# LEGUME PERSPECTIVES

## Where the global pulse beats mightiest

### Echoes of VI IFLRC + VII ICLGG in Saskatoon

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**Front cover art:**
*EELG: Earth ElectroLegumoGram*
by Aleksandar Mikić

## EDITORIAL

*Welcome to this issue of* Legume Perspectives! *The purpose of this issue is to provide a sampling of the papers presented at the International Food Legume Research Conference VI and the International Conference of Legume Genetics and Genomics VII (IFLRC-ICLGG) which were jointly held in Saskatoon, Saskatchewan, Canada July 7-11, 2014. On behalf of the Local Organizing Committee, it was a pleasure to host approximately 400 friends who arrived from many countries. In addition to the Local Organizing Committee, this event was conducted under the leadership of the International Steering Committee of IFLRC and the International Advisory Board of ICLGG, and supported by generous sponsorship from more than a dozen organizations.*

***Tom Warkentin***
*Managing Editor of*
Legume Perspectives *Issue 7*

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Carte blanche to…

…Tom Warkentin

# A meeting with pulse beating

*The IFLRC-ICLGG conference in Saskatoon was a forum for discussion of a wide array of topics of relevance to legume research internationally. Key theme areas included fundamental and applied genetics and genomics, seeds and nutrition, nitrogen fixation, plant nutrition and legume mega projects, biotic stress and plant microbe interactions, and abiotic stress and crop management. Excellent keynote presentations were provided each morning in plenary sessions followed by concurrent sessions which focused on the key themes of ICLGG and IFLRC. It was apparent to me that good progress is being made, through strong collaborations, in each of these areas, despite the fact that the legume research community is not large internationally. Many opportunities exist for legumes to contribute to humanity in terms of crop diversification, environmental stewardship, and healthy diets. Governments and industry need to be reminded of their opportunities for good investments in legume research and development.*

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# Exploring nodulation through genome resequencing and association genetics in *Medicago*

by Nevin D. YOUNG[1,2]*, Shaun CURTIN[1], Peng ZHOU[1], Diana TRUJILLO[2], Joseph GUHLIN[2] and Kevin SILVERSTEIN[3]

**Abstract:** Genome resequencing enables the discovery of candidate loci for traits of biological importance and also provides insights in to the genomic architecture of complex traits. The *Medicago* Hapmap Consortium is resequencing the genome of *Medicago truncatula* to explore the genomics of nodulation. Previously, we resequenced 320 diverse accessions of *M. truncatula* and related taxa to discover more than 6,000,000 single nucleotide polymorphisms (SNPs). Subsequent genome-wide association studies (GWAS) of nodulation uncovered previously reported genes plus several novel candidates. Functional genomics experiments are now underway to validate these novel candidates. Separately, a subset of lines from the GWAS panel is being deeply sequenced and assembled independent from the published A17 reference. This approach is essential for the discovery of DNA elements not found in the reference as well as for high confidence prediction of structural variants (SVs). These independent assemblies also lay a foundation for creating a *Medicago* "pan-genome".

**Key words:** copy number variation, GWAS, next generation sequencing, nodule-related cysteine-rich peptides, single nucleotide polymorphisms

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## Functional validation of GWAS nodulation candidates

Earlier genome-wide association studies (GWAS) analysis identified several candidate genes associated with nodulation in *Medicago truncatula* Gaertn. (4). These candidates include genes previously connected with nodulation (e.g., *CaML3, NFP, SERK2*) plus several novel candidates involved in DNA repair, ubiquination, molecular chaperones plus other nodule-upregulated loci of unknown function. To validate these associations, we have generated mutants for most candidates using a combination of tools: *Tnt1* retrotransposon (5), stable transgene hairpin knock-down, and site-directed mutagenesis with engineered nucleases (1). Of these candidates, we have characterized five mutant lines and performed preliminary nodulation phenotype analysis of mutants, showing statistically significant perturbations in nodulation in four of the candidates.

## *De novo* resequencing of *Medicago* accessions

To learn more about genome variation in *Medicago*, we sequenced and assembled 19 accessions around three nodal hubs, including A17 and R108. For all accessions, this process achieved ~90X coverage each, using a combination of short and long insert libraries, for use in Illumina next generation sequencing. This is sufficient for high-quality assemblies using the ALLPATHS-LG algorithm (2). All 19 assemblies have scaffold N50 values > 380 kbp, with some as long as 2.2 Mbp, providing an excellent set of resources for exploring *Medicago* genome structural variation, complex gene families, and pan genome.

## Map-based SNP densities are too low by a factor of two or more

One of the most interesting observations from the resequencing work has been that many SNPs in divergent or highly duplicated regions are missed if based on alignment against a reference rather than direct comparison of *de novo* assembled accessions. Difficulties aligning reads to divergent and/or repetitive regions makes the interrogation of these areas for SNPs and other variants difficult or impossible using reference-based methods alone. *De novo* assembly-based methods overcome these difficulties by anchoring syntenic diverged or duplicated regions with flanking, highly-conserved single-copy regions.

## Important gene families mediating plant-microbe interactions can be analyzed using *de novo* assembly-based approaches

The NBS-LRR (nucleotide-binding site, leucine-rich repeat) (7) and CRPs (cysteine-rich peptides) (3) gene families are important in defense response and nodule formation. Both are large gene families forming tandemly-duplicated clusters in labile genome regions. Due to highly-related gene family members and the clustered nature of these regions, alignment-based methods often fail to accurately assay these regions. High rates of structural rearrangement result in NBS-LRR gene structure changes that include gene truncation, domain swapping and gene fusion. By contrast, the smaller CRPs tend to evolve through expansion and contraction of gene family members more

often than through gene structural changes. Gene copy numbers within some CRP families are radically different among accessions, including some *Medicago*-specific subgroups. To validate these observations of CRP expansion within the *de novo* assemblies, we have supplemented Illumina-based assemblies with increasing numbers of long-read PacBio sequences.

## Genome architecture of the LEED..PEED (LP) gene family

The LP family is composed of 13 genes encoding small putatively secreted peptides with one to two conserved domains of negatively charged residues (6). This family is not present in the genomes of *Glycine max,* (L.) Merr. *Lotus japonicus* (Regel) K. Larsen, or the IRLC species, *Cicer arietinum* L. LP genes were also not detected in a *Trifolium pratense* L. draft genome or in the *Pisum sativum* L. nodule transcriptome, suggesting that the LP gene family arose within the past 25 million years. *Medicago* accession R108 and *M. sativa* L. have 11 and 10 LP gene copies, respectively. In A17, 12 LP genes are located on chromosome 7 within a 93-kb window. A phylogenetic analysis of the gene family is consistent with most gene duplications occurring prior to *Medicago* speciation events, mainly through local tandem duplications and one distant duplication across chromosomes. Synteny comparisons between R108 and A17 confirm that gene order is conserved between the two subspecies, although a further duplication occurred solely in A17. The recent expansion of LP genes in *Medicago* spp. and their timing and location of expression suggest a novel function in nodulation, possibly as an aftermath of the evolution of bacteroid terminal differentiation or potentially associated with rhizobial–host specificity.

## GWAS based on structural variant analysis

It has also been possible to use SVs such as copy number variants (CNVs) and presence-absence variants (PAVs) as a basis for GWAS analyses. Here, an association analysis conducted with TASSEL using a combined set of SNPs and SVs led to the identification of a CNV within a nodule-related cysteine-rich (NCR) peptide strongly associated with a reduction of total nodule count. This NCR deletion was validated by comparison to *de novo* assemblies. The observed CNV events had not been tagged previously by SNP calls and exhibited low Linkage Disequilibrium (LD) with nearby SNPs. These results suggest that SVs involving NCRs may play a role in nodulation variation that has not been fully characterized by SNP-only methods and hints that other SVs may also play an important role in this phenotype.

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# Chickpea translational genomics in the 'whole genome' era

by Manish ROORKIWAL[1], Mahendar THUDI[1], Pooran GAUR[1], Hari D. UPADHYAYA[1], Narendra P. SINGH[2] and Rajeev K. VARSHNEY[1,3]*

**Abstract:** Chickpea (*Cicer arietinum*) plays vital role in ensuring the nutritional food security in Asian and sub-Saharan African regions of the world. Conventional breeding efforts to elevate the yield levels and enhance crop productivity are constrained due to low level of genetic diversity present in the cultivated gene pools. Large scale genomic resources in chickpea have enabled the use of molecular breeding to develop superior chickpea varieties. In addition, efforts with an objective to exploit the available huge genetic diversity in genebank to address the issue of low productivity, ICRISAT has initiated large scale genome re-sequencing projects.

**Key words:** chickpea, genome re-sequencing, molecular breeding

## Introduction

Chickpea (*Cicer arietinum* L.), the second largest cultivated grain food legume in the world, is highly nutritious and protein rich source which contributes to income generation and improved livelihood of small-holder farmers in sub-Saharan Africa and Asia. During 2012-2013, the area, production and productivity of chickpea were 13.5 million ha, 13.1 million tones and 967 kg ha$^{-1}$, respectively (1).

Despite the efforts to increase the chickpea productivity, several abiotic (drought, heat, salinity) and biotic (fusarium wilt (FW), ascochyta blight (AB), botrytis grey mould, dry root rot, pod borer) stresses coupled with recent changes in climate have hindered the yield improvement (6). In order to fill the yield gap, there is a need to enhance precision and efficiency of selections in the segregating generations for higher and rapid genetic gains and to meet the current food and nutritional requirements.

Recent advances in genomics, especially in the area of next generation sequencing (NGS) and genotyping technologies, have reduced the cost of sequencing drastically enabling large scale genome re-sequencing to understand the genetic architecture. As a part of several global initiatives and strategic collaborations with NARS partners, large scale genomic resources including molecular markers, comprehensive genetic maps including physical map, trait mapping, and transcriptomic resources have been developed (12). In the case of chickpea, large scale molecular markers including simple sequence repeats (SSRs), hybridization-based Diversity Array Technology (DArT) and sequence based markers such as single nucleotide polymorphisms (SNPs) have become available. Use of a particular marker system for genetics research and breeding application depends on the throughput and cost of the marker assays. In order to use these markers, cost effective genotyping platforms including KASPar (2) and BeadXpress (4) system were developed. These large scale genomic resources have enabled the development of superior chickpea varieties that can sustain the yield when exposed to stress environments.

## Molecular breeding product

Advances in chickpea genomics research have made it possible to utilize genomics for enhancing the precision and efficiency. In order to use markers associated with trait of interest indentified using linkage mapping and genome-wide association studies, marker assisted backcrossing (MABC) was used to introgress the QTL/genomic region in the elite chickpea cultivars JG11. MABC has been successfully used to introgress the "*QTL-hotspot*" that harbors QTLs for drought tolerance-related traits. Introgression lines has shown improved performance with increased yield as compare to recurrent parent in rainfed as well as irrigated conditions (9). Similarly, two parallel MABC programmes were undertaken at ICRISATfor introgression of FW and AB resistance by targeting *foc1* locus and two quantitative trait loci (QTL) regions, ABQTL-I and ABQTL-II in C 214, an elite cultivar of chickpea. Screening of introgression lines for diseases identified FW and AB resistant lines (10). Efforts to pyramid the FW and AB resistance are underway. Recently, advancement in next-generation sequencing (NGS) technologies (8) have enabled the use of genome-wide marker profile/allele data for prediction of phenotype of progenies for selection to the new cycle in breeding programs using genomic selection (GS), a modern breeding approach. Efforts to deploy GS in chickpea have been initiated using training population of elite breeding lines (5).

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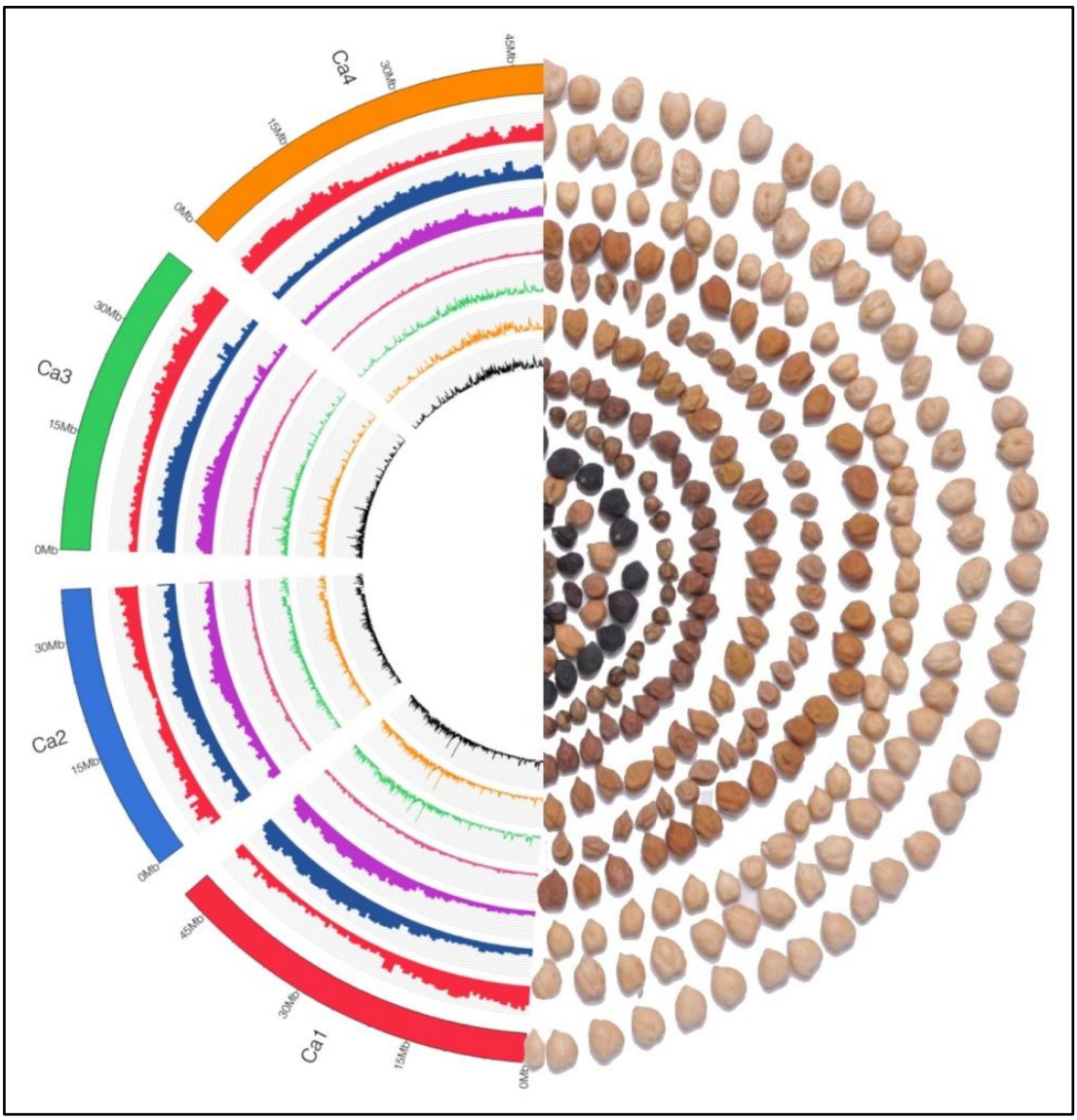


**Figure 1. An effort to show linking genome sequence diversity with trait phenotype diversity through "The 3000 chickpea genome sequencing initiative"**

## Connecting phenotype to gene(s)

The genome sequence provides the basis for a wide range of studies, from the important goal of accelerated breeding to identifying the molecular basis of key agronomic traits, in addition to understanding the basic legume biology. International Chickpea Genome Sequencing Consortium (ICGSC) completed high-quality draft genome sequence of chickpea (11). In parallel, genome sequence also became available for *desi* type (3). Draft genome alone, however, cannot address the issue of genetic diversity, therefore efforts to re-sequence the 300 chickpea lines from chickpea reference set were sequenced at 5X to 13X coverage (Fig 1). Alignment of re-sequence data on the reference genome has identified > 4 million SNPs that are being used for GWAS along with multi-season phenotyping data.

Large scale germplasm resources are available in different genebanks that can be used to explore the available genetic diversity to address the issue of low productivity and bottlenecks' associated with narrowgenetic diversity. In order to utilize these hugegermplasm collection, ICRISAT has initiated efforts to valorize the global composite collection of chickpea comprising 3000 lines selected from genebanks of ICRISAT and ICARDA (7) for identification of novel alleles. In view of above, ICRISAT launched "The 3000 Chickpea Genome Sequencing Initiative" in 2014. So far ICRISAT has re-sequenced more than 500 chickpea lines (reference set, elite varieties and parents of several mapping populations) at minimum 5X coverage.

## Summary

It is evident that recent advances in genomics, especially in the area of sequencing and genotyping technologies, have revolutionized chickpea genomics in the past decade. Few years back, chickpea used to be known as orphan crop as very limited genomic resources were available. As a part of several initiatives and strategic collaborations with several partners from different countries, large-scale genomic resources including draft genome sequence, comprehensive transcriptome assembly, high density genetic and BIN maps, QTL maps as well as physical maps have been developed. During the past decade chickpea has been transformed from genomic resources poor crop to genomic resources rich crop. These large scale genomic resources have opened the era of translational genomics in chickpea to understand the genetics of traits and as a result, approaches like MABC, and GS are being used in these crops (12).Improved lines have been developed for drought tolerance and resistance to FW and AB. Considering the revolution in chickpea genomics, it is anticipated that coming years will witness more integration of molecular breeding tools and approaches in chickpea breeding programs.

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# QTL to candidate genes: Understanding photoperiod sensitivity and flowering time in chickpea

by Amit DEOKAR, Ketema DABA and Bunyamin TAR'AN*

**Abstract:** Photoperiod insensitivity is one of the important traits for adaptation of chickpea (*Cicer arietinum* L.) to a short growing season, particularly in Western Canada where growing period is often restricted by end of season frost. Identifying QTLs/genes that regulate photoperiod insensitivity and flowering time will help to understand the genetic mechanism of photoperiod sensitivity in chickpea. Comparative analyses of flowering genes have shown that the most of flowering genes are well conserved in Arabidopsis and legumes. Based on sequence homology, we identified 130 chickpea orthologs of Arabidopsis flowering time genes. Further, combined analysis of flowering time QTLs and candidate gene mapping, we identified two chickpea gene *Ca-GI* and *Ca-ELF3* associated with days to flower and photoperiod sensitivity in chickpea. SNP markers based on the *Ca-GI* and *Ca-ELF3* candidate genes will enable efficient marker-assisted selection (MAS) of chickpea cultivars with early flowering and photoperiod insensitivity traits for better adaptation of chickpeas in short growing season areas.

**Key words:** candidate genes, chickpea, *Cicer arietinum*, photoperiod sensitivity, QTLs

Chickpea (*Cicer arietinum* L.) is one of the most important food legume crops grown over 50 countries covering around 13.5 million ha with the annual production of 13.1 million t (4). Chickpea is a quantitative long-day plant, but flowers in every photoperiod (9). This photoperiodic adaptation of chickpea has been an important factor in the wide spread of its cultivation to the Indian subcontinent, sub-tropical and tropical regions of Africa, North America and Oceania (1). Allelic variation for major adaptations traits, including photoperiod sensitivity has been identified in chickpea. Four different early flowering genes *efl-1* (identified from ICCV2), *ppd-1* or *efl-2* (ICC 5010), *efl-3* (BGD-132) and *elf-4* (ICC 16641 and ICC 16644) have been identified in chickpea (5). However, the gene sequences underline the loci has not yet been identified. In the present study, we identified quantitative trait loci (QTLs) and candidate genes associated with early flowering and photoperiod sensitivity in chickpea.

A recombinant inbred line (RIL) mapping population of 92 lines derived from a cross between the early flowering, photoperiod insensitive genotype ICCV 96029 and a photoperiod sensitive genotype CDC Frontier were used for QTL mapping. Parental genotypes and RILs were screened for response to days to flower under long-day (16 h light / 8 h dark) and short-day (10 h light / 14 h dark) conditions with a temperature of 22 °C / 16 °C in light and dark conditions, respectively. Days to flower were recorded as the number of days from emergence to the opening of the first flower. The difference in days to flower between shot-days (SD) and long-day (LD) conditions was used to determine the photoperiod sensitivity of the line.

Significant difference in parental lines and RILs for days to flower under the SD and LD conditions was observed. In both the conditions ICCV 96029 flowered 28 days earlier than CDC Frontier under LD and 63 days earlier than the CDC Frontier under SD. The photoperiod sensitivity of CDC Frontier was 37 days and ICCV 96029 was only three days; as such CDC Frontier was categorised as photoperiod sensitive; whereas, ICCV 96029 as a photoperiod insensitive genotype. RILs showed continuous variation for days to flower in SD (range 23 days - 80 days) and LD (range 22 days - 53 days), and for photoperiod sensitivity (range 9 days - 54 days).

Linkage map with 1,336 SNPs (3) was used for the QTL analysis using the ICIM-ADD (composite interval mapping) method of QTL-IciMapping 4.0.3.0 software. 11 QTLs were identified for days to flower under SD, LD conditions and photoperiod sensitivity. Four QTLs were identified for days to flower in SD condition. The amount of phenotypic variance explained by the individual QTL ranged between 4% (*qdf-SD3.2*) and 59 % (*qdf-SD5.1*), and these four QTLs together explained 81% phenotypic variation for days to flower under SD conditions. In the LD conditions, four QTLs were identified for days to flower. The percentage of phenotypic variance explained by the individual QTL ranged between 9% (*qdf-LD4.1*) and 36% (*qdf-LD8.1*), and these four QTLs together explained 75% phenotypic variation for days to flower under LD conditions. QTLs present on Chr4 (*qdf-SD4.1*, *qdf-LD4.1*) and Chr5 (*qdf-LD5.1*, *qdf-LD5.1*) were identified for both days to flower under SD and LD conditions.

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Comparative and functional genomics analyses reveal that several key genes in the Arabidopsis (*Arabidopsis thaliana* (L.) Heynh.) flowering time pathways are conserved in legumes (7). We found 130 chickpea orthologs of the Arabidopsis flowering-time pathway genes (photoperiod pathway, circadian clock, light signalling, and autonomous pathways) in the CDC Frontier genome sequence. 116 candidate genes were physically located on chickpea pseudochromosomes chr1-chr8, whereas remaining 14 genes were located on 14 different un-placed scaffolds. Candidate genes were re-sequenced to identify sequence variation between ICCV 96029 and CDC Frontier. The SNP in three candidate genes *FLOWERING LOCUS D* (*FLD*), *CRYPTOCHROME 2* (*CRY2*) and *GIGANTEA* (*GI*), and an 11-bp deletion in the coding region of early flowering 3 (*ELF3*) genes were identified between ICCV 96029 and CDC Frontier. Based on the candidate genes SNPs and insertion/deletion information, KASP assays were developed for genotyping the RIL populations. Three SNP markers (*CRY2*, *FLD* and *GI*) were mapped on Chr4 and *ELF4* on Chr5.

The candidate gene *Ca-GI* mapped in the QTL confidence interval of *qdf-SD4.1* and *qdf- LD4.1*. The Ca-GI spanning QTL explained 9% and 11% of phenotypic variation for days to flower in LD and SD condition, respectively.

The candidate gene *Ca-ELF3* mapped in the QTL confidence interval of *qdf-SD5.1*, *qdf-LD5.1*. The *Ca-ELF3* spanning QTL explained 11% and 59% of phenotypic variation for days to flower in LD and SD conditions, respectively, and 55% of phenotypic variation for photoperiod sensitivity.

The *GIGANTIA* is an important regulator of photoperiodic flowering in several monocots and dicot plants. *GI* regulates flowering by interacting with *CONSTANS* (*CO*), which then regulate flowering activator *FLOWERING LOCUS T* (*FLT*) (8). The *ELF3* is a circadian clock related gene that regulates early photoperiod-insensitive flowing. Functional analysis of pea (*Pisum sativum* L.) and soybean (*Glycine max* (L.) Merr.) ortholog of Arabidopsis *GI* genes showed that several functions of Arabidopsis *GI* gene are conserved between these species (6, 10). The loss-of-function of *ELF3* gene promotes rapid flowering under both LD and SD conditions in Arabidopsis, pea and lentil (*Lens culinaris* Medik.) (Boden et al. 2014). Overall, these reports suggest that the basic flowering pathways are likely to be conserved in Arabidopsis and other legume species. The co-localization of chickpea candidate genes *Ca-GI* and *Ca-ELF3* with QTL for early flowering and photoperiod sensitivity and conserved function of these genes across the plant species strongly suggest that the *Ca-GI* and *Ca-ELF3* regulates the photoperiod response in chickpea.

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# Regulation of legume seed size by an endosperm-expressed transcription factor

by Mélanie NOGUERO[1], Christine LE SIGNOR[1], Vanessa VERNOUD[1], Grégoire AUBERT[1], Myriam SANCHEZ[1], Karine GALLARDO[1], Jérôme VERDIER[2] and Richard D. THOMPSON[1]*

**Abstract:** There are numerous reports of transcription factors (TFs) which are implicated in the control of seed size and seed composition. We have identified, using a platform of TF sequences derived from the *Medicago truncatula* genome sequence, a class of TFs specifically expressed during the seed filling stage. One such TF, *DASH*, was shown to be confined to the developing endosperm. We investigated the role played by *DASH* through analysis of mutant alleles. These give rise to seed-lethal or near-lethal phenotypes, with degeneration of the endosperm and arrested embryo development. The relation of this phenotype to seed auxin action was investigated.

**Key words:** auxin, embryo, endosperm, *Medicago*, seed

We are studying seed development in the model legume *Medicago truncatula* Gaertn. (Mtr) as a basis for identifying key genes controlling seed size and composition in the Vicioid family of cool-season legumes such as pea (*Pisum sativum* L.), faba bean (*Vicia faba* L.) and chickpea (*Cicer arietinum* L.).

In one of the approaches used to identify candidate genes, we used a real-time PCR platform of Mtr transcription factor (TF) sequences, to reveal those expressed in the developing *M. truncatula* seed (8). The 169 gene sequences identified were subjected to a cluster analysis with profiles of expression of the major storage protein classes. This analysis permitted us to identify TFs associated with the expression of each storage protein family, flanked by 3 further classes, expressed before or after these genes during seed development (4). The number of TFs expressed preferentially in the seed filling phase was ~50. We have focused on one of these TFs, *DASH*, a DOF (DNA-binding One Zinc Finger) -type TF specifically expressed in the developing endosperm (7). This was of particular interest as related DOF TFs are required for endosperm-specific expression of storage protein-coding genes during the seed filling phase in cereals (9).

To assign a role to *DASH*, we have analyzed a stop codon-truncation mutant from a TILLING population (5), and one transposon (TnT1) insertion mutants (2). If we look at the seed complement of a pod segregating the TnT1 insertion in *DASH*, i.e. heterozygote, we see about ¼ inviable seeds displaying embryo arrest and degenerated endosperm. The embryo is retarded, and does not develop beyond the globular stage, when wild-type (WT) seeds are already at the heart stage. For the EMS mutant, whereas homozygous mutant seeds segregating on heterozygous plants show the same developmental arrest, we obtained a single homozygous mutant line out of a segregation of 200 seeds. Although this *dash* line shows normal vegetative growth, we observed pod abortion throughout most of the growth period, but pod and seed set occurred at the end of the growth cycle. Most of the resulting homozygous mutant seeds which germinated had morphological abnormalities, frequently possessing fused cotyledons.

In early attempts to recover seeds on homozygous mutant plants, we treated pods with auxin, and observed that this could partly restore the WT phenotype in terms of pod and seed size, suggesting that auxin can compensate for the absence of functional *DASH* (Fig. 1).

When auxin content was measured in developing pods, it peaked at 10 days after pollination (DAP), when the endosperm is most active, and was 36-fold higher in *dash* than WT. This suggests auxin action in the endosperm may be important for embryo development, and that auxin balance between different seed tissues may be deregulated in *dash*.

To understand better the role of *DASH*, we looked at genes potentially regulated by it, by comparing the WT and mutant transcriptomes at 8 and 10 DAP using Affymetrix arrays (1). This yielded 545 differentially expressed probes. Among the most down-regulated genes in *dash* were three sequences encoding small cysteine-rich peptides (CRP), one of which was shown by in situ hybridization to be expressed specifically in the chalazal endosperm, like *DASH* suggesting that this gene might possibly be involved in the same pathway. CRPs have been assigned diverse roles and some members of this family are implicated in processes such as fertilization, female gametophyte or seed development (6). Recently, 180 small CRPs expressed in developing seeds of Arabidopsis (*Arabidopsis thaliana* (L.) Heynh.) were identified (3). They showed a specific family of peptides, called ESF1 (Embryo Surrounding Factor 1), accumulated before fertilization in central cell gametes and thereafter in embryo-surrounding endosperm cells, required for proper early embryonic patterning by promoting suspensor elongation (3). The possible role of small peptides in early endosperm development remains to be studied.

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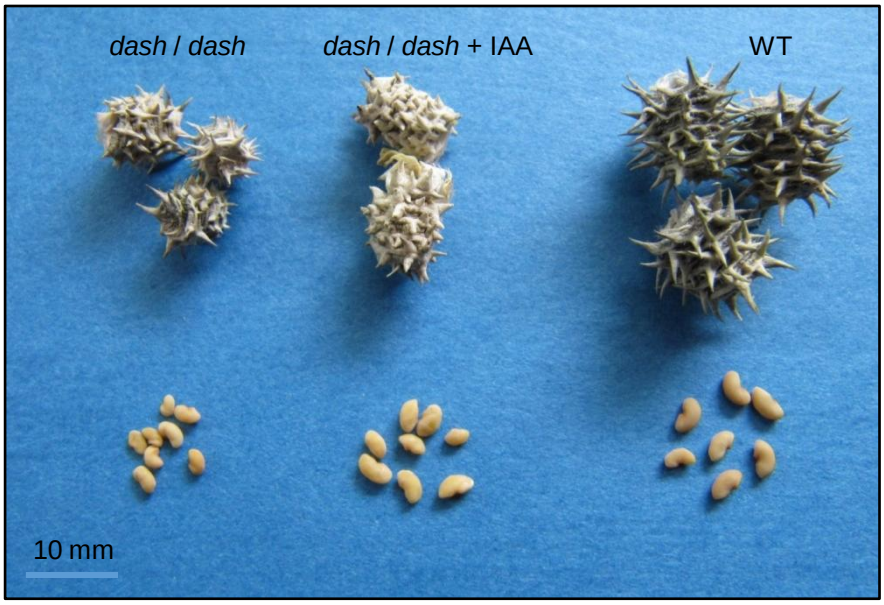


**Figure 1. Pods and seeds from dash mutant (left), *dash* mutant treated with IAA (middle) and WT plant (right); IAA treatment (solution of 100mg $l^{-1}$) was applied on small developing pods 4-5 days after flowering**

Among the most deregulated gene class in *dash* was that of auxin pathways and auxin response genes. *AUX / IAA* and *PIN* probes, implicated in auxin perception/transport, were under-expressed in the mutant. In contrast, genes related to auxin synthesis were not significantly deregulated in *dash* seeds. We thus hypothesize a defect in auxin perception / transport in *dash* seeds, which is compensated for by auxin over-accumulation. The partial complementation of *dash* by auxin addition further implies a role for auxin in *DASH* action, which is consistent with changes in expression of auxin-related genes.

In summary, an endosperm-specific DOF transcription factor, *DASH*, is required for endosperm maturation and normal embryo development. The mutant can be partially rescued by auxin, and accumulates high auxin concentrations in developing pods. Whether auxin constitutes a signal transmitted from the endosperm to the cotyledons, or whether a second signal is generated by the endosperm, will be part of future investigations.

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# Folate profiles in diverse cultivars of common bean, lentil, chickpea and pea by LC-MS/MS

by Ambuj B. JHA[1]*, Marwan DIAPARI[1] , Kaliyaperumal ASHOKKUMAR[1], Stephen J. AMBROSE[2], Haixia ZHANG[2], Bunyamin TAR'AN[1], Kirstin E. BETT[1], Albert VANDENBERG[1], Thomas D. WARKENTIN[1] and Randy W. PURVES[1,2]

**Abstract:** Knowledge of the diversity in folate profiles of pulse crop cultivars grown in contrasting locations in western Canada was not available. With this objective, folate concentration was measured in four cultivars each of common bean, lentil, chickpea and pea with a long term breeding objective to produce pulse crops rich in folates. Using liquid chromatography coupled with mass spectrometry (LC-MS/MS), six different folates were quantified in all crops. Chickpea with an average of 471 µg 100 $g^{-1}$ had the highest concentration of total folate followed by common bean (192 µg 100 $g^{-1}$), lentil (153 µg 100 $g^{-1}$), and pea (26 µg 100 $g^{-1}$). Among folates, 5-methyltetrahydrofolate was the major folate in common bean and pea, whereas 5-formyltetrahydrofolate was predominant in lentil and chickpea. Useful variation detected among the four cultivars evaluated in each crop will set the stage for wider surveys of variation and expanded breeding activities for biofortification of pulse crops.

**Key words:** cultivars, folates, liquid chromatography, mass spectrometry, pulse crops

Folates are essential vitamins and act as cofactors in many metabolic functions including the biosynthesis of nucleic acids and metabolism of amino acids (1, 8). Humans cannot synthesize folates and thus depend upon food sources (2,8). Pulse crops and other legumes are rich source of folates (USDA National Nutrient Database, http://ndb.nal.usda.gov). Deficiency of folate can cause serious health issues including neural tube defects (6). Decrease uptake of folate-rich diets during pregnancy increases the risks of preterm delivery, low birth weight, and fetal growth retardation (7). Among various strategies, biofortification is a balanced and most economical approach and could be a strategy to reduce folate deficiencies globally (3). Although studies have been conducted to measure folate concentrations in various legume crops, knowledge of the diversity in folate profiles of pulse crop cultivars grown in contrasting locations in western Canada was not available. The objective of this research was to determine concentration of folates in four cultivars of each of common bean (*Phaseolus vulgaris* L.), lentil (*Lens culinaris* Medik.), chickpea (*Cicer arietinum* L.) and pea (*Pisum sativum* L.) with a long term breeding objective to produce pulse crops rich in folates.

Seeds from field trials conducted at Saskatoon (common bean, lentil and pea), Limerick (lentil, chickpea), Rosthern (common bean), Elrose (chickpea), and Meath Park (pea), Saskatchewan in 2012 were used for analyses. These seeds were developed at the Crop Development Centre, University of Saskatchewan. Field trials were conducted in a randomized complete block design with three replicates per location. The method for sample preparation and liquid chromatography coupled with mass spectrometry (LC-MS/MS) analysis was similar to that reported by De Brouwer et al. (4, 5) with some modifications. For the trienzyme treatment, the amount of enzyme added and the length of incubation at each step were optimized.

In this study, six folate monoglutamates, folic acid (FA), 10-formylfolic acid (10-FFA), tetrahydrofolate (THF), 5-methyl-tetrahydrofolate (5-MTHF), 5,10-methenyl-tetrahydrofolate (5,10-MTHF), and 5-formyltetrahydrofolate (5-FTHF) were quantified using LC-MS/MS (Fig. 1). Significant differences ($P < 0.05$) were observed among the cultivars for all folates across the pulses, except for 5,10-MTHF in lentil, 5-MTHF in chickpea, and 5,10-MTHF and FA in pea. Significant effects for location and cultivar by location were also observed for the majority of the folates. The total folate concentration was the highest in chickpea (351 µg 100 $g^{-1}$ - 589 µg 100 $g^{-1}$), followed by common bean (165 µg 100 $g^{-1}$ - 232 µg 100 $g^{-1}$), lentil (136 µg 100 $g^{-1}$ - 182 µg 100 $g^{-1}$), and pea (23 µg 100 $g^{-1}$ to 30 µg 100 $g^{-1}$). 5-MTHF and 5-FTHF were the two major folates, representing 35% to 39% and 33% to 51% of total folate in common bean, lentil, and chickpea, respectively (Fig. 2). In pea, 5-MTHF and THF were the two most abundant folates, representing 56% and 22% of the total folate, respectively. 5-MTHF, the major folate observed in the current study, is important for plants as well as humans, and could be a target for improvement by breeders. This study was aimed at understanding variation in folate profiles of common bean, lentil, chickpea, and pea grown in western Canada as a starting point in biofortifying these crops through breeding. The useful variation detected sets the stage for wider surveys of variation and expanded breeding activities for biofortification of pulse crops.

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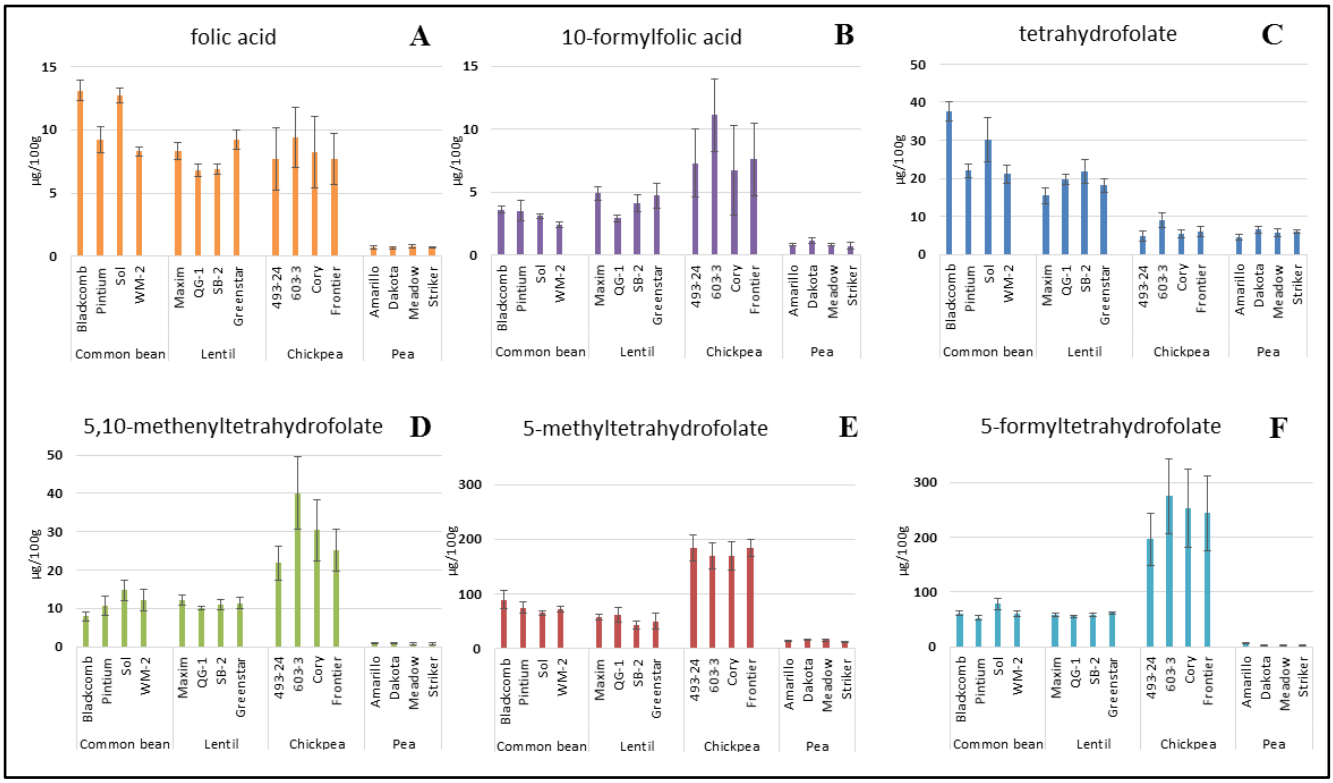


**Figure 1. Levels of six folate monoglutamates determined for four cultivars in each of common bean, lentil, chickpea, and pea; the error bars represent the standard deviation of six values for each cultivar, with three replicates at two locations**

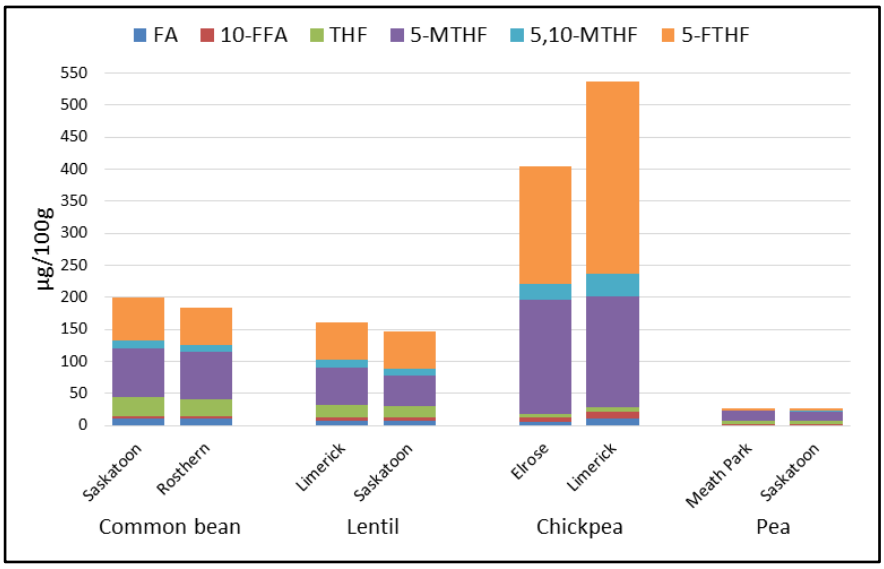


**Figure 2. Graphical representation of the mean composition of six folates in four cultivars each of common bean, lentil, chickpea, and pea grown at two locations in Saskatchewan, Canada in 2012; (FA) folic acid, (10-FFA) 10-formyl folic acid, (THF) tetrahydrofolate, (5-MTHF) 5-methyltetrahydrofolate, (5,10-MTHF) 5,10-methenyltetrahyrofolate, (5-FTHF) 5-formyltetrahydrofolate**

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# Legume recognition of rhizobia

by Yasuyuki KAWAHARADA, Simon KELLY, Anna MALOLEPSZY, Stig U. ANDERSEN, Winnie FÜCHTBAUER, Sheena R. RASMUSSEN, Terry MUN, Niels SANDAL, Simona RADUTOIU, Lene H. MADSEN and Jens STOUGAARD*

**Abstract:** Cross-inoculation groups have traditionally been used to catalogue the host specificity and selective interaction between legumes and their rhizobial microsymbionts. This classification provided a practical overview of legume-rhizobium relationships but did not provide an understanding of the host/non-host interactions. Research using the model plant *Lotus japonicus* has progressed rapidly and has led to an increased understanding of these processes. Genetic analysis of *L. japonicus* symbiotic mutants has assisted in the establishment of a gene network controlling recognition of rhizobia, infection and nodule organogenesis. A decisive feature is perception of rhizobia-produced signal molecules, Nod-factors, by *L. japonicus* LysM receptor kinases and the potential role of exopolysaccharide during symbiosis.

**Key words:** *Lotus japonicus*, plant receptors, signalling, symbiosis

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Plant genomics and genetics have progressed rapidly in recent years. A key component underlying this progress has been the utilization of model plants, which has laid the foundation for further investigations of crop plants. In legumes, *Lotus japonicus* (Regel) K. Larsen (birdsfoot trefoil) has been used as a model species for over 20 years (7) and the resulting genetic and genomic analyses in this species has led to the establishment of a large body of knowledge on molecular mechanisms and made a major contribution to our current understanding of endosymbiosis (2). One example of this is the network of around 20 genes controlling nodule organogenesis and infection that was established by genetic analysis of symbiotic mutants (13). An important feature of *L. japonicus* is that it forms determinate root nodules in association with rhizobia, a feature that it has in common with two major crop legumes, soybean (*Glycine max* (L.) Merr.) and the common bean (*Phaseolus* spp.). In addition to being a suitable plant for the study of root nodule symbiosis, *L. japonicus* can also be used to study interactions with the microbiome such as endophytes, microorganisms colonizing the rhizophere and arbuscular mycorrhizal fungi.

Many resources exist to aid the study of *L. japonicus,* including complete genome sequence, a LORE1 retrotransposon mutagenesis population, a TILLING resource (16) and recombinant inbred lines (RILs). The LORE1 population is particularly useful and it currently contains over 80,000 lines with more than 340,000 annotated insertions. Furthermore, over the next year the number of lines should increase to approximately 120,000, which would lead to over 600,000 annotated insertions and thus mutants will be available for the majority of *L. japonicus* genes (3, 19). Resources such as this not only enhance the study of *L. japonicus* but also enable translational research, the application of model legumes to wider research areas and facilitate the discovery of agronomically important legume genes.

An important line of investigation in *L. japonicus* has been how the plant perceives and responds to the various symbiotic, endophytic and pathogenic microorganisms that it encounters. Significant progress has been made in uncovering the genetic and molecular mechanisms involved in Lotus-rhizobia interactions and the functional aspects of symbiosis. The establishment of a successful rhizobia-legume interaction requires complex molecular communication between the partners in order to determine compatibility. This communication is initiated through the secretion of flavonoid compounds by the legume roots that are recognized by rhizobia and lead to the production of the major rhizobial signal, lipochito-oligosaccharides (Nod-factors) (2, 15). Nod-factors are made up of substituted β,1-4 N-acetylglucosamine (chitin) backbones and trigger various plant responses including root hair deformation, initiation of the rhizobia infection process and cortical cell divisions that ultimately lead to the formation of nodule primordia (Fig. 1). The interaction between these Nod-factors and their plant receptors is an important determinant of bacterial recognition and host specificity; as such, it is an area that continues to be actively investigated in *L. japonicus*.

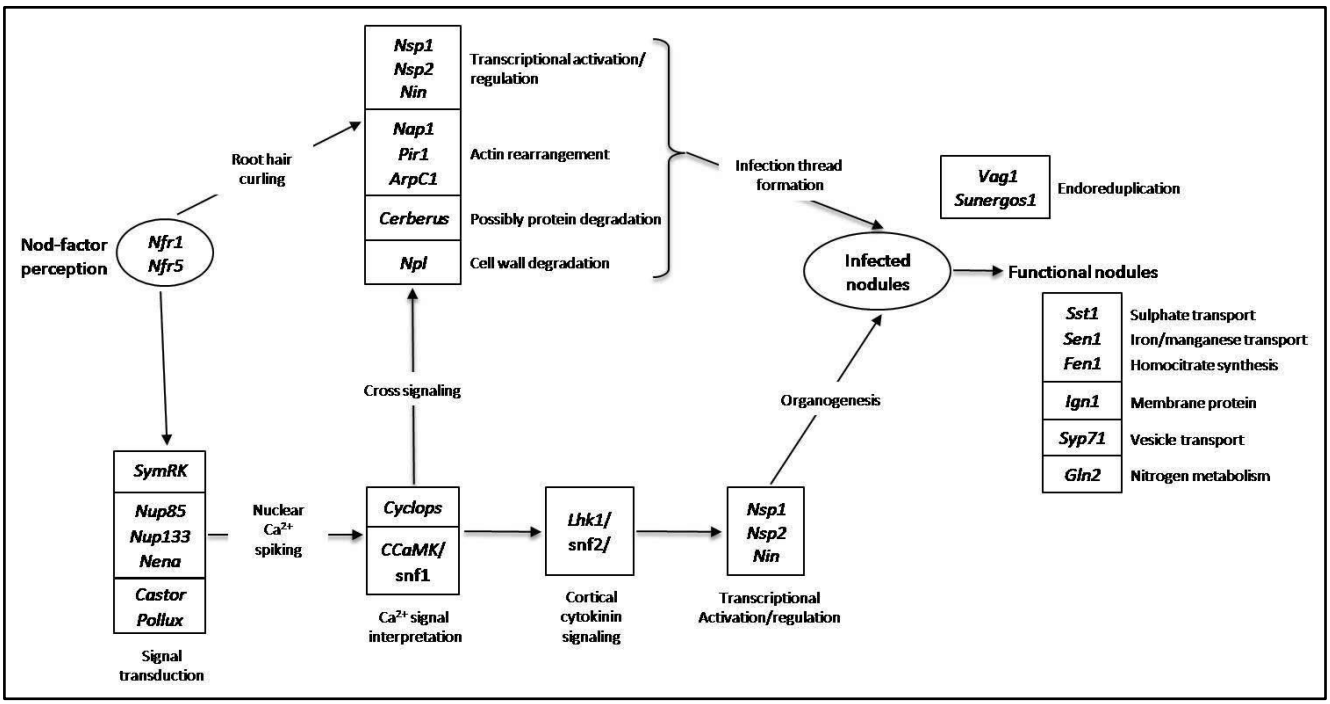


**Figure 1. Schematic of the nodule development and infection pathways in *Lotus* based on genetic analysis of symbiotic mutants: two parallel pathways facilitate infection thread formation and organogenesis, with cross signalling between the two through Cyclops-CCaMK; adapted from (13) with additions from (4), (5), (6), (8), (10), (18), (20) and (21)**

In *L. japonicus* Nod-factor perception is mediated by two LysM receptor kinases,NFR1 and NFR5 (12, 17), which have been shown to bind Nod-factor directly and with high-affinity (1) and to form a heteromeric complex (14). NFR1 and NFR5 consist of an extracellular domain made up of three LysM motifs, a transmembrane domain and an intracellular kinase domain. To better understand plant-microbe interactions much of the recent research in *L. japonicus* has been focused on other members of the LysM family. To date, 17 LysM receptor-kinases have been identified in *L. japonicus* (11) that may be involved in the perception of other symbiotic, endophytic or pathogenic microorganism derived signal molecules.

As mentioned above, Nod-factors trigger plant responses including the formation of root-hair infection threads through which rhizobia enter the nodule primordia. It has been suggested that further rhizobial signals, in addition to Nod-factors, may be required to fine-tune the infection process and ensure compatibility during rhizobia colonization of legumes (9). Exopolysaccharide (EPS) production is ubiquitous among rhizobia and the structure of the EPS produced is species and strain specific. Rhizobia affected in EPS production have been shown to be impaired in infection thread development suggesting EPS has a role to play in this infection and recognition process (9). Specifically, EPS is proposed to be a signal that regulates plant defense or developmental responses and thus allows for infection threads to develop and the bacteria to be released into the nodule primordia (9). This has led to a search for genes responding to EPS and the first results are now emerging from mutant studies using both TILLING and LORE1 mutants.

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# Plant architecture and development to control disease epidemics in legumes: The case of ascochyta blight in pea

by Alain BARANGER[1]*, Carole GIORGETTI[1], Stéphane JUMEL[1], Christophe LANGRUME[1], Anne MOUSSART[1,2], Caroline ONFROY[1,2], Benjamin RICHARD[1], Jean-Philippe RIVIÈRE[1], Pierrick VETEL[1] and Bernard TIVOLI[1]

**Abstract:** Ascochyta blight (*Didymella pinodes*) is the most encountered and damaging pea (*Pisum sativum*) aerial disease worldwide. Available resistance to this pathogen is scarce and partial. Plant and canopy height, stipule size and leaf area index as architectural traits and leaf senescence as a developmental trait are key candidates that may influence *D. pinodes* epidemics in the field. This postulates the conception of a pea plant and canopy ideotype, with rather long internodes, reduced leaf area and delayed senescence for the control of *D. pinodes* epidemics.

**Key words:** *Didymella pinodes*, microclimate, partial resistance, *Pisum sativum*, senescence

Ascochyta blight, mainly due to *Didymella pinodes* (Berk. & A. Bloxam) Petr., is the most encountered and damaging pea (*Pisum sativum* L.) aerial disease worldwide. Available resistance to this pathogen is scarce, partial, and is not always fully expressed in the field. Alternative strategies based on plant and canopy architecture and development variation effects have therefore been explored to complement partial resistance effects. Plant and canopy architecture can indeed contribute to the control of aerial diseases through the reduction of specific stages in the epidemic cycle, or by creating an environment less conducive to the development of epidemics. This control may involve specific processes, such as the modification of microclimatic conditions within the canopy (leaf wetness duration, temperature), plant and tissue ageing and their transition towards senescence (under the influence of plant stage and maturity, shade within the canopy, and/or different stresses including the one caused by the disease), and spore dispersal between organs and within the canopy (6). A strategy based on both field and controlled conditions experiments, and on modelling of the development of the plant and the pathogen, showed that architectural and developmental traits are key factors in the control of ascochyta blight epidemics.

Tissue receptivity tests under controlled conditions using inoculation of stipules, either detached or on whole plants, with a spore suspension of a *D. pinodes* monospore isolate, at different stages of plant development, showed that plant senescence increases receptivity to the disease. Disease severity was indeed lower on green stipules, and there was a shift in receptivity to the disease from the leaf yellowing phase (4).

Split-plot experiments in the field using pea cultivars showing different architectures, and/or sown at different densities and under different epidemic pressures were conducted in the field the last years. Recording of microclimatic conditions within the canopy using both leaf wetness and temperature sensors showed that the impact of canopy architecture on the microclimate mostly depends upon the weather conditions outside the canopy: during rainfall periods, leaf wetness duration (LWD) was higher within than outside the canopy. In denser canopy, LWD was slightly longer at the basis than in the middle of the canopy. During dry periods, LWD was shorter than during rainfall periods and due to dew, longer at the middle than at the base of the canopy (5). A prediction model adapted from Magarey et al. (3) showed that infection periods occurred mostly during or after rainfall periods under our field conditions. During these periods, because LWD was longer inside than above the canopy, microclimatic data were more useful to explain the infection periods than mesoclimatic data.

Under field conditions, the onset of senescence as well as microclimatic conditions at any node level within the canopy are influenced by plant height and cumulative leaf area index above this node. The decreasing gradient in disease severity from the bottom to the top of the canopy observed in the field can therefore be explained both by a decreasing gradient in leaf senescence and a decreasing gradient in leaf wetness duration during or after rainy periods from the bottom to the top of the canopy (4, 5, Fig 1).

The genetic control of architecture and development variables rely both on some major genes and QTL, that often colocalize with known factors controlling partial resistance components. We have used different biparental RIL populations segregating for these major genes and QTL, and screened these populations under controlled (climatic chambers, where architecture and development are not likely to interfere with epidemics), semi- controlled (stacked rows in the greenhouse), or field (stacked rows in a nursery) conditions to explore the links between the genetic factors controlling architectural traits and those controlling partial resistance.

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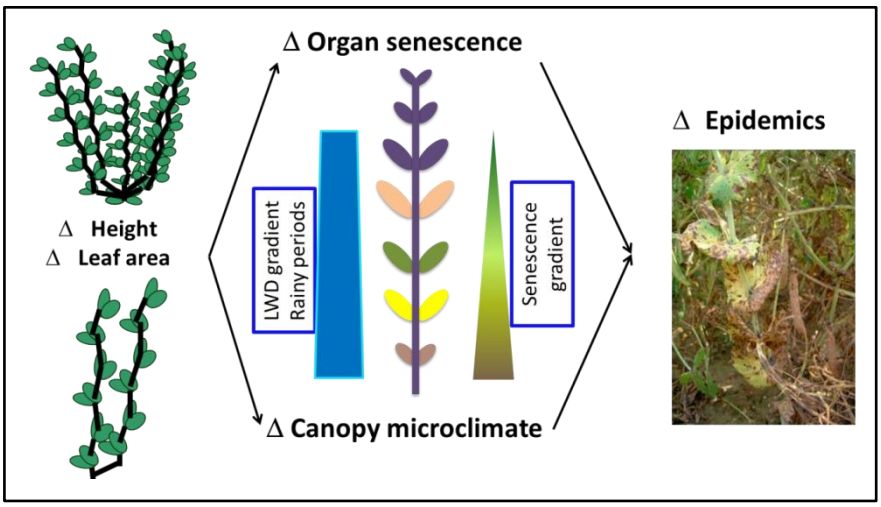


**Figure 1. Processes influenced by plant and canopy architecture likely to control *D. pinodes* epidemics**

Under controlled conditions, six colocalisation regions (CLR), highly stable across organs (stipules and stems), isolates (with differing aggressiveness levels), and inoculation methods (either a spray with a spore suspension on whole plantlets, or a deposit of a drop of spore suspension on detached stipules) were identified, between components for partial resistance, such as flecks coalescence or lesion extension, and architectural or developmental traits, such as plant height, number of nodes, number of ramifications, stipule length, and response to photoperiod (2). Five CLR, stable across organs and years of assessment, were also identified in the field, involving plant height, number of ramifications, and flowering traits. Finally two CLR were identified under semi-controlled conditions, involving plant height, stipule length, flowering traits and maturity induced senescence.

To define whether these colocalisations were due either to linkage or to pleiotropic genetic effects, we have produced near isogenic lines from heterogeneous inbred families segregating within colocalising QTL confidence intervals, and are currently checking both for segregation using molecular markers, and for partial resistance and architectural traits phenotypes in recombinant families. First insights show that, depending on CLR, both types of genetic effects (linkage and pleiotropy) may be involved (2).

Interestingly, two strategies based on one hand on epidemiology within canopies in the field, and on the other hand on genetics in plants either isolated or in stacked rows, both showed that plant and canopy height, stipule size and leaf area index as architectural traits, and leaf senescence as developmental trait, are key candidates that may influence *D. pinodes* epidemics in the field. This paves the way for the conception of a pea plant and canopy ideotype (1), with rather long internodes, reduced leaf area and delayed senescence for the control of *D. pinodes* epidemics.

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# Use of wild relatives in pea breeding for disease resistance

by Diego RUBIALES*, Eleonora BARILLI, Moustafa BANI, Nicolas RISPAIL, Thais AZNAR-FERNÁNDEZ and Sara FONDEVILLA

**Abstract:** Pea (*Pisum sativum* subsp. *sativum*) is an important cool season grain legume. Cultivation can be affected by a number of pest and diseases to some of which there is little genetic resistance available in pea germplasm. However, in occasions this is available in wild relatives that can be exploited in pea breeding. Examples of the breeding activities performed at Córdoba will be mentioned. Germplasm collections of *Pisum* have been thoroughtly screened for resistance to ascochyta blight, powdery mildew, rust, broomrape, fusarium wilt, aphid and weevil, yielding the identification of valuable sources of resistance that are bein introduced in our breeding program. Inheritance of some of the identified resistances has been studied and underlaying mechanisms characterized. As an example, a new gene (*Er3*) for powdery mildew resistance was identified in *P. fulvum* and introduced in pea, with a resistant cultivar being released. Similarly, the first two broomrape resistant cultivars are now being released. Breeding for ascochyta, rust, aphid and weevil resistance is in progress.

**Key words:** aphid, ascochyta blight, broomrape, fusarium wilt, *Pisum*, powdery mildew, resistance, rust, weevil

The cultivated pea (*Pisum sativum* L. subsp. *sativum*) is one of oldest domesticated crops. Since it domestication about 10,000 ago it has been improved for important agronomic traits such as increased seed size and quality, reduced dormancy, absence of seed dehiscence, higher seed quality or suitable flowering time among others. As a result pea is nowadays one of the most productive legumes, being the cool season grain legume most cultivated in Europe and second in the world. However, pea yield is still seriously affected by a number of diseases. Unfortunately, for several of them resistance is not available in the cultivated pea or the level or resistance identified is still insufficient for an effective control of the disease. Wild relatives of pea might be good reservoirs of resistance that can be used to improve pea crop. *Pisum sativum* cross well with all its subspecies. Crosses with *P. fulvum* Sm. and *P. abysinicum* A. Braun are more difficult but still possible. Therefore, we have taken advantage of the genetic diversity available in the first and secondary pea gene pool to improve pea resistance to diseases and pests. The main results achieved will be briefly described.

Broomrape (*Orobanche crenata* Forssk) (Fig. 1A) is a parasitic weed that constrains pea cultivation in Mediterranean Basin and Middle East. Little resistance is available in pea germplasm. Only after screening more than 3,000 accessions some levels of incomplete resistance could be indentified in a few accessions of *P. sativum* and in wild *Pisum* spp. (17). These sources of resistance were successfully crossed with pea cultivars and introduced into our pea breeding programme (18) that already yielded the registration of the first two pea cultivars resistant to broomrape (cvs. "Toro" and "Fandango") already licensed to a seed company. Accurate screening phenotyping complementing field screenings with minirhizotrons enabled identification of QTL governing specific mechanisms of resistance from *P. sativum* subsp. *syriacum* A. Berger (14). Resistance is the result of several mechanisms acting at different stages of the infection process, including low stimulation of broomrape seed germination, unsuccessful penetration of host roots, delay in post-attachment tubercle development and necrosis of the attached tubercles (16).

Aschochyta blight (Fig. 1B), caused by *Didymella pinodes* (Berk. & A. Bloxam) Petr. (syn. *Mycosphaerella pinodes* (Berk. & A. Bloxam) Vestergr.) is a widespread pea disease to which only moderate resistance is available in pea cultivars, insufficient to control the disease. Higher levels of resistance have been identified in wild species of *Pisum* (9) and introduced in our breeding program, although this has not resulted yet in the release of resistant cultivars. Good levels of incomplete resistance have been reported in *P. sativum* L. subsp. *elatius* (M. Bieb.) Asch. & Graebn. and *P. sativum* ssp. *syriacum*, but the higher levels are present in *P. fulvum*. Resistance in these wild species is characterized by a reduced success of colony establishment and lesion size. Histologically this is associated with higher frequency of epidermal cell death and protein cross-linking in infected epidermal cells (6). Resistance is a polygenic trait and QTLs associated with resistance have been identified (8, 13).

Pea powdery mildew (Fig. 1C), caused by *Erysiphe pisi* DC., is particularly important in climates with warm dry days and cool nights. Only two genes (*er1* and *er2*) conferring resistance to powdery mildew were available till the recent discovery of *Er3* in *P. fulvum*. RAPD-derived SCAR markers linked to *Er3* have been developed, allowing the identification of the different alleles of the gene in breeding material (12). The first pea cultivar ("Eritreo") carrying *Er3* has already been released. Resistance conferred by *er1* is due to a penetration barrier associated with protein-cross linking, while resistance governed by *er2* and *Er3* is mainly due to a postpenetration hypersensitive response (15). Additionally, other sources of incomplete resistance have been described in *Pisum* spp. and mechanisms of resistance acting at different steps of the infection process characterized (11).

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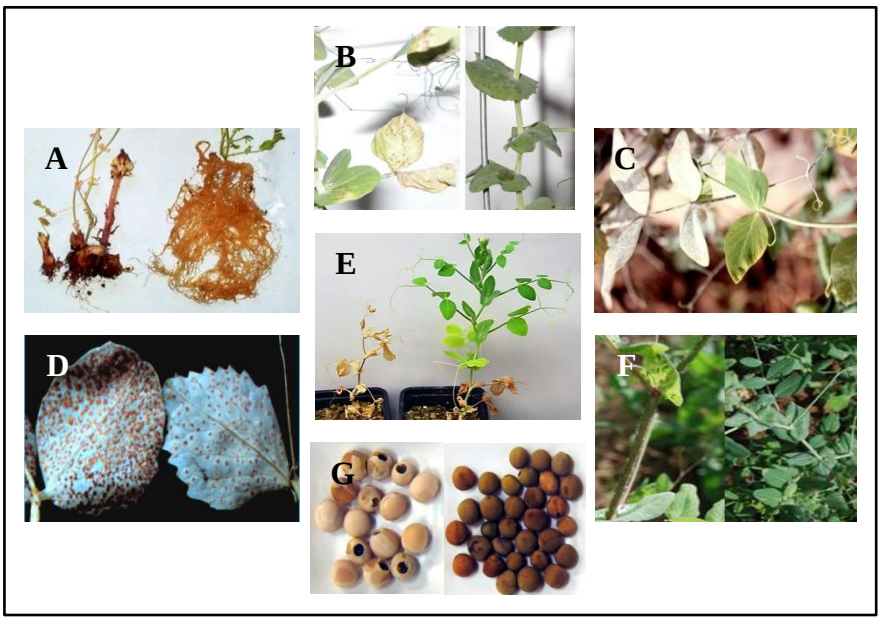


**Figure 1. Symptoms of main pea diseases or pests on susceptible (left) and resistant (right) pea accessions: (A) broomrape; (B) ascochyta blight; (C) powdery mildew; (D) rust; (E) fusarium wilt; (F) aphid; (G) weevil**

Pea rust (Fig. 1D) is caused by *Uromyces viciae-fabae* (Pers.) J. Schröt. in tropical and subtropical regions and by *U. pisi* Pers. Wint. in temperate regions (4). No complete resistance to *U. pisi* is available so far, altought incomplete resistance has been identified (2). This resistance is not associated with host cell death (3). QTLs for resistance to *U. pisi* have been identified in *P. fulvum* (5).

Pea fusarium wilt (Fig. 1E), caused by *Fusarium oxysoprum* f. sp. *pisi* W.C. Snyder & H.N. Hansen, is a recurrent soilborne disease causing important damage to pea worldwide. Although monogenic resistance has been identified and introduced in elite pea cultivar, this resistance has been overcome by the emergence of new pathogenic races (19). We identified new sources of quantitative resistance (1).

More recently we have started the identification of resistance to pea aphid (*Acyrthosiphon pisum* L.) (Fig. 1F) (5) and to pea bruchid (*Bruchus pisorum* L.) (Fig. 1G).

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# High temperature tolerance in grain legumes

by Pooran M. GAUR[1]*, Srinivasan SAMINENI[1], Laxman KRISHNAMURTHY[1], Shiv KUMAR[2], Michel E. GHANEM[2], Stephen BEEBE[3], Idupulapati RAO[3], Sushil K. CHATURVEDI[4], Partha S. BASU[4], Harsh NAYYAR[5],Veera JAYALAKSHMI[6], Anita BABBAR[7]and Rajeev K. VARSHNEY[1]

**Abstract:** High temperature stress (or heat stress) during reproductive stages is becoming a serious constraint to productivity of grain legumes as their cultivation is expanding to warmer environments and temperature variability is increasing due to climate change. Large genetic variations exist in grain legumes for heat tolerance which can be exploited for development of locally adapted heat tolerant cultivars. Heat tolerant cultivars will be more resilient to the impacts of climate change, allow flexibility in sowing dates and enhance opportunities for expanding area of grain legumes to new niches and cropping systems.

**Key words:** chickpea, common bean, faba bean, heat stress, lentil

Heat stress is increasingly becoming a serious constraint to grain legumes production in certain regions due to a large shift in area of grain legumes from cooler, long season environments to warm, short-season environments, e.g. shift in chickpea (*Cicer arietinum* L.) area from northern to southern India; increase in area under late sown conditions; and reduction in winter period and increase in temperatures due to climate change. Many countries could experience unprecedented heat stress because of global climate change (5). Heat sensitivity in grain legumes can reduce yields, product quality, and lead to restricted geographic adaptation. A high temperature of 35 °C was found critical in differentiating heat tolerant and heat sensitive genotypes in chickpea, lentil (*Lens culinaris* Medik.) and faba bean (*Vicia faba* L.), while heat sensitive lines of common bean (*Phaseolus* spp.) lose yield when night temperature is higher than 20 °C.

Effects of heat stress on grain legumes before flowering include reduction in germination percentage and increase in occurrence of abnormal seedlings; early flowering; degeneration of nodules affecting the nitrogen fixation efficiency; reduction in membrane stability, photosynthetic / mitochondrial activity and plant biomass. Heat stress during the reproductive phase affects pollen viability, fertilization, pod set and seed development leading to abscission of flowers and pods and substantial losses in grain yield. Heat stress often leads to soil moisture deficit during reproductive growth stages of grain legumes thus predisposes them to necrotrophic pathogens such as *Rhizoctonia bataticola* causing dry root rot disease.

Selection for heat tolerance can be effectively made by planting the crop at high-temperature hot spot or under late-sown conditions and selecting the plants/progenies based on number of filled pods per plant and grain yield. Pollen-based screening methods can also be used for evaluating genotypes for tolerance to heat stress. Genetic variation for heat tolerance has been identified in almost all grain legumes. Diverse sources of heat tolerance should be exploited to develop heat tolerant cultivars. The precision and efficiency of breeding programs can be enhanced by integrating novel approaches, such as marker-assisted selection, gametophytic selection and precise phenotyping.

One of the Product Lines of the CGIAR Research Program on Grain Legumes (http://grainlegumes.cgiar.org/) is on *Heat tolerant chickpea, common bean, faba bean and lentil.* It will help in establishing common sites and protocols for heat tolerance screening and comparing the levels of heat tolerance available in these legumes. It will also provide an opportunity for comparative studies on physiological mechanisms and genetics of heat tolerance in these legumes.

*Chickpea.* A field screening technique has been standardized for screening of genotypes for heat tolerance in chickpea (4). High temperatures reduced pod set in chickpea by reducing pollen viability and pollen production per flower (2). Stigma receptivity can also be affected at very high temperatures (≥ 40/30 $^0$C) leading to failure of fertilization (8). Change in level of abscisic acid was found to be associated with heat tolerance response (7), while impaired sucrose metabolism in leaves and anthers was associated with heat stress induced reproductive failure (6). Grain yield under high temperatures was found to be negatively correlated with days to flowering and days to maturity and positively correlated with plant biomass, number of filled pods per plant and number of seeds per plant (3). ICRISAT and ICARDA with national research partners in Asia and Africa have identified several heat tolerant genotypes (cultivars / elite lines / germplasm accessions) in desi (ICCV 92944, ICCV 93952, ICCV 96970, ICCV 94954, ICCV 07102, ICCV 07110, ICCV 07109, ICCV 07118, ICCV 07117, ICCV 07105. ICCV 07108) and kabuli (ICCV 95332, ICCV 92318, FLIP87-59C, Salawa, Burguieg, S051708, S00998, S03308, S03525, S051702, S051412, S03302, S02266, S051685, S051703) chickpea (Fig. 1). A heat-tolerant chickpea line ICCV 92944 has been released in three countries (JG 14 in India, Yezin 6 in Myanmar and Chaniadesi 2 in Kenya) and area under its cultivation is expanding rapidly. In Myanmar, it covered over 40,000 ha during 2012-2013 crop season (9).

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**Figure 1. A heat-sensitive (left) and a heat-tolerant (right) chickpea genotype**

*Common bean.* Heat stress is a major constraint to common bean production and breeding for heat tolerance could benefit 7.2 million ha (some of which could benefit by drought tolerance), of common bean and could increase highly suitable areas by some 54% (1). Under heat stress, the grain yield in common bean showed significant positive correlation with pod harvest index, pod partitioning index, harvest index, canopy biomass, 100 seed weight, pod number per area, and seed number per area. The heat tolerant genotypes are able to form pods and seeds and to fill seeds under heat stress Genetic variability available for heat tolerance in tepary bean (*Phaseolus acutifolius* A. Gray) has been exploited for improving heat tolerance in common bean at CIAT. Interspecific lines derived from crosses of tepary bean with common bean were found to have higher yield over common bean checks under heat stress conditions.

*Faba bean.* Being a crop of relatively high moisture areas, faba bean is very sensitive to water and heat stresses particularly in the Mediterranean region. The irrigated faba bean crop in Sudan and Egypt is severely affected by heat stress mainly during flowering and podding stages. Therefore, efforts are being made to develop faba bean genotypes that are more adapted to heat stress conditions in these areas. Preliminary evaluation of different faba bean breeding lines under heat stress was conducted at ICARDA through late and summer planting in Tel Hadya (Syria) and Terbol (Lebanon), respectively. During flowering and podding period the temperature reached 38 $^0$C for late planting and 41 $^0$C in summer planting in Terbol. The preliminary results showed that only 0.3% of the tested germplasm can be considered as tolerant to heat. Two faba bean varieties (Shendi and Marawi) with tolerance to heat were released in Sudan.

*Lentil.* Delayed sowing in field is commonly used for evaluating heat tolerance during reproductive stage in lentil. Number of filled pods per plant under heat stress showed significant positive correlation with pollen viability and has been used as a key trait for assessment of heat tolerance. Focused Identification of Germplasm Strategy (FIGS) selected germplasm for heat tolerance screening. Evaluation of germplasm under delayed planting with regular irrigation has led to identification of several heat tolerant genotypes in Morocco (ILL2181, ILL82, ILL5151, ILL5416, ILL4857, ILL956 and ILL598) and India (FLIP2009-55L, ILL2507 and ILL4248).

Improving heat tolerance in these legumes would increase yield stability, protect against global warming, and maintain and extend the geographical range of cultivation, particularly in lower elevations in many countries. Heat tolerant cultivars would enhance opportunities for expanding area of grain legumes to new niches and cropping systems, such as rice-fallows in south Asia for chickpea and lentil and maize-based systems in east and southern Africa for common bean and faba bean.

**Figure 2. A heat-sensitive (left) and a heat-tolerant line (right) of common bean**

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# Can we live only on pulses?

by Aleksandar MIKIĆ

**Abstract:** At the fortified hill of Hissar in the present southeast Serbia, dated to 11th century BC, two storages were discovered with 2572 charred grains of pea, 3031 of bitter vetch and several hundreds of various cereals, making this site rather unique in the still unexplained preference of legumes by its inhabitants. The Bible is one of the most ancient written resources where lentil and faba bean are mentioned, most remarkably in the Book of Daniel, where he and his friends ate only pulses and drank water at least for ten days. If needed, we can live only on pulses that will always remain an essential part of our everyday diet.

**Key words:** archaeobotany, Bible, Book of Daniel, ethnology, grain legumes, pulses

## Introduction

Pulses have been used by Neanderthal (2) and modern man, in both Paleolithic (1) and Neolithic (8). Among the first domesticated plant species in the world, in Fertile Crescent during 9th millennium BP, were chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* Medik.), pea (*Pisum sativum* L.) and bitter vetch (*Vicia ervilia* (L.) Willd.) (10).

## Material evidence

It is archaeology with archaeobotany that are the most significant material sources of determining the importance of some crop in the past. There are viewpoints that the legumes could have predated cereals, although their remains are much scarcer, due to a high protein content and more prominent degradability of the grains (3).

One of such curious archaeological findings is the fortified hill of Hissar near the modern town of Leskovac in the present southeast Serbia, dated to 11th century BC and defined as the northernmost point of a developed culture of a Greek extraction (4).

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There, at Hissar, two storages were discovered with charred grains of various crops and wild plant species. One of them contained about 340 grains of several cereal species and 2,572 grains of pea, apart from more than 30 grains of lentil, faba bean (*Vicia faba* L.) and bitter vetch. In the other, there were about 300 grains of cereals and 3,031 grains of bitter vetch, with several ones of lentil and faba bean. This is one of the most unique findings in the world, since, as a rule, it is the cereals that regularly outnumber the remains of pulses.

It is noteworthy that the first known ancient DNA in legumes was extracted from both pea and bitter vetch charred grains, with a genetic similarity between the charred pea from Hissar and the present population of *P. sativum* L. subsp. *elatius* (M. Bieb.) Asch. & Graebn. 150 km southwards, despite a time gap of more than three millennia (7). The question why the Hissar population obviously preferred pulses to cereals remains open, but what is sure is that this tradition lives on, since this region has been renown for preparing the best meals of the *Phaseolus* spp. immature pods and mature grains in the region.

## Oral tradition

Both historical linguistic and ethnology may belong to this category. Historical linguistics or, as named by non-mainstream groups of language experts, palaeolingistics brings forth rather valuable comparative analyses resulting in attesting the proto-words in diverse ethnolinguistic families relating to pulses and thus providing us with an insight on their role in everyday lives of our ancestors (6). There is also a possibility, although with a certain amount of risk of miscomprehension, to go that far into the past as more than 10 millennia and attempt to attest the proto-word for pulses in general, that subsequently was diversified into the forms denoting individual pulse crops (5) In parallel, ethnology contributes to the same goal by attesting myths, legends, folk stories, fairytales, customs and ethnomedicine of diverse human communities worldwide and throughout the mankind history.

## Written records

The Bible, namely its part considered Old Testament by the Christians, is one of the most ancient written resources where several pulse crops are mentioned, such as lentil, in Genesis (25:34) and the Second Book of Samuel (23:11), and both lentil and faba bean, in the Second Book of Samuel (17:28) and the Book of Ezekiel (4:9).

The most impressive record on pulses may be found in the Book of Daniel, dated to 2nd century BC. Having robbed the Solomon's Temple in Jerusalem, the Babylonian king Nebuchadnezzar inducted in his services some young members of the Judean nobility, including Daniel and his three companions, Hananiah, Mishael and Azariah. Deciding to remain faithful to his own faith, Daniel refused to eat meat and drink wine at the king's table and suggested a ten-day diet: 'Prove thy servants, I beseech thee, ten days; and let them give us pulse to eat, and water to drink' (1:12) (Fig. 1). Although their overseer Melzar was afraid their health would deteriorate, Daniel and his friends appeared healthier than the others and were allowed by Melzar to continue with their pulse- and water-based diet (1:16).

Interestingly enough, there is no mention of the pulses in the New Testament, neither in Qur'an. However, there are numerous Christian saints whose only feed were pulses, such as Venerable James the Solitary of Syria from 5th century and Venerable John the Silent from 6th century, who ate only lentil, and Saint Mary of Palestine and Venerable Mastridia of Jerusalem from 6th century and Venerable John of Rila, from 9th century, who lived only on faba bean (9).

## Instead of a conclusion

Yes, we can live only on pulses, that will surely always keep their essential place in our diets, in the same way as we, if needed, may live on other food. Because, one may always remember a Christ's reply to various Satan's temptations: 'But he answered and said, It is written, Man shall not live by bread alone, but by every word that proceedeth out of the mouth of God.' (Matthew 4:4).


**Figure 1. The line 12 of the chapter 1 of the Book of Daniel, 'Prove thy servants, I beseech thee, ten days; and let them give us pulse to eat, and water to drink', in (from above to below and from left to right) Hebrew, Aramaic, Koine Greek, Vulgate Latin, Armenian, Old Church Slavonic, Coptic and Early Modern Welsh**

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*I Howard.*

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# Second International Legume Society Conference (ILS2) *2016: Legumes for a Sustainable World*

Tróia, Portugal, 12-14 October 2016

The International Legume Society and the Instituto de Tecnologia Química e Biológica of the Universidade Nova de Lisboa cordially invite you to join us at the Second International Legume Society Conference, scheduled from 12-14 October, 2016 at Tróia resort, in the vicinity of Lisbon, Portugal.

In a world urgently requiring more sustainable agriculture, food security and healthier diets the demand for legume crops is on the rise. This growth is fostered by the increasing need for plant protein and for sound agricultural practices that are more adaptable and environmentally sensitive. Food, feed, fiber and even fuel are all products that come from legumes – plants that grow with low nitrogen inputs and in harsh environmental conditions. The Second Legume Society Conference will be held during 2016 - the United Nations' International Year of Pulses. The goals of this UN International Year include: the encouragement of connections throughout the food chain that would better utilize pulse based proteins; increase global production of pulses; better utilization of crop rotations; and to address challenges in the trade of pulses.

The conference will address the following themes: Legume Quality and Nutrition; Farming Systems/Agronomy; Abiotic and Biotic Stress Responses and Breeding; Legume Genetic Resources; and New "Omics" Resources for Legumes. The health and environment benefits, as well as, the marketing of legumes will be transversal topics throughout the conference. Special attention will be given to foster the interaction of researchers and research programs with different stakeholders including farmers and farmer associations, seed/feed and food industries, and consumers. For this, the conference will also be the site of the Final Meeting of the EU-FP7 ABSTRESS project, the Annual Meeting of EU-FP7 LEGATO project; and final dissemination events of EU-FP7-ERANets MEDILEG and REFORMA. The results and conclusions from these four important research programs will be shared with conference attendees.

Please join us in beautiful Tróia, Portugal from 12-14 October, 2016! Plan now to include the Second ILS Conference in your busy agenda. Kindly share this information with any colleagues dealing with legumes.

*Diego Rubiales, on behalf of the Scientific Committee*
*Pedro Fevereiro, Carlota Vaz Patto and Susana Araújo, on behalf of the Organizing Committee*

**Local Organizers**
The Instituto de Tecnologia Química e Biológica / Universidade Nova de Lisboa (ITQB/UNL) will be responsible for the organization of the Conference, in cooperation with the International Legume Society. The official language of the Conference will be the English.

INSTITUTO DE TECNOLOGIA QUÍMICA E BIOLÓGICA ANTÓNIO XAVIER /UNL
Knowledge Creation

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## Venue

The conference will be held in Tróia in the vicinity of Lisbon, Portugal. Tróia is a beautiful sand peninsula dividing the Sado River from the Atlantic Ocean.

The nearest airport is the Lisbon International Airport, about 50 Km away. Shuttles will be made available from and to Lisbon International Airport.

During the period of Roman occupation, date from the 1st century to the 6th century AD, Tróia was an island of Sado delta, called Ácala Island.

Sado Estuary Nature Reserve, where dolphins swim, and the Serra da Arrábida Natural Park, where a full developed Mediterranean forest can be seen, are two of the main natural attractions nearby Tróia peninsula.

The Tróia Golf Championship Course is considered the best course in Portugal in the categories of difficulty and variety. It also stands in 20th place in the list of the best golf courses in Europe drawn up by the Golf World magazine.




**Tentative Programme**

October 11th, 2016
Morning-Afternoon: Satellite projects meetings
Evening: Conference Registration

**October 12th, 2016**
08:00 Registration; 09:00 Welcome addresses;
09:45 Session 1 (Opening plenary)
11:15 Coffee break
11:45 Sessions 2 & 3
12:45 Lunch
14:30 Sessions 2 & 3
16:30 - 19:00 Sessions 4 & 5
20:45 Third International Legume Football Cup

**October 13th, 2016**
9:00 Session 6
11:15 Coffee break
11:45 Sessions 7 & 8
12:45 Lunch
14:30 Sessions 7 & 8
16:00 Coffee break
16:30 International Legume Society Assembly
20:45 Third International Legume Football Cup

**October 14th, 2016**
09:00 Session 9
11:15 Coffee break
11:45 Sessions 10 & 11
12:45 Lunch
14:30 Sessions 10 & 11
16:00 Coffee break
16:30 Session 12 (Closing plenary)
20:00 Farewell Dinner

**October 15th, 2016**
Satellite projects meetings

*Bem vindos a Tróia, amigos das leguminosas!*

# International Year of Pulses - 2016

Global Pulse Confederation (CICILS-IPTIC)

CICILS – IPTIC, shortly to be renamed Global Pulse Confederation is head quartered in Dubai and licenced under the Dubai Government authority, Dubai Multi Commodity Centre (DMCC). CICILS is the not for profit peak body for the whole global pulses industry value chain. As the sole international confederation for the industry it enjoys membership from 18 national associations (federations) and over 600 private sector members in an industry worth over $100 billion at the retail level and over 60 million tonnes in pulse production and distribution in over 55 countries. The organisation represents the common good of all sectors of the global pulse industry value chain from growers and researchers, through input and logistics suppliers, traders, exporters and importers to government bodies, multilateral bodies, processors, canners and consumers. CICILS works for transparency and sustainability in all sectors and aspires to contribute in as many ways possible to global food security and improved health and nutrition. The CICILS Executive Board consists of up to 30 members from all over the world elected from the membership. Board positions are voluntary, non-profit and carry no remuneration.

## OUR VISION

To create an inclusive global pulse organization recognized for its integrity, professionalism and ability to work together across the entire pulse value chain to resolve issues and grow the industry.

## OUR MISSION

To lead the global pulse industry to major crop status by facilitating free and fair trade and increasing production and consumption of pulse crops worldwide.

## OUR GOALS

- To expand the permanent membership of CICILS to include the broadest base of organisations and companies involved both directly and indirectly in the global trade of pulses.
- To ensure a reliable, consistent and safe pulse value chain delivering pulses that meet the requirements of the industry's existing and future customers and consumers - and to encourage all industry sectors that impact on production, marketing and service delivery for Pulses to operate ethically and at world's best practice.
- To identify, select, fund and/or otherwise support approved research and development activity that leads to increased production and consumption of pulse crops to address the critical health, sustainability and food security issues around the world.
- To work towards harmonisation of the global pulse trade and removal of all barriers to trade for pulses world wide, and where possible develop new markets.
- To hold annual conventions of the highest calibre, that unite CICILS-IPTIC global membership in friendship, provide a focus for exchange of ideas and information, and a forum for discussion and amicable resolution of industry issues.
- To support national and regional member associations through active participation in local country activities by local CICILS members ("Ambassadors").

## Themes

CICILS and its IYOP partners have identified a series of thematic areas that will be the focus for activities during the International Year. These areas represent the key issues where new and increased efforts could help make a difference in promoting sustainable agriculture and livelihoods, as well as healthy diets, through increased production, trade and consumption of pulses.

We are working on more than 100 activities and projects related to 2016, four of them have already been launched in the areas of branding, school programs, recipes, and market access. Fifteen external partners have been recruited to work on the year, from major science centres, health institutes, academia to farm groups. Additionally, a total of 30 national committees have begun activities in every continent.

These activities will be built around four thematic areas:

### 1) Creating Awareness

IYOP 2016 is an opportunity to increase awareness and global demand for pulses. We aim to reach an audience of 20-40 million people worldwide using social media, websites and global media outreach.

### 2) Food & Nutrition Security & Innovation

IYOP has set the ambitious targets of helping initiate:

- 20 governments to commit to including pulses as part of their food security policies.
- 100 research projects substantiating the ability of pulses to combat nutrition and health issues.
- 100 research projects into functional and nutritional properties for food product advancement.

### 3) Market Access & Stability

IYOP is an excellent opportunity to open a dialogue on improving the regulatory framework in which trade occurs. We hope to reduce trade barrier costs that are borne by farmers, processors, traders and consumers while introducing greater efficiencies to enhance food security, reduce price volatility and enhance the return to growers.

### 4) Productivity & Environmental Sustainability

IYOP 2016 is a perfect chance to draw the focus of the scientific community. We hope to see the completion of a 10-year plan of action on pulse research by the end of 2016 and the genome sequencing of three pulse crops by 2018.

## National Committees

CICILS has convened a worldwide network of promotional teams to ensure wide-reaching and global coordination of activities on the 2016 International Year of Pulses. The National Groups are made up of experts with “great ideas” who plan and coordinate the most important activities of IYoP outreach, from the ground up. Their work is essential to the successful dissemination of the key thematic areas of the Year.

The Groups will meet via a conference call every two months. The purpose of the calls is to provide an update on activities, exchange ideas, identify gaps and coordinate a global approach on the key themes of the IYoP. As of February 2015, there were 30 countries on the National Promotions Group mailing list and additions to this list will follow over the course of 2015 and 2016.

## Join Us

We know you all love pulses, which is why we want to give you 10 ideas on what you and/or company can do to help promote the 2016 International Year of Pulses.

1. Include a link to iyop.net in your website.
2. Spread the word! Have your communications team promote pulse stories in the media. Messages like: "What Are Pulses and Why Are They Important?" can help.
3. Donate your recipes to the global collection, and feature the recipes on your web site. Send your recipes to IYOP@emergingag.com.
4. Donate your photos to our Photo Gallery.
5. Be social and talk about us! Follow us on Twitter and use the hashtag#IYOP2016.
6. Make use of your own connections to get more supporters. Do you know a local company who could be a sponsor? Perhaps you know someone in the Agricultural Department in your country? We are here to coach you and to provide you materials on how to get them on board.
7. Share your news. Send us your pulse related news to include in the News pages of iyop.net.
8. Submit your event to iyop.net to include on our Event Calendar.
9. Translate materials on iyop.net into your national language.
10. And finally… to welcome the Year, have an Event on January 5th, 2016 and serve pulses!


**Mendel's Legacy - 150 years of the Genius of Genetics**
**Brno, Czech Republic, 7-10 September 2015**
**http:// www.mendelgenius.com/**


**IIth International Plant Breeding Congress and EUCARPIA Oil and Protein Crops Section Conference**
**Antalya, Turkey, 1-5 November 2015**
**http:// http://www.intpbc2015.org/**


**North American Pulse Improvement Association Biennial Meeting**
**Niagara Falls, Canada, 5-6 November 2015**
**http://www.eventbrite.com/e/napia-2015-biennial-meeting-tickets-5457734230**


**26th General Meeting of the European Grassland Federation**
**Trondheim, Norway, 5-8 September 2016**
**http://www.egf2016.no**


**10th World Soybean Research Conference**
**Savannah, USA, 10-16 September 2017**
**http:// www.wsrc10.com/**

*Legume Perspectives* is an international peer-reviewed journal aiming to interest and inform a worldwide multidisciplinary readership on the most diverse aspects of various research topics and use of all kinds of legume plants and crops.

The scope of *Legume Perspectives* comprises a vast number of disciplines, including biodiversity, plant evolution, crop history, genetics, genomics, breeding, human nutrition, animal feeding, non-food uses, health, agroecology, beneficial legume-microorganism interactions, agronomy, abiotic and biotic stresses, agroeconomy, sociology, scientometrics and networking.

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