Probor´ the lowest Met contents in 2013 (0.69 vs. 0.64 g 100 g^{-1} CP, $p < 0.05$). Contrary to 2012, S fertilization affected the mean Met content of the lupins in 2013 (-S: 0.67, +S: 0.69 g 100 g^{-1} CP). Furthermore, S fertilization led to increased Cys contents of ´Boruta´ and ´Sonet´ in both years and ´Boregine´ in 2012 ($p < 0.05$). This reaction of the SAAs was already observed in grain legumes (Schumacher *et al.* 2011). The crops of ´Sonet´ in both years and of ´Boruta´ in 2013 showed significantly lower Lys contents ($p < 0.05$) when fertilized with S. This might be due to an altered storage protein ratio (Blagrove *et al.* 1976). ´Probor´ was not affected by S fertilization in both years. In conclusion, S fertilization can positively affect the SAA contents of blue lupins to the detriment of the amount of Lys. Further effects of environment and variety are considered to alter the reaction.

Table: Crop yield (t ha^{-1}, 86% DM) and contents of crude protein (CP in g · 100 g^{-1} DM) and some amino acids (g · 100 g^{-1} CP) in four varieties of blue lupines (*L. angustifolius* L.) with and without sulfur fertilization (S) in 2012

Variety	S	Yield	CP	Lysine	Methionine	Cysteine
Boregine	-	3.73 ^{a}	32.56 ^{b}	5.13 ^{b}	0.58 ^{b}	1.10 ^{c}
	+	3.53 ^{a}	33.38 ^{b}	4.91 ^{bc}	0.62 ^{b}	1.27 ^{b}
Boruta	-	2.25 ^{c}	30.44 ^{d}	4.63 ^{d}	0.64 ^{b}	1.28 ^{b}
	+	2.10 ^{c}	30.61 ^{d}	4.68 ^{cd}	0.62 ^{b}	1.49 ^{a}
Probor	-	2.89 ^{b}	37.96 ^{a}	4.51 ^{d}	0.51 ^{c}	1.21 ^{bc}
	+	2.52 ^{b}	38.68 ^{a}	4.67 ^{cd}	0.53 ^{c}	1.21 ^{bc}
Sonet	-	2.41 ^{bc}	28.32 ^{c}	5.42 ^{a}	0.67 ^{a}	1.27 ^{b}
	+	2.31 ^{bc}	28.72 ^{c}	4.98 ^{b}	0.70 ^{a}	1.59 ^{a}

$^{a, b, c, d}$ Significant differences within columns ($p < 0.05$)

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Tags: lysine, methionine, cysteine

(P40)

A rapid HPLC-UV assay of γ-conglutin in *Lupinus albus* seeds and extracts

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State of art and aim: Because it has less fat content and high levels of dietary fiber and protein, lupin seeds are considered to be of high quality in food culture. Moreover, their health promoting properties have been investigated, in particular as anti-hyperglycemic product. It has been reported that the γ-conglutin, which is a minor protein of the mature lupin seed, is the promoter of this property. In fact, it has been discovered in *in vitro* assays the insulin-binding and insulin-mimetic properties of γ-conglutin [1]. *In vivo* assays have been shown that γ-conglutin lowers blood glucose in hyperglycemic rats and has a hypoglycemic effect in healthy humans and rats [2]. γ-Conglutin is hence a potential antidiabetic agent.
Therefore, defining a suitable analytical method to identify and quantify γ-conglutin in lupin seeds and in its related products is an important goal to control high quality seeds selection and the process development of a nutraceutical extract enriched in γ-conglutin.
Some analytical methods to measure γ-conglutin content in lupin seeds have been developed [3], but the proposed solutions seem to be unsuitable for an industrially-oriented quality control, because of the time consuming sample preparation and the high costs of sophisticated technology to be used.
The entire production chain should be controlled: the field selection of the plant material, manufacturing in-process control, quality control of the extracts. The present work describes a rapid HPLC-UV method for the identification and quantitation of the γ-conglutin in *Lupinus albus* seeds and its related extracts.

Results and discussion: A one day exhaustive extraction of γ-conglutin from milled lupin seeds has been developed. Several solubilisation solvents for enriched extracts have been investigated. A rapid 30 minutes solution has been selected. Basing on a reverse phase HPLC separation (30 minutes run time), detection and quantification have been carried out against γ-conglutin reference standard by UV-detection. The method has been assessed for accuracy, linearity, precision and stability of the sample solution. An alternative columns overview has been also taken into consideration.

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Acknowledgements: Prof. M. Duranti team's for purification and supply of γ-conglutin reference standard.

Tags: *Lupinus albus*, γ-conglutin, HPLC-UV assay.

(P41)

Quality of Lupin Seeds of Three Varieties of White Lupin Grown in the Czech Republic

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State of art and aim: The purpose of the study was to assess whether there are any differences in the nutritional value of seeds of three varieties of white lupin, namely ´AMIGA´, ´DIETA´ and ´ZULIKA´, grown in the Czech Republic.

Results and discussion: Despite the fact that the varieties belonged to the same white lupin species, we observed considerable variety differences, mainly in the levels of nitrogenous substances (376.00 g/kg for ´AMIGA´, 391.40 g/kg for ´DIETA´, and 429.10 g/kg for ´ZULIKA´, dry matter basis) and the level of fat (112.40 g/kg for ´AMIGA´, 86.00 g/kg for ´DIETA´ and 72.00 g/kg for ´ZULIKA´) in the seeds. Lupin seeds contained significantly less starch than cereals. The oil levels affected total gross energy of the seed (21.30 MJ/kg for ´AMIGA´ variety, 20.80 MJ/kg for ´DIETA´, and 20.70 MJ/kg for ´ZULIKA´). Considerable differences were further found in the levels of individual minerals, Ca, P and Mg (g/kg) as well as in their mutual proportions. Analysis of individual amino acid levels showed a deficit of sulphurous amino acids in the lupin protein. The highest amino acid levels were measured for glutamic acid (namely 73.10 g,/kg, 79.20 g/kg, and 84.60 g/kg of seed, respectively), followed by arginine (47.50 g/kg, 48.20 g/kg, and 54.60 g/kg, respectively). As for fatty acid (FA) levels in the lupin oil, the individual varieties ´AMIGA´, ´DIETA´, and ´ZULIKA´ contained 8.35 g of FA/100 g of oil, 13.75 g of FA/100 g of oil, and 16.12 g of FA/100 g of oil, respectively. The lupin oils were a rich source of unsaturated FA, especially MUFA (49.90 g/100 g of oil, 49.86 g/100 g of oil, and 42.74 g/100 g of oil, respectively). The lupin oil also showed relatively favourable levels of PUFA, namely 24.80 g/100 g of oil, 19.72 g/100 g of oil, and 23.15 g/100 g of oil, respectively. Very favourable levels were found for n-6 PUFA and n-3 PUFA groups. Lupin varieties showed n-6 FA levels of 16.06 g/100 g of oil, 11.76 g /100 g of oil, and 13.76 g /100 g of oil, respectively, while n-3 FA levels were 8.74 g /100 g of oil, 7.96 g of FA/100 g of oil, and 23.15 g /100 g of oil, respectively. From nutritional point of view, the ratio of n-3/n-6 PUFA may be seen as very positive. It was similar in the oils of all three varieties, namely 1: 1,84, 1 : 1.48, and 1 : 1,47.The results show that lupin seeds may be considered a significant source of arginine being one of the essential amino acids, important especially for poultry. Lupin seeds also represent a suitable protein component of farm animal diets with very high-quality oil. As is apparent from the results above, due to great variability among varieties, diets have to be formulated with regard to the nutritional composition of the particular lupin variety.

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Tags: white lupin, amino acids, fatty acids, n-3, n-6, n-9 fatty acids.

(P42)

Replacement of Soybean Meal with Lupin Meal in Duck Diet

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State of art and aim: Considering the increasing prices of soybean meal, the objective of the study was to evaluate the effect of complete replacement of soybean meal with white lupin seed meal of AMIGA variety on growth performance and carcass indicators in Cherry Valley Pekin meat-type ducks. A total of 139 one-day old ducklings were randomly divided in the control (32 males and 38 females) and experimental group (31 males and 38 females). The ducks of the control group were fed diets with soybean extracted meal (47%) as the basic protein component of the feed, while the ducks of the experimental group were fed diets with lupin meal as its basic protein component.
Results and discussion: For both genders, differences between the average BW at the end of fattening on day 42 were not significant ($P > 0.05$). For the whole period of fattening (42 days), the experimental birds consumed less feed per 1 kg of live weight gain. Mean FCR values in males were 2.11 kg/kg and 2.30 kg/kg in the experimental and control group, respectively. In females, the values were 2.21 kg/kg and 2.30 kg/kg, respectively. Regarding the carcass indicators, higher carcass yield was found in ducks of the experimental group compared to the control group (63.0 vs. 60.4%), the difference was found significant only in females ($P < 0.01$). Females of the experimental group also showed significantly higher proportion of abdominal fat and lower proportion of skinless legs, heart and liver ($P < 0.05$). In males at slaughter age, only higher proportion of heart yield in the experimental group ($P < 0.05$) was found, 0.68% compared to 0.57% in the control group. In our study, the complete replacement of soybean meal with lupin seed meal of AMIGA variety in diets for Cherry Valley Pekin ducks fattened for the period of 42 days did not impair their growth performance and carcass composition. At the same time, lower feed consumption per unit of weight gain in the experimental group was observed. However, when formulating the diets, it is necessary to optimize their ingredient composition, especially during the first half of the fattening period. Differences in nutritional composition of the particular lupin varieties have to be taken into consideration.

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Tags: white lupin, Amiga, Pekin duck, yield, carcass value.

(P43)

Characterization of β-conglutin proteins in Lupin (*Lupinus angustifolius* L.)

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State of art and aim: Lupin is an important PULSE, which displays a wide range of benefits. In agriculture it acts as a disease break for crops in rotation, and at molecular level, lupin seed proteins may also be involved in possible plant pathogen suppression[1].
Furthermore, lupin seed proteins promote different positive health aspects, preventing cardiovascular disease, and reduction of glucose and cholesterol blood levels[2].
Sweet lupin seeds seem to be particularly promising as a source of innovative food ingredients due to averaged protein content similar to soybean and an adequate composition of essential amino acids. Thus, lupin seeds may be important source of proteins (mainly from conglutin protein families) for human and animal consumption. However, with the rapid introduction of novel foods and new ingredients in traditional foods, the number of allergic people is also rising, becoming a serious and a growing problem in the Western world[3].
The aim of the current work was to evaluate different functional features of a particular family of lupin seed proteins, β-conglutins, focusing in their properties involved in plant-pathogen defense and allergy.

Results and discussion: The results obtained point to a protective role of the β-conglutins, a major class of lupin seed proteins, against plant-pathogen attack, particularly in the case of fungal pathogens.
In a parallel study concerning the implications of β-conglutins as allergenic proteins, we identified multiple forms of β-conglutins polypeptides ranging from 15-80kDa, with IgE-binding characteristics in patients with lupin specific allergy. Thus, β-conglutins might be considered as major allergen in different species of lupin, including the "sweet lupin" group, since several of these polypeptides were recognized by human IgEs, having the potential to trigger an immune response leading to allergy symptoms. Therefore, based on current molecular data, lupin allergy might be more complicated than previously thought because of the involvement of a large number of polypeptides.

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Tags: conglutins, lupin species, narrow-leafed lupin, plant-pathogen defense, food allergy, diagnosis.

(P44)

Diversity of *Lupinus* sp. from the Mediterranean region in the VIR collection

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State of art and aim: The collection of the Vavilov Institute of Research (VIR) comprises the world diversity of cultivated species of lupin and its wild relatives. The Mediterranean region is the center of origin and domestication of cultivated lupin species. Wild relatives of cultivated lupins in flora of the Mediterranean are also presented. The germplasm from this region is very valuable. Species of *Lupinus* L. from the Mediterranean region have a great diversity of morphological, phenological, physiological and other traits. The main task of our work is to search and evaluate samples with economically important traits. The aim of the study is to find the source of commercial important characteristics.

Results and discussion: Twelve species of *Lupinus* in the Mediterranean region were described. Among them narrow-leafed lupin (*L. angustifolius* L.), yellow lupin (*L. luteus* L.) and white lupin (*L. albus* L.) have important economic significance (Kurlovich, 2002). 2919 accessions of 51 species in the VIR collection of lupin are presented. 9 species of them have the center of origin and morphogenesis in the Mediterranean region. Accessions of the Mediterranean species in the collection are distributed as follows: *L. albus* – 494, *L. angustifolius* – 841, *L. atlanticus* Glads. – 9, *L. cosentinii* Guss. – 25, *L. digitatus* Forsk. – 6, *L. hispanicus* Boiss. et Reut. – 18, *L. luteus* – 800, *L. micrantus* Guss. – 5 and *L. pilosus* Murr. – 15 samples. The accessions from the Mediterranean region represent more than 10% in the VIR lupin collection. Native accessions were received from Spain (87), Egypt (53), Greece (28), Morocco (36), Italy (22), Israel (15) and so on. Wild and landrace types in the VIR lupin collection from the Mediterranean region account about 30% and 70 %, respectively. Valuable traits are often found in wild and landrace types of the cultivated species. N.I.Vavilov collected lupins in the Mediterranean countries in 1926-27. Accessions of *L. albus* with early maturity were found among landraces collected by him into Palestine. Many samples with different morphological, physiological and biochemical traits have been introduced to the VIR collection later. The gene pool from the Mediterranean countries is widely used in the breeding programs. Source of economically valuable characters in the VIR collection are presented. Among agriculturally important species early maturity (accessions from Egypt and Israel), early maturity and high seed yield (from Egypt, Greece and Yugoslavia), high protein content (from Palestine, Greece, Egypt, Spain and Italy) and high oil content (from Palestine, Yugoslavia and Spain) (Chmeleva, 1989) were detected for *L. albus*. Some accessions of *L. angustifolius*: with resistance to gray mold (from Morocco), high seed yield (from Algeria, Corsica), early maturity (from Palestine), and resistance to drought (from Turkey, Crete, and Greece) were selected. *L. luteus* accessions with high seed yield and resistance to drought (from Turkey), early maturity, high seed yield, resistance to virus diseases (from Italy) and with high protein content (from Italy) were discovered. Also one accession of *L. digitatus* with high protein content have been found.

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Tags: collection, lupin, Mediterranean region, early maturity, protein.

(P45)

Indirect ELISA test for quantitation of conglutin γ in *Lupinus albus,* L. seeds and extracts (PRO-GAMMA™)

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State of art and aim: Like other legumes, lupin is a good source of proteins, whose importance for humans is related not only to their nutritional value, but also to their nutraceutical properties for the treatment and prevention of cardiovascular diseases, type II diabetes and obesity. Conglutin γ, a homo-tetrameric glycoprotein from lupin seed, has been shown to reduce plasma glucose levels both in experimental animals and healthy humans. After the inclusion of lupin in EU food allergen list, some ELISA commercial kits have been made available for detection and quantification of even minimal doses of lupin proteins in foods, but all of them lack specificity for conglutin γ from *Lupinus albus*. Therefore, aim of this study is the development of a new ELISA assay, based on an antibody specifically arisen against conglutin γ in *Lupinus albus*, to quantitate this protein in lupin seeds and extracts thereof (PRO-GAMMA™: registered trade mark for Indena lupin extract).

Results and discussion: An antibody specifically arisen against conglutin γ from *Lupinus albus* has been previously developed by Prof. Duranti group for immunocytochemical applications. The specificity of this antibody was checked by western-blotting in PRO-GAMMA™, comparing the result both with that of a Total Protein Extract from lupin and with Conglutin γ Reference Standard, obtained with high purity by a published chromatographic procedure. The ELISA test was conducted according to Indirect (plate coated with antigen) Noncompetitive technique. Linearity was tested in the range 0-125 ng/ml. As expected, the response was linear and the precision highest in the range 0-15.6 ng/ml, according to the results of other authors. Extraction of conglutin γ from the seeds was complete using 0.1 M TRIS + 0.5 M Glycine buffer (pH 8.7) at 45° C under stirring overnight, according to the conditions selected for optimal extraction of lupin proteins by other authors, who tested several buffers at different pH and ionic strength. A lower extraction time (2 hours) lead to uncompleted extraction. For PRO-GAMMA™, in case of protein precipitation by organic solvents, stronger denaturing conditions (7 M urea) were needed for complete solubilisation of conglutin γ. When the test was conducted using antibodies raised against conglutin γ of different lupin species, e.g. *Lupinus luteus*, the response and the fitting of calibration points were lower. A medium-binding plate (more hydrophobic) bound conglutin γ more effectively than a high-binding one, usually suggested for binding of antibodies. The dependence of the binding extent from the pH of the buffer was investigated and found highest for pH>7. According to the dependence of conglutin γ ionisation and its aggregation status from pH, it can be concluded that the protein is best bound to the plate as a tetramer. The best concentration of primary antibodies was determined as 0.3 µl/ml.

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Tags: lupin, *Lupinus albus*, conglutin γ, ELISA.

(P46)

Dry heat exposure reduce anthracnose infection in Andean lupin seed

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State of art and aim: The lupin, *Lupinus mutabilis* Sweet, is a native Andean legume with high nutritional value. Anthracnose is considered to be the most devastating disease of lupin in Ecuador and around the world. Anthracnose is caused by the seed-borne pathogen *Colletotrichum acutatum* (1). In Ecuador small farmers select the commercial seed for sale, leaving poor quality seed for future sowing. In addition, they exchange lupin seeds within local networks increasing the risk of anthracnose dissemination. Fungicide seed treatments reduce transmission of anthracnose from seed, but it usually does not eradicate seed-borne infection. Exposure of infected seed to 60 °C for periods of up to 1 week reduce anthracnose infection in three lupin species, but the effects of heat treatment varied on seed germination across the lupin species (2). Dry heat treatment is necessary to study the reduction or eradication of seed infection in *L. mutabilis*.

Results and discussion:
The effect of dry heat (60±2.0 °C) was investigated by using exposure periods of 0, 1, 2, 4 and 8 hours in four lupin genotypes. The effect of dry heat was evaluated by the percentage of disease incidence and germination on the seed. Exposure of infected seed to 60 °C showed to reduce anthracnose infection in the four genotypes. Seed infection level steadily reduced as the exposure time increased from 1 to 8 h. In the four lupin genotypes infection was more than 50 % lower after 4 h exposure (Table 1). Weimer (3) reported that anthracnose seed infection in lupins was reduced by 80 % after hot air treatment for 7 h at 70 °C. The exposure time of 4 and 8 h resulted in lower seed germination and varied among genotypes (Table 1). Exposure to 60°C or greater temperature for 1 week significantly reduced seed germination (Thomas and Adcock 2004). Our results will facilitate transfer of good quality seed within Ecuador and the Andean countries.

Table 1. Effect of 1, 2, 4, or 8 hours exposure to 60°C on anthracnose infection and seed germination of four *Lupinus mutabilis* genotypes

Exposure	Seed infection (%)				Seed germination (%)			
Time	Cotopaxi	Chimborazo	ECU-2658	I-450	Cotopaxi	Chimborazo	ECU-2658	I-450
Original	7.3	7.5	7.7	7.5	89	87	75	75
1h	5.8	6.5	7.0	3.5	89	87	75	71
2h	4.8	4.8	4.8	3.0	88	87	69	71
4h	3.5	3.2	3.3	2.8	81	75	69	62
8h	3.0	2.5	3.0	1.2	73	75	62	62
LSD (P=0.005)*	1.72	2.11	2.15	2.34	7.23	5.42	5.69	5.84

*LSD for seed infection and seed germination of each genotype

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Acknowledgements:
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Tags: Anthracnose, dry heat, seed pathology

(P47)

Diversity of *Lupinus albus* L., *Lupinus angustifolius* L. and *Lupinus luteus* L.

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State of art and aim: The aim of this study was investigation of the biodiversity of three annual species of lupin: *Lupinus albus* L., *Lupinus angustifolius* L. and *Lupinus luteus* L. Many years of research (1973-2014) by means of expedition missions in many countries, by the study collection of the N. I. Vavilov Institute (2500 accessions) in various geographic regions, and by generalization of the data obtained by other researchers, have enabled as to find out new regularities in the variability of characters depending on genetic features of the species and accessions, and also on ecological and agronomic conditions of their growth (Kurlovich *et al.* 1990). Intraspecific taxonomic and eco-geographical classifications have identified as a result.

Results and discussion: Different subspecies, varieties, subvarieties and forms of lupins were classified on the basis of Vavilov's concepts: the law of homologous series in hereditary variation, studies on the problem of the spesies as a system, differential systematic and geographical method of plant studies (Vavilov, 1965). Each form of lupin can be assesed as an object fitting in with a number of consecutive taxa. We recognize subspecies (**subsp**.) as an isolated group of individual plants within a population of a species. They occupy certain part of the area of a species, constitute together a mobile system are able to cross among themselves and with plants growing in other parts of the area of this species, produce prolific progeny, possess distinctive morphological and inheritable characters in vegetative and generative organs with the uniform genetic base, and incorporate transient forms (e.g. subsp. *graecus*, *termis* and *albus* within the limits of *Lupinus albus* L.). Allelism and character complementarity tests have shown that the colour of seed is correlated with the colour of the corolla in lupins. This linkage reflects the stability of genetic system, which corresponds to the rank of varieties (**var**.). A good diagnostic character is the colour of vegetative parts, and the absence or presence of anthocyan in particular. Being less stable, it could be used in indentifying subvarietas (**subvar**.). Considerable practical interest for breeders may be generated by the plants with determinate branching, fascicular stem and other characters of breeding value. Such forms are theoretically possible in all varieties and subvarieties systematized by as. Therefore, it seems justified to regard them in the rank of forma (**f**.). We also arranged the accessions of different origin with ecologically and geographically influenced differences in biological, physiological biochemical and other properties into genotypes, eco-geographic group of ecotypes, separate ecotypes and concultivars (varietal types). This approach allowed us to develop a more detailed intraspecific taxonomic and eco-geographical classification and outlined efficient ways of lupins genetic resources utilization in breeding.

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Acknowledgements: I wish to express my gratitude to Prof. **M. Agaev** and Dr. **A. Stankevich** for participation in the development of taxonomic and eco-geographical classifications and to all my Colleges from many countries for their kind help in organizing the plant collecting missions.

Tags: biodiversity, classification, ecotype, genetic resources, varieties, Vavilov.

(P48)

Where, when, how many times and from what progenitors were lupins first domesticated?

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State of art and aims: The ancient origins of domestication of lupin crop species in the Mediterranean remain shrouded in mystery in terms of where, when, how many times and from what progenitors, lupin species were first domesticated. It is thought that *L. albus* was cultivated and likely domesticated in the Balkans, possibly in Greece, but the origin of domesticated *L. luteus* remains unknown, and it is unclear to what extent other large-seeded Old World lupin species might have also been cultivated in ancient times. We are investigating these questions using new DNA sequence data alongside new syntheses of the available archaeological and historical data.

Results and discussion: For the Old World species, historical and archaeological data suggest that lupins were used and cultivated in Greek and Roman times, but that it was undomesticated material (with shattering pods and bitter seeds) that was used. In terms of genetic data, we have generated a large RADseq data set including all but one species with multiple accessions and dense sampling of accessions from across the Mediterranean for the two putative domesticates *L. albus* and *L. luteus*. The resulting phylogeny is well-resolved and robustly supported and all species except *L. hispanicus*/*L. gredensis* form reciprocally monophyletic species clades. Within *L. albus* and *L. luteus*, there is no clearcut wild/domesticated transition on the phylogeny, but instead domesticated accessions are scattered geographically and intermingled phylogenetically, and in the case of *L. albus* a domesticated accession is sister to a clade containing the remaining accessions. There appear to be several possible explanations that individually or in combination could account for these results: (i) the true wild progenitor of *L. albus* was not sampled and remains to be discovered, i.e. that all the material sampled is either domesticated or feral derivatives (see below) of domesticated varieties; (ii) true domestication happened only relatively recently following a long period of cultivation with extensive diffusion of material across the Mediterranean complicating inferences of domestication; (iii) the intermingling of domesticated and putatively wild accessions could be the result of feralisation and even possible de-domestication, whereby domesticated forms spread and naturalized following cultivation, and potentially lost their domestication traits in the process. The long history of human disturbance across the Mediterranean and the prevalence of lupin populations in disturbed, ruderal populations mainly in agricultural landscapes is in line with these ideas. More extensive fieldwork and denser sampling will be needed to shed more informative light on the domestication history of the Mediterranean lupin crop species.

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Tags: domestication, RADseq, archaeology, *L. albus*, *L. luteus*

(P49)

Comparative genomics of lupin rhizobial symbionts

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Native bradyrhizobial symbionts of lupins thriving in the Iberian Peninsula were studied. The analysis included five species that preferred neutral or acid soils: *L. angustifolius, L. luteus, L. hispanicus, L. micranthus* and *L. cosentinii,* and the unique, recently described lupin species *L. mariae-josephae*, endemic of alkaline Chromic Luvisol soils ("terra rossa") in Eastern Spain. Draft genome sequences of at least one strain from each lupin species were obtained by Illumina (PE, 2x300 bp, MySeq v. 3.0) sequencing, assembled with SPAdes, and annotated with the RAST server. Pairwise Average Nucleotide Identities (ANI) between genomes were calculated using JSpecies. The results showed a clear separation between strains nodulating *L. mariae-josephae* and those that nodulate lupins from acid soils. These results are in agreement with the observed host specificity, *i. e.* bradyrhizobia from *L. mariae-josephae* don't nodulate most of the lupins from acid soils and any bradyrhizobia from acid soil lupins do nodulate, although inefficiently, *L. mariae-josephae*. In contrast, acid soil lupins are efficiently nodulated by bradyrhizobia isolated from any of these lupins. Consistent with this, comparison of symbiotic regions (*nod*, *fix*, *nif* genes), showed maximum differences between *L. mariae-josephae* strains and those from other lupins. Another genomic features were also compared and results will be discussed.

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(P50)

Intestinal epithelial Caco-2 cells responses to lupin seed protein fractions

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State of the art and aim: In the recent years biological active proteins have been found in lupin seeds, such as bowman-birk inhibitor and *gamma*-conglutin, which showed anticarcinogenic and hypo-glycemic activity respectively (Duranti *et al*, 2008). Beside, a certain number of bioactive proteins and peptides have been described in soybean and other legumes. However, little is known about the responses at immunological level after the interactions of seed proteins with intestinal mucosae. The relationship between the consumption of certain foods and the reduction or the establishment of immunological diseases has been demonstrated. For example, from bovine milk can originate a number of casein hydrolysates with positive immunomodulatory properties (Sandré *et al*, 2001). Therefore, the aim of this study was to investigate the immune response of intestinal epithelial Caco-2 cells to different lupin seed protein fractions and assess possible other cellular effects.

Results and discussion: Three protein fractions, namely *alpha*, *beta* and *gamma* -conglutin, were purified from lupin seeds through a well-established procedure (Magni *et al*, 2007). Stable transfected Caco-2 cell monolayers were incubated with the protein fractions (0,5 mg/mL) and interleukin 1b (2 ng/mL) for 4 hours. This cell line, containing the plasmid pNiFty2-Luc, expresses the nuclear factor (NF)-kB-binding sites on membrane, whose activation by pro-inflammatory molecules can be monitored measuring light emission. The results showed that *gamma*-conglutin, by interacting with NF-kB receptor, activated significantly the inflammatory response of the transfected Caco-2 cells. A lower response was measured with *alpha* and *beta* -conglutins. The effects of *gamma*-conglutin on cell protein expression were also investigated in hepatic cells, a possible target of *gamma*-conglutin. HepG2 cells were incubated for 4 hours with or without the integer and the pepsin/pancreatin hydrolyzed *gamma*-conglutin. The 2D electrophoretic maps of cell protein extracts did not show differences in the protein patterns. These preliminary results show that only *gamma*-conglutin negatively modulated the immune response of transfected Caco-2 cells after 4 hours of incubation but this protein did not induced major changes in the protein pattern of HepG2 cells as monitored in the 2D maps. Further researches will be necessary to investigate the cellular and immune responses of cell models to *gamma*-conglutin.

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Tags: intestinal Caco-2 cells, immune-modulation, lupin proteins

(P51)

Technical basis for wild lupin management in quinoa producing areas in Southern Highlands of Bolivia

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State of art and aim: In recent years in the Highlands of Bolivia, quinoa has become very important because of its economic value achieved through the export market. This situation has led to a large increase of cultivated area, from 10000 to 100000 hectares in 10 years, expanding production acreage at the expense of grazing areas and generating serious problems of soil erosion and decay of soil fertility. The Bolivian Altiplano is a very fragile area, located at 4000 m altitude with 250 mm of rainfall and less than 1% of organic matter. In this scenario, the only commercial crop is quinoa, but, its increased cropping area together with changing climate are generating difficulties to quinoa sustainable production. In this context, an alternative that deserves to be studied is to identify the wild plant species of the area, to take advance of them in the quinoa production systems. In this regard, wild lupins represent an excellent alternative. Currently, lupins are not fully exploited due to a number of biological and technological aspects inherent to the species (seed dormancy). Therefore, it has been proposed to assess the adaptation of wild lupin species in the Bolivian highlands, study their reproductive aspects and adapt technological components oriented to their multiplication and massive repopulation.

Results and discussion: For the Andes, there are more than 80 species within the *Lupinus* genus, being the tarwi *Lupinus mutabilis* the only domesticated species. The wild species found in Southern Altiplano are *Lupinus subacaulis, L. antomontanus, L. otto-buchtienii* and *Lupinus* sp. The interesting aspects of the wild lupins are its ecological adaptation and production of abundant organic matter in an arid environment (8 tons of dry matter per hectare), in addition to its good N2 fixation capacity. The seed germinates in December and January (Summer), develops large tap root in Autumn, passes vegetative growth phase in Winter (cold and dry), enters reproductive phase in Spring and sets seed in Summer. The above shows that these species remain green and keep growing during the frigid winter when there is no crop in the field. This particular adaptation is important for quinoa production system; it could be included in crop rotation with quinoa, improved fallow and cover crop during dry seasons with high speed winds. Seed treatments were performed to break achieving 85% of germination, and semi-mechanized planting methods have been adapted. The pilot plots planted with wild lupin showed encouraging results and it constitutes a practical option to make the quinoa production system of the Southern Altiplano sustainable.

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Acknowledgments: The McKnight Foundation, USA, and PROINPA Foundation, Bolivia

Tags: wild lupin, organic matter, cover crop.

(P52)

Allergy to lupin proteins: a review of clinical cases

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State of art and aim: Lupin (*Lupinus* spp.) is a member of the *Fabaceae* family, which includes the well-known allergen peanut (*Arachis hypogaea*). Used to add protein and fibre and to improve texture, lupin is also an useful alternative to soybean when non-genetically modified food ingredients are sought, for example, in baby foods. In Italy, lupin flour and protein concentrates are being increasingly used in bakery and pasta formulations, in particular in gluten-free products, where lupin derivatives produce better elasticity, texture, and flavour than soybean derivatives. The first described case of allergy to lupin involved a girl, who experienced urticaria and angio-oedema after eating a pasta fortified with sweet lupin flour. Since then, the number of reports on allergic reactions to lupin in the form of flour, seed or dust are rising in number. The aim of this study was to review the cases of lupin allergy described in the scientific literature, analyzing the symptomatology reported by patients and the major allergens identified as responsible for the allergic reactions.

Results and discussion: The search of the scientific literature in PubMed/MEDLINE revealed about 200 cases of lupin allergy. The broad symptoms included asthma/shortness of breath, oral allergy syndrome, generalized urticaria, allergic rhinitis, angioedema, gastro-intestinal pain and anaphylactic shock. The cases of allergy were recorded mainly in France, Italy, the Netherlands, Spain and Australia. Allergic reactions involved both children and adults; in a significant percentage of cases, a pre-existing allergy to peanut was documented. Data from the French Allergy Vigilance Network revealed that the relative frequency of asymptomatic sensitization to lupin in patients of all ages with atopic disease indicates a possible increase of primary allergy to lupine in the next future. Alpha-conglutin and beta-conglutin were recognized as the major lupin allergens; in addition, beta-conglutin is also involved in the cross-reactivity with peanut proteins.
Literature data showed that there are different routes of lupin sensitization. Sensitization *via* inhalation of lupin seed flours due to occupational exposure is well known, while evidences for sensitization by pollen inhalation are limited. Some authors investigated also the possibility of transcutaneous and intaruterine sensitizations but, at present, no scientific evidences have been reported.

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Tags: lupin, allergy, symptoms, allergens, sensitization.

(P53)

Inclusion of narrow-leafed lupin (*Lupinus angustifolius*) meal in poultry diets to evaluate productive performance and meat quality.

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State of art and aim: The aim of this work was to evaluate the performance (feed intake, weight and feed conversion) and meat quality (carcass yield, abdominal fat and meat color) of broilers fed with diets containing 0%, 5% and 10% of Haags Blaue cultivar (*Lupinus angustifolius).* One day old chickens were arranged in three groups of 100 animals each with five replicates, under a complete randomized design experiment. The diets were isoproteic and isoenergetic and were labeled as follow, control (C) a soybean basal diet, 5% *L. angustifolius* diet (E1), and 10% *L. angustifolius* diet (E2). At the end of the productive performance, the E1 and E2 groups had a higher feed consumption ($P < 0.05$) than C group. The weight and feed conversion values were similar among C, E1 and E2 groups ($P > 0.05$). On the other hand broilers carcass yield was also similar among groups ($P > 0.05$).In addition E2 broilers showed a lower abdominal fat compared with E1 and C groups, while E1 and E2 groups resulted with a higher meat pH values ($P < 0.05$). At the end the meat color test showed that broiler C group had differences with the other groups ($P < 0.05$) but all groups showed results that indicate dark firm and dry meat. The inclusion of 5 and 10% of *L. angustifolius* meal doesn't affect weight and feed conversion and also can improve chicken meat quality.

Results and discussion: At the end of the experiment feed intake values were of 5703, 5560 and 5332g for the E2, E1 and C groups, respectively. In regards to weight and feed conversion were similar in all groups with 2771g, 2.00; 2800g, 1.98 and 2776g, 1.94, respectively. These results are in agreement with those obtained by Ravindran *et al.,* (2002) who fed chickens with *L. angustifolius.* Carcass yield in all groups was similar and ranged between 82-83%, however the abdominal percentage fat showed differences among groups (C=1.1%, E1= 1% and E2= 0.5%). This feature can be related to the fiber content in *L. angustifolius* meal, similar results were reported by Khempaka *et al.,* (2009) who employed dried cassava pulp with high content of fiber. The meat pH values were 6.41, 6.49 and 6.47 for C, E1 and E2, respectively; while the color test showed more luminosity for the meat of broilers in group C compared with E1 and E2 groups. Furthermore, it must be said that values for dark firm and dry meat in all groups ended with similar that values were reported by Fanatico *et al.,* (2007), it could have happened because of a fatigue of the chickens before slaughter.

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Aknowledgements
The complete experiment was funded by the Departamento de Producción Animal.

Tags: lupin, poultry, meat quality

Workshop 1:

Is lupin a real and valuable source of nutrients and ingredients for the food industry?

(co-organizers and moderators: Peter Eisner and Udo Prins)

Contributions:

1. Beany and bitter - how to avoid sensorial hurdles in consumer acceptance (Peter Eisner, Fraunhofer Institute, D)

2. Lupin: from traditional Mediterranean food to trendy and innovative 'super-food' (Udo Prins, Louis Bolk Institute, NL)

3. Lupin as an alternative to meat and soybean (Alexander Bauer and Karl Selg-Mann, Purvegan,D)

4. Heinz: a long experience in lupin flour for gluten free products (Nicola Ferrari and Alice Vailati, Heinz Group)

5. Nutraceutics and Production: the case of MOPUR (Renny Elia, Mopur, I)

6. Promotion of *tarwi* product from the Province of Huaylas (Perù) to national and international markets in the context of sustainable rural development" (Idoia Ortiz, Fondazione L'albero della vita, NGO)

Workshop 2:

Lupin Genomic Workshop

(co-organizers and moderators: A. Aïnouche and M. Nelson)

Contributions:

1. Lessons from the NLL genomic project" and "the NLL genetic /genomic website (Karam Singh / Lars Kamphuis, CSIRO, AUS)

2. Natural selection during adaptive radiation in New World lupins (Dmitry Filatov, Oxford, UK)

3. Discovering domestication syndrome genes in lupin (Matthew Nelson, Univ. of Western Australia)

4. Genotyping by sequencing of white and narrow-leafed lupins (Rychel S IPG Poznan, PL)

5. The evolutionary dynamics of the repetitive compartment in the lupine genomes (A. Aïnouche, Univ. Rennes, F)

6. Comparative genomics of lupine rhizobial symbionts (Luis Rey and Tomas Ruiz, Univ. Polytech. Agric., Madrid, E).

Workshop contributions

Lessons learned from the Narrow-leafed Lupin genome sequencing project

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With the narrow-leafed lupin genome sequencing project nearing completion, we will present an overview of the critical steps in generating a high-quality lupin genome assembly. Our team consisted of a strong bioinformatics component, which is essential to all genome sequencing projects, and molecular biology/genetics members to assist in the generation of quality DNA and RNA and to follow up biological questions derived from the generated sequence information. The team has a strong knowledge base of the species we sequenced, which proved invaluable in some of the decision making during the project and is linked in with strong international partners involved in other legume genome sequencing projects. We will outline the steps used to sequence, assemble and annotate the genome, address some key hurdles we had to overcome, and if we were to initiate a project for another lupin species what we would recommend doing differently.

Once a genome sequence has been generated, it will also need to be made available to a wide range of scientist and interest groups, in a user friendly and informative manner. An outline of how we have tried to achieve this by making our datasets freely available on the Lupin Genome Portal (http:www.lupinexpress.org) will be presented.

Molecular adaptation during a rapid adaptive radiation in andean lupins

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Andean lupins include over 80 closely related species that formed in a surprisingly short evolutionary time (<2 million years). Such 'explosive' adaptive radiations of species remain one of the most puzzling evolutionary phenomena and the evolutionary genetic processes behind such radiations remain unclear. Rapid morphological and ecological evolution during 'explosive' adaptive radiations suggests that many genes may be under fairly strong selection, although this remains untested. We tested this hypothesis by comparing synonymous (d*S*) and non-synonymous (d*N*) substitution rates in rapid radiation in the Andes and less species rich plant groups that did not undergo 'explosive' adaptive radiations. Under neutrality d*N* is expected to be equal to d*S* (i.e. d*N*/d*S* = 1), while purifying and adaptive selection reduce or inflate d*N*, respectively (i.e. under strong adaptive selection d*N*/d*S* > 1). To estimate substitution rates in thousands of genes we conducted high-throughput transcriptome sequencing in multiple species of Andean and non-Andean lupins. The resulting transcriptome sequences were assembled into multispecies alignments and the sequence differences between the species were used for phylogenetic analyses. This allowed us i) for the first time to reconstruct fully-resolved phylogeny of the Andean lupins and ii) measure the d*N* and d*S* substitution rates in over 2 thousand genes. Positive selection was detected in 143 (6.4%) and 52 (2.4%) genes in the Andean and non-Andean lupins respectively. Re-analysis of publicly available transcriptome data from other plant genera shows that the proportion of genes under selection in non-Andean lupins is high but comparable to other non-lupin genera. Conversely, Andean lupins exhibit 2-3 times more genes under selection than any other group analysed. These results support the conjecture that rapid adaptation during 'explosive' adaptive radiations is accompanied by fairly strong adaptive selection in many protein coding genes.

Discovering domestication syndrome genes in lupin

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Plant domestication involved the accumulation of domestication traits that made the plants increasingly more useful and productive to people. The founding father of the discipline, Nikolai Vavilov, described this as the 'homologous series in inherited variation' and which is now known as the 'domestication syndrome' (Hammer 1984). Narrow-leafed lupin followed this same pattern of fixation of domestication syndrome alleles: altered time of flowering and maturity (*Ku*), reduced fruit dehiscence (*lentus* and *tardus*), removal of seed dormancy (*mollis*) and reduced seed alkaloid content (*iucundus*). In Australia, the *leucospermus* allele (affecting plant-wide pigmentation, most notably white flower colour) is used as a visual marker to distinguish domesticated varieties from wild types and can therefore also be considered a domestication trait. Until recently, the identity of the genes underlying domestication traits remained obscure except for a few notable examples in major crop species such as maize. But now, with the advent of low-cost genome sequencing technologies and accumulation of plant genetic resources, we have the necessary tools to identify domestication syndrome genes in lupin species. In this presentation, I will provide an overview of progress towards identification of lupin domestication genes with a focus on narrow-leafed lupin. I will also discuss how this information can help alleviate the problem of low genetic diversity in domesticated lupin.

Reference:

Hammer K. 1984. Das Domestikation syndrom. Die Kulturpflanze 32(1): 11-34.

Genotyping by sequencing of white and narrow-leafed lupins

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State-of-the-art and aim:
Over the last years significant progress in generation of publicly available sequence resources has occurred, including three crop lupin species: white (*Lupinus albus*), yellow (*L. luteus*), and the narrow-leafed (*L. angustifolius*) (Książkiewicz et al. 2015). Flagship achievements encompass: transcriptome assemblies and gene indices (all three species), linkage maps (white and narrow-leafed lupin), and a draft genome assembly (narrow-leafed lupin). These resources, together with DNA-based molecular markers, can facilitate the gene-based prediction of phenotype. Marker assisted selection (MAS) has recently become an integral part of plant breeding programs and has accelerated new cultivar development. However, the most of the marker systems provided for lupins are ineffective due to high cost of scoring and low level linkage with particular traits. Advances in sequencing technologies have driven down sequencing costs and launched increasing interest in genotyping by sequencing (GBS), which generates ready to use markers based on sequence polymorphism.

Results and discussion: The aim of our ongoing research is to generate a technology of monitoring the narrow-leafed and white lupin lines carrying particular QTLs (quantitative trait loci) conferring agricultural traits. It will enable allele-directed selection in breeding programs. This target will be reached by determination of relationships between variability of white and narrow-leafed lupin phenotype quantitative traits, genetic polymorphisms of markers linked to these traits, genetic information contained in genome regions carrying these loci, and transcriptional activity of genes encoded by these sequences. The narrow-leafed lupin accessions from the world germplasm collection, together with recombinant inbred lines (RILs) from two mapping populations, 112 of narrow-leafed lupin and 193 of white one, are currently being profiled by GBS. Polymorphisms of obtained markers will be correlated with agricultural traits by association mapping. Phenotype observations include plant morphology and physiology, content of alkaloid and other non-nutrient and nutrient components, and resistance to biotic and abiotic stresses. Consensus, high density linkage maps containing both newly developed GBS and existing gene-based PCR and AFLP markers (Phan et al. 2007 and Vipin et al. 2013) will be constructed to support the identification of genomic regions in the marker-trait association study. Obtained linkage maps, composed mainly of sequence-based markers, will be also used for synteny survey in comparative mapping approach to sequence legume genomes (*Arachis duranensis*, *Arachis ipaensis*, *Cajanus cajan*, *Cicer arietinum*, *Glycine max*, *Lotus japonicus*, *Medicago truncatula*, *Phaseolus vulgaris*, and *Vigna radiata*).

References:
Phan *et al.* (2007) *DNA Research*, 14, 59-70
Vipin *et al.* (2013) *Breeding Science*, 63, 292-300
Książkiewicz *et al.* (2015) *Plant Mol Biol Rep*, 33, 84-101

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Tags: GBS, white lupin, narrow-leafed lupin, molecular markers, next-generation sequencing

The evolutionary dynamics of the repetitive compartment in the lupin genomes

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The evolutionary dynamics of the repetitive compartment is investigated in the legume genus *Lupinus* to identify the repeats that are involved in the noteworthy genome size variation (970 to 2400 Mb) which accompanied diversification of this plant system. For this purpose, particular attention is focused on comparative genomic analyses of closely related lupine species, which have remarkable genome size differences, and are adapted to contrasted ecological conditions in the Mediterranean and North African regions. Based on Illumina HiSeq sequence data, an accurate evaluation of the different classes and families of repeats was performed on five lupines species with small and large genomes, using appropriate bioinformatics pipeline dedicated to repeats detection and annotation in non-model organisms. The results revealed: (1) that the repetitive compartment may represent 14 to 60 % of the lupin genomes; (2) that different patterns of repeat dynamics have been involved in genome size variation, with lineage-specific amplifications of different classes of repeats in Mediterranean and African species. The results indicate that, rather than retrotransposons, the SSRs played an important role in genomes during the recent (< 2 MY) radiation / fragmentation of Old World lupins in North Equatorial Africa, which suggests that different mechanisms were involved in the genome evolutionary dynamics along with the diversification and adaptation of lupines in various Mediterranean and African ecogeographical conditions.

Comparative genomics of lupin rhizobial symbionts

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Native bradyrhizobial symbionts of lupins thriving in the Iberian Peninsula were studied. The analysis included five species that preferred neutral or acid soils: *L. angustifolius, L. luteus, L. hispanicus, L. micranthus* and *L. cosentinii,* and the unique, recently described lupin species *L. mariae-josephae*, endemic of alkaline Chromic Luvisol soils ("terra rossa") in Eastern Spain. Draft genome sequences of at least one strain from each lupin species were obtained by Illumina (PE, 2x300 bp, MySeq v. 3.0) sequencing, assembled with SPAdes, and annotated with the RAST server. Pairwise Average Nucleotide Identities (ANI) between genomes were calculated using JSpecies. The results showed a clear separation between strains nodulating *L. mariae-josephae* and those that nodulate lupins from acid soils. These results are in agreement with the observed host specificity, *i. e.* bradyrhizobia from *L. mariae-josephae* don't nodulate most of the lupins from acid soils and any bradyrhizobia from acid soil lupins do nodulate, although inefficiently, *L. mariae-josephae*. In contrast, acid soil lupins are efficiently nodulated by bradyrhizobia isolated from any of these lupins. Consistent with this, comparison of symbiotic regions (*nod*, *fix*, *nif* genes), showed maximum differences between *L. mariae-josephae* strains and those from other lupins. Another genomic features were also compared and results will be discussed.

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