BOOK OF ABSTRACTS

ICLGG 2017

8th International Conference on Legume Genetics and Genomics
18-22 September 2017
Siófok, Hungary

Supported by:

The congress has received financial support from the European Union's FP7 RTD Programme under grant agreement no 613551 LEGATO

ISBN 978-615-5270-39-0

This book is working material for the 8th ICLGG Conference.

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Abbreviations:

PL	Plenary lecture (by invitation)
OL	Oral lecture (15 min long, contributed)
P	Poster (ordinary and also with flash talk, the number in this case refer the poster code)

ISBN 978-615-5270-39-0

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ISBN 978-615-5270-39-0

ISBN 978-615-5270-39-0

ABSTRACTS OF TALKS

Book of abstracts
ISBN 978-615-5270-39-0

Physiological limits to legume genetic improvement

T.R. Sinclair

North Carolina State University, Raleigh, USA

Increases in mass and nitrogen accumulation by legumes to achieve increased yields ultimately require improved physiological activity that results in more effective use of available resources. The critical resources in grain legume production are light and CO_2 for photosynthesis, gaseous nitrogen for symbiotic N_2 fixation, and water to avoid stress. While genetic variability in photosynthesis has been identified in legumes, increased cellular or leaf photosynthetic capacity has not had any major impact on yield. Therefore, it is concluded that photosynthesis is not a major approach to crop yield increase. On the other hand, the unique capability of legumes to fix N_2 opens the possibility of overcoming the high nitrogen input required for yield formation in these species. Since it now appears that N_2 fixation activity is mainly regulated by the host plant, plant genetic selection appears to be a major opportunity for yield increase. However, N_2 fixation is vulnerable to soil-water deficit, especially in warm-season species such as soybean, cowpea and common bean. Identification of genotypes with more tolerant N_2 fixation to soil drying has been a major advance in developing higher yielding cultivars in some legume species.

How to refer your abstract:
T. R. Sinclair (2017) Physiological limits to legume genetic improvement; ICLGG 2017 - Book of abstracts, ICLGG2017/PL/102

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Statistical models for genetic improvement: towards genotyping guided global analysis of multiple families

L. Moreau, **A. Charcosset**

INRA, UMR Génétique Quantitative et Evolution – Le Moulon, Gif sur Yvette, France

In the "pre-genotyping era", plant breeding mostly relied on the phenotypic comparison of individuals within segregating families of limited size. Statistics were important to optimize phenotyping experiments and analyze results but global analyses of data obtained over time for multiple families were rare. This contrasted with the routine use of the BLUP (Best Linear Unbiaised Predictor) model based on pedigree in animal breeding, especially for large dairy cattle populations. A switch towards a more global treatment of information started in the 1990s with the implementation of multiparental QTL mapping designs that make it possible to compare diverse alleles segregating in the population of interest. We will present advances achieved with these approaches and how they can benefit from dense parental genotyping. We will then present how genomic prediction, which proved particularly adapted to highly polygenic traits, now extends the utility of global statistical models and discuss some key issues related to the choice of the training population. Finally we will discuss some complementarities of multiparent QTL mapping and genomic prediction to manage diversity in breeding programs.

How to refer your abstract:
L. Moreau, A. Charcosset (2017) Statistical models for genetic improvement: towards genotyping guided global analysis of multiple families; ICLGG 2017 - Book of abstracts, ICLGG2017/PL/137

Book of abstracts
ISBN 978-615-5270-39-0

Harvesting crop wild relatives to improve chickpea cultivation in food-insecure countries

E.J.B. von Wettberg, P.L. Chang, A. Greenspan, S. Moenga, B. Alford, E. Dacosta-Calheiros, M.A. Yilmaz, A. Cakmak, K.S. Moriuchi, N. Carrasquila-Garcia, B. Erena Mamo, V. Singh, M.A. Cordeiro, L. Balcha, L. Vance, E. Bergmann, E.J. Warschefsky, K. Negash Dinegde, S.G.A. Shah Sani, J. Rose, A. Migneault, C.P. Krieg, F. Basdemir, K. Raiz, R.M. Atif, S. Yimer, D. Bekele, R. Mufti, T. Getahun, G. Sefara, S. Ijaz, M. Yildirim, B. Tanyolac, L.P. Henao, A.Y. Zhang, Z. Damtew, M. Chichaybelu, R. Immareddy, B. Sarma, E. Marques, F. Assefa, A. Surendrarao, S. Singh, B. Patil, S. Saylak, H. Temel, N.V. Noujdina, M.L. Friesen, E. Siler, D. Lindsay, H. Ozelik, J. Kholova, H. Sharma, P. Gaur, V. Vadez, K. Tesfaye, A.F. Woldemedhin, B. Tar'an, A. Aydogan, B. Bukun, R.V. Penmetsa, J. Berger, A. Kahraman, S.V. Nuzhdin, **D.R. Cook**

University of California-Davis; University of Southern California; University of Vermont; Ethiopian Institute for Agricultural Research; Addis Ababa University; University of Saskatchewan; International Crops Research Institute for the Semi-Arid Tropics; Dicle University; Turkish Agricultural Research System; CSRIO Plant Industry; Harran University; Florida International University; Quaid-i-Azam University; University of Agriculture Faisalabad; Banaras Hindu University; University of Agricultural Sciences Dharwad; Punjab Agricultural University; Ege University

Chickpea is a pulse legume of critical importance in low-income food insecure countries, in advanced developing economies, and in developed countries. Paradoxically, countries with the highest nutritional demand for chickpea are also those with the lowest yields, often ½ to ¼ of yields found in the developed world. Whole genome sequencing reveals that ~95% of genomic variation was lost from modern elite cultivars during domestication. This has profound implications, because corresponding reductions to trait variation limit the ability to adapt the crop to changing environments and to meet emerging needs, raising an urgent need for new sources of diversity. We address this need by harnessing the expanded genetic potential of chickpea's wild relatives, focusing on traits related to tolerance to biotic and abiotic stress, improved seed nutrient density and symbiotic nitrogen fixation. We have built and are characterizing a large and systematic collection of wild *Cicer* species from a representative range of natural environments. Genomic technologies have been used to develop an improved genome of the cultivated species, and two new genomes of wild relatives. We have characterized genetic diversity among ~1,100 accessions of the wild progenitor and nominated particular plant accessions as targets of pre-breeding, phenotyping and breeding. We have identified trait variation for flowering time, pest resistance, nitrogen fixation, heat tolerance, plant architecture, seed phenotypes, yield, and drought tolerance, among others. A pre-breeding population involves twenty-six diverse wild donor accessions crossed into five cultivated elite varieties, with ~10,000 independent segregating progeny. The outcomes of this project are intended to be high-yielding, climate-resilient chickpea varieties within the context of user-preferred traits: seed quality and nutrient density, reduced inputs due to climate resilient nitrogen fixation, and biotic stress resistance among them. Parallel projects on microbial symbionts have characterized ~1,500 *Mesorhizobium* genomes, identifying domestication-associated shifts in genome content, with a systematic effort to develop commercial-grade inoculants for use in the developing and developed world. Finally, similar activities involving both culture independent and culture dependent on the chickpea microbiome has

identified taxa that are highly enriched on and within chickpea roots that are candidates for improving plant health.

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E.J.B. von Wettberg, P.L. Chang, A. Greenspan, S. Moenga, B. Alford, E. Dacosta-Calheiros, M.A. Yilmaz, A. Cakmak, K.S. Moriuchi, N. Carrasquila-Garcia, B. Erena Mamo, V. Singh, M.A. Cordeiro, L. Balcha, L. Vance, E. Bergmann, E.J. Warschefsky, K. Negash Dinegde, S.G.A. Shah Sani, J. Rose, A. Migneault, C.P. Krieg, F. Basdemir, K. Raiz, R.M. Atif, S. Yimer, D. Bekele, R. Mufti, T. Getahun, G. Sefara, S. Ijaz, M. Yildirim, B. Tanyolac, L.P. Henao, A.Y. Zhang, Z. Damtew, M. Chichaybelu, R. Immareddy, B. Sarma, E. Marques, F. Assefa, A. Surendrarao, S. Singh, B. Patil, S. Saylak, H. Temel, N.V. Noujdina, M.L. Friesen, E. Siler, D. Lindsay, H. Ozelik, J. Kholova, H. Sharma, P. Gaur, V. Vadez, K. Tesfaye, A.F. Woldemedhin, B. Tar'an, A. Aydogan, B. Bukun, R.V. Penmetsa, J. Berger, A. Kahraman, S.V. Nuzhdin, D.R. Cook (2017) Harvesting crop wild relatives to improve chickpea cultivation in food-insecure countries; ICLGG 2017 - Book of abstracts, ICLGG2017/PL/131

Book of abstracts
ISBN 978-615-5270-39-0

Exploiting systematic mutagenesis to identify targets for gene editing

S.U. Andersen

Department of Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark

CRISPR/Cas9 genome editing has enabled targeted modification of specific genomic loci, offering a new strategy for quick elimination of undesirable alleles in elite cultivars. Complex multigenic traits are not currently amenable to modification by genome editing. In contrast, genes underlying qualitative Mendelian traits are attractive targets. However, the identification of such genes remains a major challenge in most crops, and although a number of Mendelian traits with agronomic potential have been identified in legumes, the corresponding causal alleles have only been identified in relatively few cases. Genetic mapping approaches based on natural variation are hampered by large numbers of possible causal polymorphisms, and systematic mutagenesis coupled with phenotypic screening therefore presents an attractive alternative. Strategies for causal gene identification facilitated by systematic chemical or retrotransposon mutagenesis coupled with next-generation sequencing will be discussed [1-2]. In this context, retrotransposon mutagenesis offers unique opportunities through gene-tagging and development of annotated mutant collections. These will be illustrated drawing on examples from the Lotus japonicus LORE1 resource [3-4], and possible approaches for developing similar resources in other legumes will be outlined.

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How to refer your abstract:
S.U. Andersen (2017) Exploiting systematic mutagenesis to identify targets for gene editing; ICLGG 2017 - Book of abstracts, ICLGG2017/PL/130

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Genetic behavior and genome diversity in *Arachis hypogaea*

D.J. Bertioli[1,2], S.C.M. Leal-Bertioli[1,3], B. Abernathy[1], C. Chavarro[1], J. Clevenger[1], C. Ballen[1], J. Jenkins[4], J. Grimwood[4], J. Schmutz[4], B. Scheffler[5], P. Ozias-Akins[6], S.A. Jackson[1]

[1] *Center for Applied Genetic Technologies, University of Georgia, Athens, USA*
[2] *University of Brasília, Institute of Biological Sciences, Campus Darcy Ribeiro, Brasília, Brazil*
[3] *Embrapa Genetic Resources and Biotechnology, Brasília, Brazil*
[4] *HudsonAlpha Institute for Biotechnology, Huntsville, USA*
[5] *Genomics and Bioinformatics Research Unit, Stoneville, USA*
[6] *Department of Horticulture, University of Georgia, Tifton, Georgia, USA*

Cultivated peanut (*Arachis hypogaea* L.) is an oilseed and grain legume that is widely cultivated and important both in international trade and as an energy and protein source for smallholder farmers. It is an allotetraploid (genome type AABB) with closely related component genomes that diverged only 2-3 million years ago. This makes the assembly of the *A. hypogaea* genome very challenging. Fortunately, it ancestors are well-defined; *A. duranensis* and *A. ipaënsis*, which contributed the A and B component genomes respectively. Additionally, since polyploidy, the ancestral component genomes have remained substantially distinct and intact. However, during meiosis, chromosomes from different subgenomes occasionally do interact and exchange genetic information. This leads to a genetic behaviour that is not completely as expected for a classic allotetraploid. Furthermore, it has provided a drive for genome diversification.

How to refer your abstract:
D. J. Bertioli, S.C.M. Leal-Bertioli, B. Abernathy, C. Chavarro, J. Clevenger, C. Ballen, J. Jenkins, J. Grimwood, J. Schmutz, B. Scheffler, P. Ozias-Akins, S.A. Jackson (2017) Genetic behavior and genome diversity in Arachis hypogaea; ICLGG 2017 - Book of abstracts, ICLGG2017/PL/132

Book of abstracts
ISBN 978-615-5270-39-0

The pea genome

J. Kreplak, M.A. Madoui, K. Labadie, G. Aubert, P. Bayer, P. Capal, A. Klein, A. Kougbeadjo, J. Vrana, K.K. Gali, C. Fournier, L. d'Agata, B. Taran, C. Belser, M.C. Le Paslier, A. Bendahmane, H. Bergès, V. Barbe, R. McGee., J. Lichtenzveig, C. Coyne, T. Warkentin, J. Batley, J. Macas, D. Edwards, J. Dolezel, P. Wincker, **J. Burstin**

The International Pea Genome Consortium

Pea (*Pisum sativum* L.) has long been a model for plant genetics. It is also a widely grown pulse crop producing protein-rich seeds in a sustainable manner. Thanks to large national and international programs, and driven by innovations in sequencing technology, informatics and biotechnology, many genomic resources are now available for pea. An atlas of the expression of its genes in many tissues, high density genetic mapping, and the ongoing sequencing of its genome have provided useful tools for dissecting traits of interest. We will present how the pea genome draft sequence opens the way to explore genetic diversity of pea.

How to refer your abstract:
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Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Lentil genomes: weird and wonderful wildlings

K.E. Bett[1], L.D. Ramsay[1], K. Koh[2], C.T. Caron[1], G. Ronen[3], A. Vandenberg[1]

[1] *Department of Plant Sciences, University of Saskatchewan, Canada*
[2] *Global Institute for Food Security, University of Saskatchewan, Canada*
[3] *NRGene Technologies, Israel*

Lentil (*Lens culinaris* L.) is becoming an increasingly important food crop globally, but due to its large genome (~4 Gb) and limited research funding, few resources have been available to the breeding community until recently. In 2016 we released a draft assembly of the genome of the cultivated species, *L. culinaris*, which led to the development of several useful molecular markers for the breeding program and resources for further investigation into this interesting genome.

Within our breeding program at the University of Saskatchewan (USASK) and within other groups around the world, wild *Lens* species are of interest as sources of useful genetic variation. *Lens ervoides* has been used in the USASK breeding program for many years and improvements in disease resistance and overall plant vigour are noticeable. *Lens lamottei, L. odemensis* and *Lens tomentosus* also have genetic variability of interest to lentil breeders. We sequenced *L. ervoides* using both paired-end (42 x coverage) and mate-pair (54 x coverage) libraries and produced a crude assembly of the genome. For *L. odemensis* and *L. lamottei* we partnered with NRGene and produced two very high quality genome assemblies using second and third generation sequencing. Structural genomic variation among and within species is evident. We are using these data to map traits in intraspecific populations to track introgressions and to identify candidate genes associated with traits of interest for the breeding program.

How to refer your abstract:
K.E. Bett, L.D. Ramsay, K. Koh, C.T. Caron, G. Ronen, A. Vandenberg (2017) Lentil genomes: weird and wonderful wildlings; ICLGG 2017 - Book of abstracts, ICLGG2017/OL/110

Book of abstracts
ISBN 978-615-5270-39-0

A reference genome sequence of cowpea

S. Lonardi[1], M. Muñoz-Amatriaín[2], S.I. Wanamaker[2], Q. Liang[1], T. Zhu[3], M.C. Luo[3], D.M. Goodstein[4], S. Shu[4], **T.J. Close**[2]

[1] *Department of Computer Science and Engineering, University of California Riverside, California, USA*
[2] *Department of Botany and Plant Sciences, University of California, Riverside, California, USA*
[3] *Department of Plant Sciences, University of California, Davis, California, USA*
[4] *U.S. Department of Energy, Joint Genome Institute, Department of Energy, Walnut Creek, California, USA*

Cowpea, *Vigna unguiculata* L. *Walp*, is a diploid warm-season legume with a genome size of ~620 Mb. Cowpea, known as blackeyed pea among other common names, is relevant as a grain legume in the USA and Europe, and as a fresh vegetable in China and elsewhere, but is of major importance as food and fodder in sub-Saharan Africa. Here we describe the production of a reference genome sequence of an elite African variety, IT97K-499-35, based on single molecule real-time sequencing (91x coverage; Pacific Biosciences) together with two optical maps (BioNano Genomics) and ten genetic linkage maps containing a total of 44,003 SNPs. The v1.0 cowpea pseudomolecules contain 519 Mb of sequence, derived from superscaffold sequences with N50 = 16.4 Mb and L50 = 12. Synteny between cowpea and other warm-season legumes has been clarified, including common bean (*Phaseolus vulgaris* L.), which provided the basis of new cowpea chromosome numbering. A total of 29,773 gene models were annotated using a combination of *ab initio* and transcript (RNA-Seq and Sanger EST) evidence, providing a measure of 95.9% plant completeness using BUSCO v2. This reference genome sequence, which is accessible through Phytozome (www.phytozome.net), constitutes an important resource to understand its unique genome features for the improvement of cowpea and related species. This work was conducted mainly under the NSF BREAD project "Advancing the Cowpea Genome for Food Security" with partial support from the Feed the Future Innovation Lab for Climate Resilient Cowpea.

How to refer your abstract:
S. Lonardi, M. Muñoz-Amatriaín, S.I. Wanamaker, Q. Liang, T. Zhu, M.C. Luo, D.M. Goodstein, S. Shu, T.J. Close (2017) A reference genome sequence of cowpea; ICLGG 2017 - Book of abstracts, ICLGG2017/OL/87

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Development of genomic resources for narrow-leafed lupin, including a reference genome and pan-genome and identification of candidate genes for domestication traits

L.G. Kamphuis[1,2,3], G. Garg[1], P. Bayer[4], R.C. Foley[1], L.-L. Gao[1], M.N. Nelson[2,5], J.K. Hane[3], D. Edwards[4], K.B. Singh[1,2,3]

[1] *CSIRO Agriculture & Food, Floreat, WA, Australia*
[2] *UWA Institute of Agriculture, University of Western Australia, Crawley, Australia*
[3] *Centre for Crop and Disease Management, Curtin University, Bentley, Australia*
[4] *School of Biological Sciences, University of Western Australia, Crawley, Australia*
[5] *Natural Capital and Plant Health, Royal Botanic Gardens, Kew, Wakehurst Place, Ardingly, UK*

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.

We generated a high quality reference genome assembly (609Mb), which has captured >98% of the gene content [1]. Furthermore in-depth RNAseq datasets from five different tissue types, being roots, stems, leafs, flowers and seeds have been generated [2]. These datasets were used to develop gene-based molecular insertion/deletion (indel) and SNP markersin and in addtion DArTSeq data was generated to create a dense reference genetic map (n=9,972 markers across 20 chromosomes). The transcriptome datasets, the novel gene-based molecular markers and improved genetic map are housed on the lupin genome portal [3], which also has BLAST and Gbrowse interface to assess the genome and transcriptomes. Current research focuses on the generation of a pan-genome for the species using 40 genetically diverse NLL accessions. These resources have lead to the identification of potential candidate genes for a number of important traits. In conclusion the developed resources will significantly improve and accelerate NLL breeding programmes, especially since NLL has only been 'domesticated' for little more than 50 years.

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L.G. Kamphuis, G. Garg, P. Bayer, R.C. Foley, L.-L. Gao, M.N. Nelson, J.K. Hane, D. Edwards, K.B. Singh (2017) Development of genomic resources for narrow-leafed lupin, including a reference genome and pan-genome and identification of candidate genes for domestication traits; ICLGG 2017 - Book of abstracts, ICLGG2017/OL/27

Book of abstracts
ISBN 978-615-5270-39-0

The *Lathyrus sativus* genome project

A. Sarkar, P.M.F. Emmrich, A. Edwards, C. Martin, T.L. Wang

The John Innes Centre, Norwich, UK

Grasspea (*Lathyrus sativus*) is a hardy legume grown by poor and marginal farmers in the Indian subcontinent and Africa for animal feed, food and fodder, often on impoverished soils with minimal inputs. The presence of a neurotoxin (beta-ODAP), which can cause neurolathyrism in people subsisting on a predominantly grass pea diet for an extended length of time, is a major factor preventing wider adoption of this promising crop. It is a diploid (2n=14) with an estimated haploid genome size of 6.9 Gbp [1].

We report on the progress of sequencing the grass pea genome of a European line. A *de novo* shotgun sequencing strategy has been adopted based on the construction of a PCR-free library for paired end sequencing and several mate-pair libraries for sequencing on the Illumina platform. This has been supplemented by long read sequencing using MinION (Oxford Nanopore Technologies) to improve higher order assembly in the absence of good genetic or physical maps. The draft genome is being annotated using transcriptome data from this and two Indian lines, as well as data from genome and transcriptome sequences of related legumes.

The draft genome sequence will aid in the identification of the genes in the beta-ODAP biosynthesis pathway, and also of genes for various traits of interest. The data will help in the development of high quality genetic and physical maps for marker-assisted and genomic selection strategies for agronomic improvement. Additionally, the draft genome will aid in gene function analysis by TILLING, as well as enable a genome editing platform for grass pea.

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How to refer your abstract:
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Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Genetic diversity and strategies for seed quality enhancement in pea

C. Domoney[1], T. Rayner[1], C. Moreau[1], M. Ambrose[1], A. Clemente[2], N. Ellis[3], P.G. Isaac[4]

[1] *John Innes Centre, Norwich, Norwich Research Park, UK*
[2] *Estación Experimental del Zaidín, Granada, Spain*
[3] *Department of Biology Sciences, University of Auckland, Auckland, New Zealand*
[4] *IDna Genetics Ltd, Norwich Research Park, Norwich, UK*

High-throughput screening methods have been deployed to identify natural variation and induced mutations in genes which control seed composition and visual traits. For example, a *Pisum elatius* accession was identified as an extremely rare null trypsin-chymotrypsin inhibitor mutant, where both closely-linked genes which encode the major seed inhibitors showed deletion of coding sequence [1]. Combining this variant with a fast neutron-derived null mutation for seed lectin [2] and a natural variant lacking pea albumin 2 [3] provides opportunities for considerable gain in nutritional quality in pea seeds. A series of deletion mutations is being used to generate seeds lacking the major seed protein, vicilin, leading to major changes in protein composition and functionality.

Mutations affecting the concentration of resistant starch in wrinkled-seeded pea seeds are being used to investigate the benefits conferred by such starch to human health that are relevant to the prevention of Type 2 diabetes; one rare wrinkled-seeded phenotype has been shown to be maternally determined, affecting metabolism in the seed coat [4]. Variation within a range of metabolites accumulated in wrinkled seeds can be defined genetically.

Besides seed composition, visual traits can also influence the economic value of seeds for food crops. Variation in the control of colour loss from seeds and leaves in pea relates to the regulation of the chlorophyll degradation pathway, which may be controlled genetically while avoiding perturbations in chlorophyll turnover which impair plant performance and yield [5].

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How to refer your abstract:
C. Domoney, T. Rayner, C. Moreau, M. Ambrose, A. Clemente, N. Ellis, P.G. Isaac (2017) Genetic diversity and strategies for seed quality enhancement in pea; ICLGG 2017 - Book of abstracts, ICLGG2017/PL/70

Book of abstracts
ISBN 978-615-5270-39-0

The role of MADS-box genes in the evolution of fruit morphology and seed dispersal strategies

C. Ferrandiz, C. Fourquin, I. Martínez-Fernandez, A. Berbel, F. Madueño

Spanish National Research Council (CSIC)- Instituto de Biologia Molecular y Celular de Plantas, Valencia, Spain

Fruits are a major evolutionary acquisition of Angiosperms. Fruits evolved to protect the developing seeds and to ensure seed dispersal, and for that, they have adopted a huge morphological and functional diversity, greatly responsible for the evolutive success of flowering plants. In addition, fruits are of major economic importance, representing the edible part of many crops as well as being a source for production of seed, oil and other compounds. Fruit patterning depends in great extent from carpel patterning, the process of specification, differentiation and spatial arrangement of different functional compartments in the carpels, the ovule-bearing floral organs organized into the female reproductive structure of the flower, or gynoecium. Our long-term goal is to understand how fruit patterning is established, and what is the molecular basis of the morphological and functional diversity found between species.

A robust model explaining genetics of seed dispersal has been proposed in Arabidopsis, involving the transcription factors *FRUITFULL* (*FUL*), *SHATTERPROOF1* and *2* (*SHP1, SHP2*). A key question we need to address is how well these genetic pathways are conserved among the flowering plants, and how modifications on these routes have contributed to generate fruit morphological and functional diversity. To serve this purpose, we focus our study on the Leguminosae family. First, we have evaluated the functional conservation of this genetic network in two legume species, *Pisum sativum* and *Medicago truncatula*, possessing highly different fruit morphologies. Our studies include functional and molecular characterization of the *FUL* and *SHP* orthologues in these species, including expression studies, heterologous complementation, characterization of mutants in pea and *M. truncatula*, etc. Second, we have tested if variations of the Arabidopsis model can be related to morphological and functional fruit diversity. For this purpose, we have studied the *Medicago* genus, which presents a large range of fruit morphologies, from straight and long pods to highly coiled and spiny fruits. All together, our data point to a key role of the FUL/SHP genetic route in controlling pod morphology in *Medicago*, and thus, unveiling the importance of the variation in this genetic network to generate fruit diversity. Furthermore, our results provide insights on possible mechanisms of domestication of pod indehiscence in grain legumes that will be discussed.

How to refer your abstract:
C. Ferrandiz, C. Fourquin, I. Martínez-Fernandez, A. Berbel, F. Madueño (2017) The role of MADS-box genes in the evolution of fruit morphology and seed dispersal strategies; ICLGG 2017 - Book of abstracts, ICLGG2017/PL/93

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Nodule-specific plant peptides control intracellular accommodation of symbiotic bacteria

J. Montiel[1], Q. Wang[2], S. Yang[2], A. Downie3, B. Balint[4], A. Gombár[5], J. Liu[2], E. Ábrahám[1], A. Farkas[1], P. Bihari[4], Á. Domonkos[5], T. Wang[1], P. Mergaert[6], L. Fodor[5], L. Mao[7], Z. Fei[7], E. Kondorosi[1], P. Kaló[5], H. Zhu[2], **A. Kereszt**[1]

[1] *Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary*
[2] *University of Kentucky, Lexington, USA*
[3] *John Innes Centre, Norwich, United Kingdom*
[4] *Seqomics Biotechnology Ltd., Mórahalom, Hungary*
[5] *National Agricultural Research and Innovation Centre, Gödöllő, Hungary*
[6] *Institute for Integrative Biology of the Cell, Gif-sur-Yvette, France*
[7] *Cornell University, Ithaca, USA*

In the nodules of the Inverted Repeat-Lacking Clade (IRLC) legumes, antimicrobial-like molecules called nodule-specific cysteine-rich (NCR) plant peptides are produced and delivered to the developing symbiotic bacteria termed bacteroids. As a consequence, rhizobia encounter a so-called terminal differentiation during which their genome is multiplied via endoreduplication cycles, their size increases, they loss their reproductive capacity, and the bacteroids end up with different morphologies that can be swollen, elongated, spherical, and elongated–branched, depending on the host plant. In the model legume Medicago truncatula, more than 700 genes are predicted to code for NCRs and the expression of 639 members of the family could be detected in nodules. Despite the high number of NCR genes, deletions of certain individual genes (NCR169, NCR211) result in the failure of the symbiotic interaction.

To investigate the evolution of bacteroid differentiation and the NCR peptides we studied the morphology and cell division capacity of bacteroids in a number of legumes representing different subclades of IRLC, then identified the predicted NCR proteins from these legumes housing distinct bacteroid morphotypes. Via the analysis of their expression and predicted sequences, we were able to establish correlations between the composition of the NCR family and the morphotypes of bacteroids. Phylogenetic analysis revealed that NCRs have a single origin, however, their evolution has followed different routes in individual lineages, and enrichment and diversification of cationic peptides has resulted in the ability to impose major morphological changes on the endosymbionts. The wide range of effects provoked by NCRs such as cell enlargement, membrane alterations and permeabilization, as well as biofilm and vesicle formation is dependent on the amino acid composition and charge of the peptides.

Interestingly, studies on the incompatible interaction between M. truncatula cv. Jemalong and Sinorhizobium meliloti strain RM41 that form effective symbioses with other partners, revealed that allelic forms of two NCR peptides are responsible for the elimination of the developing bacteroids from the nodules.

How to refer your abstract:
J. Montiel, Q. Wang, S. Yang, A. Downie, B. Balint, A. Gombár, J. Liu, E. Ábrahám, A. Farkas, P. Bihari, Á. Domonkos, T. Wang, P. Mergaert, L. Fodor, L. Mao, Z. Fei, E. Kondorosi, P. Kaló, H. Zhu, A. Kereszt (2017) Nodule-specific plant peptides control intracellular accommodation of symbiotic bacteria; ICLGG 2017 - Book of abstracts, ICLGG2017/PL/134

Book of abstracts
ISBN 978-615-5270-39-0

Comparative genetic analysis of flowering time adaptation in legumes

J.L. Weller[1], R. Ortega[1], J.K. Vander Schoor[1], V. Rajandran[1], O. Williams[1], V. Hecht[1], E.C. Perez-Wright[1], S. Ridge[1], A.J.S. Rubenach[1], R. Lee[2], D.M. Bond[2], R.C. Macknight[2], R.V. Penmetsa[3], D.R. Cook[3], K.E. Bett[4], T. Millàn[5], A. Gonzalez[6], M. Santalla[6]

[1] *School of Biological Sciences, University of Tasmania, Hobart, Tasmania, Australia*
[2] *Department of Biochemistry, University of Otago, Dunedin, New Zealand*
[3] *Department of Plant Pathology, University of California, Davis, USA*
[4] *Department of Plant Sciences, University of Saskatchewan, Saskatoon, Canada*
[5] *Department of Genetics, University of Cordoba, Cordoba, Spain*
[6] *Misión Biológica de Galicia-CSIC, Pontevedra, Spain*

A better understanding of flowering genes in legume crops will be valuable in understanding their prehistoric expansion from regions of initial domestication, in breeding for new environments and in accessing wider genetic diversity present in wild crop relatives. We are using a comparative approach to explore the genetic network controlling flowering time adaptation in a number of legume species. In addition to the use of induced mutants in pea (*Pisum sativum*) and barrel medic *(Medicago truncatula*), recent work has focused on characterization of natural variation in crop species including pea, lentil (*Lens culinaris*) and chickpea (*Cicer arietinum*). We have performed comparative phylogenetic analyses of many of the major flowering gene families in legumes, and examined the expression patterns of key genes, including members of the *FT* family of florigen genes. A positional candidate gene approach has enabled the identification of putative causal genes for major flowering loci and shown a striking conservation in certain genomic regions conferring flowering time adaptation across several species. Evidence on the molecular and physiological basis for adaptive changes at these loci will be presented and possible reasons for their prominence will be discussed.

How to refer your abstract:
J.L.Weller, R. Ortega, J.K. Vander Schoor, V. Rajandran, O. Williams, V. Hecht, E.C. Perez-Wright, S. Ridge, A.J.S. Rubenach, R. Lee, D.M. Bond, R.C. Macknight, R.V. Penmetsa, D.R. Cook, K.E. Bett, T. Millàn, A. Gonzalez, M. Santalla (2017) Comparative genetic analysis of flowering time adaptation in legumes; ICLGG 2017 - Book of abstracts, ICLGG2017/OL/49

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

MtSOC1a promotes flowering and elongation of the primary shoot axis in the reference legume *Medicago truncatula*

M. Jaudal[1], C. Che[1], L. Zhang[1], J. Wen[2], K.S. Mysore[2], J. Putterill[1]

[1] *Flowering Lab, School of Biological Sciences, University of Auckland, New Zealand*
[2] *Plant Biology, Samuel Roberts Noble Foundation, Ardmore, Oklahoma, USA*

The *SOC1* gene is an important integrator of different flowering time pathways in Arabidopsis. *SOC1-like* genes regulate flowering in some plants while others have different functions. However, no *soc1* mutants have been characterized yet in legumes. Flowering of the reference legume Medicago, like Arabidopsis, is promoted by vernalisation and long day (LD) photoperiods. However, different mechanisms of flowering time control seem to be involved because Medicago lacks *FLC-like* genes and *CO* function. In this study, three Medicago *SOC1-like* genes (*MtSOC1a-c*) were characterised. *MtSOC1a* and *MtSOC1c* transcript levels were elevated in the shoot apex just prior to flowering in LD indicating a possible involvement in the floral transition, while *MtSOC1b* increased in the shoot apex after flowering. All the *MtSOC1-like* genes depended on a *FT-like* gene, *FTa1*, for the magnitude and timing of their expression. Overexpression in Arabidopsis indicated that *MtSOC1a* was the most effective at promoting flowering. The *Mtsoc1a Tnt1* insertion mutant line flowered late in LD and short days (SD) with a very short primary axis and reduced expression of *MtSOC1b-c and FUL-like* genes. *Mtsoc1a* mutants with *35S:MtSOC1a* transgene showed a precocious increase in primary shoot axis height. However, loss of *MtSOC1b* had no effect on flowering time and architecture. This study indicates that *MtSOC1a* is regulated by *FTa1* and has an important function in promotion of flowering and regulation of primary shoot axis elongation in *Medicago*.

How to refer your abstract:
M. Jaudal, C. Che, L. Zhang, J. Wen, K.S. Mysore, J. Putterill (2017) MtSOC1a promotes flowering and elongation of the primary shoot axis in the reference legume Medicago truncatula; ICLGG 2017 - Book of abstracts, ICLGG2017/OL/36

Book of abstracts
ISBN 978-615-5270-39-0

Complex interactions in the rhizosphere: interplay between rhizobia, mycorrhizae, and the microbiome across *Medicago* genotypes

C.A. Friel[1], M.E. Afkhami[2], M.F. Friesen[1,3]

[1] *Department of Plant Biology, Michigan State University, East Lansing, USA*
[2] *Department of Biology, University of Miami, Miami, USA*
[3] *Department of Plant Pathology and Department of Crop and Soil Sciences, Washington State University, Pullman, USA*

Increasing agricultural sustainability is of utmost importance in the face of climate change and population increase. Although synthetic fertilizers have fueled a boom in agricultural productivity, manufacture and use of these fertilizers is environmentally damaging [1]. An alternative source of essential mineral nutrients comes from interactions between plants and microbial resource mutualists. Arbuscular mycorrhizal fungi (AMF) supply phosphate (P) and nitrogen (N) from the soil, while rhizobial bacteria fix N out of the atmosphere in exchange for photosynthetic carbon (C). These symbionts compete with the rest of the rhizosphere microbiome for a limited supply of plant C, but are frequently studied in isolation.

To investigate the interactions between microbial mutualists, plant genotype, and the larger soil microbial community, we factorially manipulated the presence of rhizobia, AMF, and a native soil microbiome across 16 genotypes of the model legume *Medicago truncatula*, representing its full genetic diversity. We used qPCR to mutualist population sizes and to quantify nutrient transfer. We also used 16S sequencing to assess the composition of the rhizosphere microbiome. This data will allow us to answer three questions: 1) How does the presence of a native soil community affect the symbiotic function of resource mutualists? 2) How does the presence of commercial-level inocula of resource mutualists affect the makeup of the rhizosphere microbiome? And 3) Does the presence of resource mutualists mediate the effect of plant genotype on microbiome composition?

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How to refer your abstract:
C.A. Friel, M.E. Afkhami, M.F. Friesen (2017) Complex interactions in the rhizosphere: interplay between rhizobia, mycorrhizae, and the microbiome across Medicago genotypes; ICLGG 2017 - Book of abstracts, ICLGG2017/OL/77

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Common bean microRNAs: unraveling novel players for the control of rhizobia nitrogen fixing symbiosis

G. Hernández[1], D. Formey[1], J.A. Martín-Rodríguez[1], J.L. Reyes[2], L. Cárdenas[2], L. Girard[1], B. Nova-Franco[1], L.P. Íñiguez[1], A. Leija[1]

[1] *Centro de Ciencias Genómicas – Universidad Nacional Autónoma de México (UNAM), Cuernavaca, Mexico*
[2] *Instituto de Biotecnología – UNAM, Cuernavaca, Mexico*

This work aims to identify the whole set of microRNAs (miRNAS) from common bean (*Phaseolus vulgaris*) and to functionally characterize new regulators especially for the rhizobia symbiosis (RS). Based in sRNA and degradome RNA-seq data we have performed two genome-wide analyses of the common bean sRNAome: one includes libraries from different plant organs and the second was done in root hairs –a single-cell model- induced with pure *Rhizobium etli* nodulation factors –a unique signal molecule. Precursors and mature miRNAs and their target genes were identified, including more than 100 novel miRNAs. We constructed weighted correlation networks of miRNAs that describe the pairwise relationship among miRNAs that differentiate the nodule library from other libraries; novel miRNAs from identified networks are proposed to act in the regulation of RS. We demonstrated the key role of the node miR172c/APETALA2-1 (AP2) in the common bean RS. Increased expression of miR172c improves rhizobial infection, nodulation, SNF, expression of AON genes and decreased sensitivity to nitrate inhibition of nodulation. We are analyzing two novel miRNAs included in the identified networks: a novel isoform of the miR319 family and miRNov270. These miRNAs showed differential expression in bean nodules and opposite expression of their proposed targets: the TCP transcription factor and a LRR-kinase, respectively. The current analysis of the symbiotic phenotype of composite bean plants overexpressing or silencing each of these candidates, would allow deciphering their roles in the RS.

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G. Hernández, D. Formey, J.A. Martín-Rodríguez, J.L. Reyes, L. Cárdenas, L. Girard, B. Nova-Franco, L.P. Íñiguez, A. Leija (2017) Common bean microRNAs: unraveling novel players for the control of rhizobia nitrogen fixing symbiosis; ICLGG 2017 - Book of abstracts, ICLGG2017/OL/26

Book of abstracts
ISBN 978-615-5270-39-0

Using *Medicago truncatula* to tackle disease issues in legumes with a focus on soil-borne fungal pathogens and insect pests

K.B. Singh

CSIRO Agriculture and Food, Floreat, Western Australia, Australia
Centre for Crop and Disease Management,Curtin University, Floreat, Australia

We are interested in using the model legume *Medicago truncatula* to help dissect important pest and pathogen problems facing legume crops. One area of activity is around plant defence to aphids and related phloem-feeding insects, which cause severe plant damage, through feeding activities and as vectors of plant viruses. Our group also uses *M. truncatula* to look at plant resistance mechanisms and fungal pathogenicity strategies for soil-borne fungal pathogens. One is *Rhizoctonia solani* AG8, a devastating pathogen causing bare patch of cereals, brassicas and legumes. The other is Fusraium oxysporum which causes wilt diseases on many crops, including most legumes. In both cases a combination of approaches on both the pathogen/pest and plant side of these interactions is helping provide valuable insight and opening up opportunities to generate enhanced resistance in crops and important leads to follow to probe for weaknesses in the pathogen.

How to refer your abstract:
K.B. Singh (2017) Using Medicago truncatula to tackle disease issues in legumes with a focus on soil-borne fungal pathogens and insect pests; ICLGG 2017 - Book of abstracts, ICLGG2017/PL/143

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Quantitative resistance for durable management of *Aphanomyces* root rot of pea

M-L. Pilet-Nayel[1,2], C. Lavaud[1,2], A. Desgroux[1,2], A. Lesné[1,2], G. Boutet[1,2], A. Quillévéré-Hamard[1,2], C. Le May[1,2,3], A. Moussart[1,2,4], A. Baranger[1,2]

[1] *INRA, Institut de Génétique, Environnement et Protection des Plantes, Le Rheu, France*
[2] *PISOM, Unité Mixte Technologique INRA/Terres Inovia, Le Rheu, France*
[3] *Agrocampus-Ouest, Institut de Génétique, Environnement et Protection des Plantes, Le Rheu, France*
[4] *Terres Inovia, Thiverval Grignon, France*

Quantitative resistance is of growing interest in plant breeding for pathogen control in low-input cropping systems, due to its high durability potential. For more than 15 years, we have explored pea quantitative resistance to the major soil-borne pathogen *Aphanomyces euteiches*.

From pea partially resistant germplasm, QTL (Quantitative Trait Loci) and GWA (Genome-wide Association) mapping studies identified main genomic regions controlling quantitative resistance, together with closely-linked markers and favorable haplotypes[1,2]. Marker assisted back-cross-introgressions were performed to create NILs (Near Isogenic lines) at single or combinations of these genomic regions, to validate effects and identify combinations of QTL contributing to higher levels of quantitative resistance[3]. QTL affecting different steps of the pathogen life cycle were identified from the NILs[4], opening the way to the pyramiding of QTL with different action modes to achieve a more effective and durable control [5]. We further plan to fine-map resistance QTL, analyze their genomic conservation between legume hosts and estimate their effects on resistance to other pathogens of the root complex. Work is in progress to analyze the effect of resistance QTL deployment, combined to cultural control methods and rotations, on pathogen populations structure, soil inoculum potential evolution and on plant yield preservation.

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Book of abstracts
ISBN 978-615-5270-39-0

Insertion mutagenesis of *Medicago truncatula* and its utilization to identify novel sources of resistance against Asian soybean rust

U. Gill, Y. Ishiga, S.R. Uppalapati, S. Mittal, H-K. Lee, J. Wen, **K.S. Mysore**

Noble Research Institute, Ardmore, USA

Tobacco retrotransposon, *Tnt1*, has been used to mutagenize and tag the whole genome of a model legume, *Medicago truncatula*. *Tnt1* is very active and transpose into, on average, 25 different locations during *M. truncatula* tissue culture [1]. We have generated over 20,000 independent *Tnt1*-containing lines encompassing approximately 500,000 insertion events. Over 400,000 *Tnt1* flanking sequence tags (FSTs) have been recovered and a database has been established. We have pooled genomic DNA from all the lines for customized reverse-genetic screening, and the frequency of insert identification in this pool for average-sized-gene is approximately 85% percent [2]. The range and diversity of mutant phenotypes obtained to date suggest that *M. truncatula* offers a great opportunity to dissect symbiotic and developmental pathways for comprehensive understanding of legume biology. A forward genetics approach using *Tnt1* tagged *M. truncatula* lines has been established to identify genes that confer nonhost resistance to Asian Soybean Rust pathogen, *Phakopsora pachyrhizi*. Several *M. truncatula Tnt1* mutants with altered response to *P. pachyrhizi* have been identified and being characterized. *irg1* (inhibitor of rust germ-tube differentation1) mutant inhibited pre-infection structure differentiation of *P. pachyrhizi* and several other biotrophic pathogens [2]. *IRG1* encodes a Cys(2)His(2) zinc finger transcription factor, PALM1 that also controls dissected leaf morphology in *M. truncatula* [3]. Characterization of other mutants will be presented.

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Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Co-x, a non canonical disease resistance gene of common bean to the fungus *Colletotrichum lindemuthianum*, the agent of anthracnose

M.M.S. Richard[1], S. Blanchet[1], V. Thareau[1], P.N. Miklas[2], A. Gratias-Weill[1], C. Meziadi[1], S. Pflieger[1], W. Marande[3], H. Berges[3], **V. Geffroy**[1]

[1] *Institute of Plant Sciences Paris Saclay IPS2, CNRS, INRA, Université Paris-Sud, Université Evry, Université Paris Diderot, Université Paris-Saclay, Orsay, France*
[2] *USDA ARS, Grain Legume Genet & Physiol Res. Unit, Prosser, USA*
[3] *INRA, CNRVG, Castanet Tolosan, France*

Plant resistance to microbial pathogens is a complex process relying on different layers of resistance. Specific resistance relies on the specific recognition of pathogen-derived effectors, called Avirulence (Avr) proteins, by plant resistance (R) proteins encoded by *R* genes. Strikingly, the majority of cloned *R* genes encodes Nucleotide Binding-Leucine Rich Repeat (NB-LRR) proteins. Anthracnose, caused by the phytopathogenic fungus *Colletotrichum lindemuthianum*, is one of the most important diseases of common bean. Various specific resistance (*R*) genes, named *Co-*, conferring race-specific resistance to different strains of *C. lindemuthianum* have been identified. The *Co-x R* gene is interesting for both applied and academic reasons. Agronomically, *Co-x* confers resistance to an extremely virulent strain of *C. lindemuthianum*. From a fundamental point of view, preliminary mapping data suggested that *Co-x* gene is not a canonical plant disease *R* gene encoding a NB-LRR protein. In order to identify the atypical molecular basis of *Co-x*, we used a map-based cloning strategy, based on a RILs population and locus-specific markers developed thanks to the access to the complete genome sequence of the Andean genotype G19833. This allowed us to restrict the target region to 58kb in G19833. In this report, we will present the molecular basis of *Co-x*, a non-canonical resistance gene, and its peculiar evolutionary history in legume.

How to refer your abstract:
M.M.S. Richard, S. Blanchet, V. Thareau, P. N. Miklas, A. Gratias-Weill, C. Meziadi, S. Pflieger, W. Marande, H. Berges, V. Geffroy (2017) Co-x, a non canonical disease resistance gene of common bean to the fungus Colletotrichum lindemunianu, the agent of anthracnose; ICLGG 2017 - Book of abstracts, ICLGG2017/OL/64

Book of abstracts
ISBN 978-615-5270-39-0

An RNAseq approach towards deciphering mechanisms involved in bruchid tolerance in faba bean

J. Kreplak[1], M. Terezol[1], C. Desmetz[1], B. Raffiot[2], O. Bouchez[3], P. Marget[1], J. Burstin[1], G. Aubert[1]

[1] *UMR Agroécologie, INRA, Dijon, France*
[2] *Terres Inovia, Dijon, France*
[3] *GeT-PlaGe, INRA, Castanet-Tolosan, France*

Broad bean weevil (Bruchus rufimanus) is a major pest of faba bean. Once eggs are laid on the pods, larvae penetrate, develop in the seeds and create damage that affects the quality of the beans. This renders them unsuitable for the human consumption market. Therefore, in the context of reducing pesticide use and in order to develop faba bean varieties resistant to bruchid, the search for tolerant accessions is an important issue.

A germplasm screen has identified two accessions with good levels of tolerance, suggesting that these genotypes are less attractive to the insects and/or that their seeds contain compounds toxic for the larvae. In order to understand the underlying molecular mechanisms, we used an RNAseq transcriptomic approach on different plant tissues (leaf, flower, young pod and developing seed) of these two tolerant accessions and one additional sensitive cultivar.

As the Vicia faba genome has not yet been sequenced, a *de-novo* assembly was performed to build a set of genes to be used for differential expression analyses: individual assemblies per tissue and genotype have been done and clustered to eliminate redundancy. A SuperTranscript [1] of 30825 genes (average size of contigs 1945bp) has been obtained with good completeness (97% of BUSCO [2]) representing the transcriptome from the four organs of each of the three genotypes. Differential expression studies using this Unigene have highlighted contrasted response of the three genotypes for specific pathways and will help identifying regulated genes.

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Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Identifying genomic regions associated with disease resistance using GWAS: some real breeding examples in common bean

J.M. Osorno[1], J.S. Pasche[2], P.E. McClean[1], S. Schroder[1], S.M. Moghaddam[1], A. Soltani[1], K. Zitnick-Anderson[2], K. Simons[2], J.E. Vasquez[1], K. Gishing[1], C. Agarwal[1]

[1] *Department of Plant Sciences, North Dakota State University, Fargo, USA*
[2] *Department of Plant Pathology, North Dakota State University, Fargo, USA*

Common Bean (*Phaseolus vulgaris* L.) is the most important legume for direct human consumption worldwide1. Biotic stresses mostly in the form of fungal, bacterial, and viral diseases are among the most important limiting factors for achieving potential seed yields across all production areas2. The identification of genomic regions harboring disease resistance genes and the design of reliable DNA markers is of critical importance to continue the progress towards disease resistance. High-throughput genotyping and phenotyping tools in common bean in combination with Genome Wide Association Studies (GWAS) are powerful tools for both genetics and breeding3. Here we show several examples of how GWAS allowed the identification of important genomic regions controlling disease resistance to Halo Blight, Common Bacterial Blight, Root Rots, Rust, and Anthracnose. Some well-known genomic regions have been mapped more accurately, while in some other cases, new genomic regions have been discovered. In addition, some breeder-friendly markers have been developed in order to facilitate the selection process across multiple populations. In contrast with markers obtained from biparental mapping, GWAS markers appear to be more robust/reliable across multiple genetic backgrounds.

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Book of abstracts
ISBN 978-615-5270-39-0

Assessment of genetic purity of inter-specific F_1 hybrids involving *V. radiata* and *V. umbellata*

A.N. Bhanu, P. Kumar, M.N. Singh, K. Srivastava

Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India

Mungbean (*Vigna radiata* (L.) Wilczek) (2n=22), third in the series of important pulse crop, is an excellent source of easily digestible proteins. Using inter-specific hybridization, the useful traits of ricebean (*V. umbellata*) can be interogressed in mungbean to develop improved varieties in biotic stress-prone areas [1, 2]. Genetic purity test of true hybrids from controlled crosses before further generations of selfing or crossing and selection is essential for Mungbean improvement.

The present study was conducted to transfer mungbean yellow mosaic (MYM) disease resistance in mungbean from ricebean and to assess the genetic purity of developed inter-specific F_1 hybrids using morphological features and microsatellite markers. One ricebean genotype (RBL1) was hybridized as male with two genotypes of mungbean (K 851 and TM 96-2).

Significant difference in the crossability of ricebean genotype with greengram genotype was observed. Crossability was recorded 8.2% (TM 96-2 × RBL 1) and 4.6% (K 851 × RBL 1). Pollen fertility was recorded1.6% and 3.4% in TM 96-2 × RBL 1 and K 851 × RBL 1, respectively. Morphological features such as epicotyl colour, hypocotyl length, petiole length, germination habit, etc., were used as indicators of true hybridity. Molecular and morphological characterization verified the genetic purity of the developed hybrids. These hybrids exhibited resistance against mungbean yellow mosaic disease under natural epiphytotic field conditions. The present study will help in developing improved varieties or lines of mungbean coupled with stable MYMV resistance.

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A.N. Bhanu, P. Kumar, M.N. Singh, K. Srivastava (2017) Assessment of genetic purity of inter-specific F1 hybrids involving V. radiata and V. umbellata; ICLGG 2017 - Book of abstracts, ICLGG2017/OL/91

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Successful aflatoxin mitigation in peanut using HIGS and transgenic approaches: technology and translation

P. Bhatnagar-Mathur, K.K. Sharma

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad, Telangana, India

Aflatoxins are the most important foodborne carcinogenic contaminants of groundnut that are of important health and economic concerns. Combining the three independently inherited components of resistance to *Aspergillus flavus* infection and concomitant aflatoxin production has not yielded much success due to poor understanding of the host resistance mechanisms. Since, aflatoxin contamination in groundnut mostly occurs pre-harvest in the field, the control of *A. flavus* and aflatoxin contamination is critical and most effective prevention strategy. We have successfully induced genetic variability in groundnut to confer significant resistance to pre-harvest *A. flavus* infection and aflatoxin contamination by, (1) prevention of fungal infection by boosting the innate plant immunity, (2) prevention of subsequent fungal growth and, (3) inhibition of aflatoxin production in scenarios where fungal infection is difficult to eradicate. An altered host system biology with regards to peanut/*Aspergillus* pathosystem by differentially regulating the expression of candidate genes for altered specific host-pathogen interactions and subsequent activation of defense pathways. Fungal bioassays using mature seed cotyledons showed significantly lower toxin accumulation (0.1-4.0 ppb) against the inoculated untransformed control samples that accumulated >2000 ppb aflatoxin in the untransformed controls, and over 600 ppb in the best available resistant peanut cultivar. Our studies provided better understanding of the molecular-genetic mechanisms of different types of resistances for very low to non-existent levels of aflatoxin contamination that have significant potential to contribute to the current global efforts in developing peanut with very low to non-existent levels of aflatoxin contamination. This offers the possibilities of identifying resistance mechanisms that inhibit the fungal growth and aflatoxin biosynthesis. Efforts to translate this knowledge to introduce resistance to regionally adapted varieties of peanut are ongoing for wider adoption.

How to refer your abstract:
P. Bhatnagar-Mathur, K.K. Sharma (2017) Successful aflatoxin mitigation in peanut using HIGS and transgenic approaches: technology and translation; ICLGG 2017 - Book of abstracts, ICLGG2017/OL/47

Book of abstracts
ISBN 978-615-5270-39-0

Cross-species eQTL mapping: a new genetic approach to reveal causal interactions between symbionts

D. McKenzie Bird[1], D.M. Nielsen[1], V.M. Williamson[2]

[1] *NC State University, USA*
[2] *University of California-Davis, USA*

Research on parasitism by root-knot nematode (RKN: *Meloidogyne* spp.) has been hampered by the lack of a *bona fide* genetic platform. To redress this, we developed a QTL-based mapping strategy to attribute phenotypic differences in the host plant (Medicago) to genotypic differences in the parasite (*M. hapla*). Parental lines VW9 and LM exhibit differences in many agronomic traits. Exploiting the facultative meiotic parthenogenesis of *M. hapla* permitted construction of a mapping population of essentially homozygous F2 lines. Medicago was individually inoculated with each of the 98 nematode RILs (6 replicates), and RNASeq performed individually on the ~600 samples: hundreds of plant genes showing differential regulation, dependent of the nematode genotype, were revealed. These genes were broadly distributed across the Medicago genome. In contrast, the responsible nematode loci were typically in clusters. One such locus, regulating more than 60 Medicago genes, was delineated to 84kb by recombination breakpoints, and is predicted to encode 15 proteins, but we are yet to infer function. This region is highly polymorphic between LM and VW9. To infer processes more broadly, we performed Network Inference Analysis, enabling interactions and pathways to be deduced. Initial analyses point to defined RKN loci with a role in regulation of plant methyl and acetyl transferases. It was recently published that a soybean nematode R-gene (*RHg4)*, also encodes a methyl transferase. The mode of action conferring resistance remains unclear.

How to refer your abstract:
D. McKenzie Bird, D.M. Nielsen, V.M. Williamson (2017) Cross-species eQTL mapping: a new genetic approach to reveal causal interactions between symbionts; ICLGG 2017 - Book of abstracts, ICLGG2017/OL/135

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Drought response of nodulated roots in pea: from ecophysiological to transcriptomic analyses

M. Prudent[1], C. Salon[1], S. Girodet[1], C. Jeudy[1], N. Rossin[1], K. Boucherot[1], F. Jacquin[1], G. Aubert[1], S. Pateyron[2], A. Moing[3], S. Balzergues[2], V. Vernoud[1]

[1] *UMR1347 Agroécologie, AgroSup Dijon, INRA, Univ. Bourgogne Franche-Comté, Dijon, France*
[2] *POPS transcriptomic platform, Institute of Plant Sciences Paris-Saclay IPS2, CNRS, INRA, Univ Paris Sud, Univ Evry, Univ Paris-Diderot, Sorbonne Paris-Cite, Univ Paris-Saclay, Orsay, France*
[3] *Métabolomic Plateform Bordeaux−MetaboHUB, Centre de Génomique Fonctionnelle Bordeaux, IBVM, Centre INRA Bordeaux, Villenave d'Ornon, France*

In the context of climate change, more frequent episodes of water stress are expected, which will negatively impact symbiotic N_2 fixation and consequentely plant nitrogen nutrition, growth and productivity. This emphasizes the need to select drought tolerant pea genotypes. In this study, the physiological and transcriptional responses of both roots and nodules to a drought event, followed by a recovery period were investigated.

The hybridization of a 40k pea microarray indicated that, as a result of drought, ~390 and ~380 genes were at least 2-fold differencially regulated in roots and nodules, respectively. After rewatering, most of these genes were regulated in an opposite manner to drought effect. This analysis allowed to identify common and specific metabolic regulatory processes involved in drought tolerance and recovery. The most highly deregulated genes in response to drought (including LEA family members, delta-1-pyrroline-5-carboxylate synthase, SWEET family members...) were subsequently analysed for their expression patterns in response to several drought events each followed by a recovery period. We will discuss the behavior of these genes in terms of kinetics and intensity of their expression.

Acknowledgement: *this study was supported by the Burgundy & Franche-Comté Region (FABER program), Terres Inovia, the FP7-ABSTRESS project and its grant FP7-613551, the FP7-LEGATO project and its grant agreement FP7-289562.*

How to refer your abstract:
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Book of abstracts
ISBN 978-615-5270-39-0

The Yin and Yang of nodulation: regulatory peptides that positively and negatively regulate root and nodule development in response to nitrogen availability

N. Imin[1,2], N. Mohd-Radzman[1], A. Ivanovici[1], N. Patel[1], C. Delay[1], M. Taleski[1], K. Chapman[1], M.A. Djordjevic[1]

[1] *Research School of Biology, The Australian National University, Canberra, Australia*
[2] *School of Biological Sciences, University of Auckland, Auckland, New Zealand*

Plants - having to process information without brains - have evolved complex intercellular regulatory systems including regulatory peptide signalling to regulate growth, development and responses to their environment. Here, we describe regulatory peptides that is involved in the modulation of root, nodule and shoot development in response to nitrate limitations. In Arabidopsis, upon overexpression or exogenous application of CEP (C-TERMINALLY ENCODED PEPTIDE) peptides reduce root growth in a receptor dependent manner. A T-DNA insertion mutant shows the opposite phenotype, producing a larger root system under nitrate limitations through regulating G1 to S phase transition of the cell cycle in the root meristem. In *Medicago truncatula*, CEP peptide suppresses lateral root development and enhance root nodule formation and nitrogen fixation. The CEP peptide mediated enhancement of nodulation is partially tolerant to nitrate levels that suppress nodulation. We have isolated and identified several CEP peptides *in vivo* and have found that peptide activity differs according to the peptide's post-translational modifications. On the other hand, nodule specific CLE (CLAVATA 3/ENDOSPERM SURROUNDING REGION related) peptides inhibit nodulation in a receptor dependent manner mediated through long distance signalling. Overall, our work suggests signalling peptides are important positive and negative regulators of root development and nodulation, and provides a link between nitrogen demand signalling and developmental programs.

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N. Imin, N. Mohd-Radzman, A. Ivanovici, N. Patel, C. Delay, M. Taleski, K. Chapman, M.A. Djordjevic (2017) The Yin and Yang of nodulation: regulatory peptides that positively and negatively regulate root and nodule development in response to nitrogen availability; ICLGG 2017 - Book of abstracts, ICLGG2017/PL/50

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Drought response and genetic diversity in *Pisum fulvum*, a wild relative of domesticated pea

E. Naim-Feil[1], M. Toren[1], G. Aubert[2], A. Sherman[3], R. Ophir[3], Y. Saranga[1], **S. Abbo**[1]

[1] *The Hebrew University, Israel*
[2] *INRA, France*
[3] *Agricultural Research Organization, Volcani Center, Israel*

Productivity of grain crops in semi-arid areas is often affected by drought, which is likely to increase due to climate changes. A collection of 160 wild pea (*Pisum fulvum*, Pf) accessions was assembled from across its ecological range in Israel (350-850 mm annual precipitation) and used to assess genetic diversity in this taxon. A range of penology and other morpho-physiological traits was documented. We hypothesized that native species evolving under east Mediterranean climate carry adaptive traits to cope with drought stress. Accessions were classified according to SNP variation and habitat ecogeography. Significant differences were found between accession groups, but grouping in both systems did not match. Fifty-two Pf accessions and 3 domesticated (*Pisum sativum*) genotypes were evaluated, during two seasons, under well-watered (600mm) and water-limited (350mm) conditions. Total dry matter, grain yield, harvest index and average grain weight were higher in domesticated pea than wild Pf, however several Pf accessions exhibited lower drought susceptibility indices (i.e. greater stability across environments) than domesticated pea. Of special interest were a number of Pf genotypes in which low susceptibility to water stress was coupled with relatively high productivity. Sampling habitats of the low susceptibility - high productivity accessions are not the driest ones but are rather characterized by mild (400-530mm) annual precipitation. Further sampling and evaluation of Pf from such locations may improve our understanding of pea drought adaptation and yield physiology.

How to refer your abstract:
E. Naim-Feil, M. Toren, G. Aubert, A. Sherman, R. Ophir, Y. Saranga, S. Abbo (2017) Drought response and genetic diversity in Pisum fulvum, a wild relative of domesticated pea; ICLGG 2017 - Book of abstracts, ICLGG2017/OL/13

Book of abstracts
ISBN 978-615-5270-39-0

The interplay between sulfur nutrition and the drought response in pea: a focus on seed development and composition

C. Henriet[1], A. Kilandamoko[1], D. Aimé[1], J. Kreplak[1], T. Balliau[2], M. Zivy[2], V. Vernoud[1], K. Gallardo[1]

[1] *Agroécologie, AgroSup Dijon, INRA, Université Bourgogne Franche-Comté, Dijon, France*
[2] *INRA, CNRS, PAPPSO, UMR 0320 / UMR 8120 Génétique Végétale, Gif-sur-Yvette, France*

Pea (*Pisum sativum* L.) produces seeds rich in proteins, but seed yield and quality remain unstable due to various stresses, including drought and sulfur deficiency that interact in the context of climate change and reduced sulfur deposition. To investigate the interplay between sulfur nutrition and drought, sulfate-deprived pea plants were subjected to a short water-stress of 9 days during the early reproductive phase. While drought alone did not impact seed yield, sulfur deficiency alone or combined with drought decreased it by 38% and 65% respectively. An analysis of the seed protein composition revealed differences in the accumulation of sulfur-rich (11S) and sulfur-poor (7S) globulins in response to individual or combined stresses. While the 11S/7S globulin ratio did not vary in response to drought alone, it decreased by 88% in response to sulfur deficicency, but only by 47% under combined stress conditions. To decipher the strategy used by plants to regulate the 11S/7S ratio, the partitioning of carbon, nitrogen and sulfur between the different plant compartments was studied. Next, to pinpoint the mechanisms by which seeds adjust their metabolism under multi-stress conditions, developing seeds were collected at three time points and subjected to shotgun proteomics. A total of 2237 proteins were identified and quantified and the data were used to build a co-abundance protein network, which enabled the reconstruction of the metabolic pathways governing early seed development in pea and highlighted the impact of drought combined with sulfur deficiency on these pathways.

How to refer your abstract:
C. Henriet, A. Kilandamoko, D. Aimé, J. Kreplak, T. Balliau, M. Zivy, V. Vernoud, K. Gallardo (2017) The interplay between sulfur nutrition and the drought response in pea : a focus on seed development and composition; ICLGG 2017 - Book of abstracts, ICLGG2017/OL/54

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Building the base: widening the genetic & adaptive diversity of chickpea

J. Berger[1], A. Kahraman[2], C. Toker[3], B. Aydin[2], A. Aydogan[4], B. Bukun[5], C. Ludwig[1], P. Smykal[6], E.J. von Wettberg[7], R.V. Penmetsa[8], A. Greenspan[8], S. Nuzhdin[9], D.R. Cook[8]

[1] *CSIRO Agriculture and Food, Wembley, Australia*
[2] *Department of Field Crops, Harran University, Turkey*
[3] *Department of Plant Protection, Akdeniz University, Turkey*
[4] *Ministry of Agriculture (CRIFC), Ankara, Turkey*
[5] *Dicle University, Diyarbakir, Turkey*
[6] *Palacký University in Olomouc, Czech Republic*
[7] *Florida International University, Miami, USA*
[8] *University of California Davis, Davis, USA*
[9] *USC University of Southern California, USA*

The narrow base of chickpea is a constraint to further improvement, reflecting the crop's unique evlutionary history [1]. The annual wild relatives have long been recognized as an excellent source of biotic and abiotic stress resistance [2], but were themselves constrained by extremely limited collection. Thus, the wild progenitor (C. reticulatum) and its close relative (C. echinospermum) were represented by only 16 and 10 independent accessions in the world collection [3]. These numbers were too low to characterize adaptive potential, let alone exploit this for base broadening of chickpea.

Comprehensive surveying/collection of annual wild Cicer populations across altitude, temperature and to a lesser extent rainfall gradients throughout SE Anatolia has resolved this constraint. Populations were identified at flowering and leaves collected on a single plant basis to expedite population genetics. Mature seeds were similarly collected, site seasonal climates and soils characterized. The world collection has been increased by > 1200 single plant accessions from 84 locations covering the Anatolian distribution range: C. reticulatum (n=589, 42 sites), C. echinospermum (n=282, 17 sites), C. pinnatifidum (n=252, 25 sites), C. bijugum (n=85, 6 sites). These accessions are the basis of an international effort to phenotype adpative traits and introgress these into domesticated chickpea.

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Book of abstracts
ISBN 978-615-5270-39-0

Development of the alfalfa breeder's toolbox: a resource for genomic, genetic and germplasm resources for alfalfa improvement

M.J. Monteros[1], C. He[1], J. Choi[1], P.X. Zhao[1], N. Tayeh[1], X. Dai[1], A.D. Farmer[2], J. Mudge[2], H. Tang[3], J. Chang[1], N. Krom[1], J.N. Vaughn[4], P. Mehta[1], C.M. Motes[1], M. Trammell[1], B. Motes[1], S. Sullivan[5], I. Liachko[5], E.C. Brummer[6], N.D. Young[7], C.D. Town[8], M.K. Udvardi[1]

[1] *Noble Research Institute, Ardmore, USA*
[2] *NCGR, Santa Fe, USA*
[3] *University of Arizona, Tucson, USA*
[4] *USDA, Athens, USA*
[5] *Phase Genomics, Seattle, USA*
[6] *University of California, Davis, USA*
[7] *University of Minnesota, St. Paul, USA*
[8] *J. Craig Venter Institute, Rockville, USA*

The availability of genomic and genetic resources has facilitated the implementation of translational approaches to accelerate genetic gains in plant breeding programs. Alfalfa (*Medicago sativa* L.), also known as lucerne, is a perennial forage legume with global agronomic importance.

Strategies to enhance alfalfa improvement include approaches to sequence and assemble the diploid and tetraploid alfalfa genomes and integrate them with multiple datasets as part of the Alfalfa Breeder's Toolbox. The diploid genome was generated using PacBio sequencing, while Illumina and PacBio sequencing approaches were used for the tetraploid alfalfa genome. These technologies resulted in a highly fragmented tetraploid genome assembly due to high levels of heterozygosity and the complexity associated with haplotype variation. Integrating a Hi-C based proximity guided assembly enabled the *de novo* assembly of chromosome-scale scaffolds corresponding to the eight alfalfa chromosomes. The diploid genome includes a gene annotation pipeline that enabled its use to anchor additional datasets in the Alfalfa Breeder's Toolbox (ABT).

The Alfalfa Breeder's Toolbox was developed as a user-friendly, web-based portal to facilitate utilization of genomics information for practical breeding applications. The ABT provides access to and visualization of (1) the alfalfa genome sequence, (2) gene models, (3) gene expression profiles in response to abiotic stress conditions presented in the gene expression atlas, (4) molecular markers, (5) shifts in allele frequencies obtained through cycles of selection, and (6) phenotypic data from field-based germplasm evaluation trials. Specific functionalities of the ABT will be addressed using test case scenarios that aim to address practical plant breeding applications.

The long-term goal of the ABT is to generate a resource for the community to store, query and visualize curated alfalfa datasets to enable genomics-based breeding approaches to develop enhanced alfalfa cultivars. Lastly, the ABT can also be useful to translate the considerable genomics resources developed in *Medicago truncatula* and other legumes to address challenges limiting alfalfa productivity.

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M.J. Monteros, C. He, J. Choi, P.X. Zhao, N. Tayeh, X. Dai, A.D. Farmer, J. Mudge, H. Tang, J. Chang, N. Krom, J.N. Vaughn, P. Mehta, C.M. Motes, M. Trammell, B. Motes, S. Sullivan, I. Liachko, E.C. Brummer, N.D. Young, C.D. Town, M.K. Udvardi (2017) Development of the Alfalfa Breeder's Toolbox: A Resource for Genomic, Genetic and Germplasm Resources for Alfalfa Improvement; ICLGG 2017 - Book of abstracts, ICLGG2017/PL/133

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Recent advances in the regulation of seed protein composition in legumes: from genome-wide studies to new seed protein profiles

C. Le Signor[1], J. Buitink[4], N.D. Young[2], J.-M. Prosperi[3], V. Vernoud[1], C. Henriet, G. Aubert[1], O. Leprince[4], R.D. Thompson[1], J. Burstin[1], **K. Gallardo**[1]

[1] *Agroécologie, AgroSup Dijon, INRA, Univ. Bourgogne Franche-Comté, Dijon, France*
[2] *Department of Plant Pathology, University of Minnesota, St. Paul, USA*
[3] *AGAP, INRA, CIRAD, Montpellier SupAgro, Montpellier, France*
[4] *IRHS, INRA, Agrocampus-Ouest, Univ. Angers, SFR 4207 QuaSaV, Beaucouzé, France*

Seeds of legumes such as pea (*Pisum sativum* L.) provide proteins for animal feed and human nutrition. Although the so-called "antinutritional factors" have been reduced in these seeds, offering globally good nutritional value, amino acid balance needs to be improved and stabilized, notably regarding sulfur amino acid content. By integrating omics data, notably from protein quantity loci and genome-wide association studies (GWAS using the HapMap platform[1]) on the abundance of storage proteins in *Medicago truncatula* seeds, we provide a repertory of genes involved in transcription, post-translational modifications, transport, or targeting of globulins to storage vacuoles [2]. Inference of a gene co-expression network between GWAS-derived transcription factors and globulin genes revealed key regulators of seed protein composition. A systematic search for orthologous sequences in the pea gene atlas[3] enabled us to transfer the knowledge to the target pea crop. The potential of this translational genomics approach for revealing genes important for seed nutritional quality improvement will be presented through pea TILLING lines[4] for selected candidate genes that exhibit seed protein profiles with increased abundance of sulfur-rich proteins. Because sulfur nutrition enables sulfur-rich protein accumulation[5], we further studied the leaf transcriptome of pea subjected to sulfur deficiency during the reproductive period. Genes involved in the supply of sulfur for accumulation of sulfur-rich proteins have been characterized by analysis of TILLING mutants.

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Book of abstracts
ISBN 978-615-5270-39-0

Sustainable intensification of grain legumes with smallholders in Africa through nitrogen fixation: highlights from the N2Africa project

E. Wolde-meskel[1], J. van Heerwaarden[2], B. Abdulkadir[1], K. Giller[2]

[1] *International Livestock Research Institute, Addis Ababa, Ethiopia*
[2] *Plant Production Systems, Wageningen University, Wageningen, The Netherlands*

Improving Nitrogen Fixation in grain legumes is central to the sustainable intensification of agriculture in sub-Saharan Africa (SSA) and inoculation with effective rhizobia can make an important contribution to this goal. Genetic and phenotypic studies have identified large taxonomic diversity and differences in symbiotic effectiveness between isolates from SSA soils, suggesting that there is potential for developing more effective inoculants from native bacteria. The N2Africa project has pursued two approaches in this regard: First, identification of elite strains from native rhizobial collections with the aim of developing inoculants for local production in SSA and second, promotion of inoculation with effective bacterial strains at scale. Here, we report the genetic and symbiotic diversity of indigenous isolates, success with the search for elite strains and achievements of the project in getting the inoculant technology out to farmers at a larger scale through Private Public Partnership (PPP). Response of crops to inoculation across a large number of smallholder's farms, covering diverse soil fertility and agro-ecological conditions, was evident. Commonly, increased grain yield of >10% over yield on control plots (a yield level assumed to be visible to farmers) was realized for most farmers. However, observed grain yields on control plots and responses to inoculation on individual farms varied greatly with a relative yield responses ranging from 3% - 100%. The additive benefits and possibilities for a wide scale promotion of inoculant technology to smallholders through a PPP approach will be discussed.

How to refer your abstract:
E. Wolde-meskel; J. van Heerwaarden; B. Abdulkadir, K. Giller (2017) Sustainable intensification of grain legumes with smallholders in Africa through Nitrogen Fixation: Highlights from the N2Africa project; ICLGG 2017 - Book of abstracts, ICLGG2017/PL/136

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

The CGIAR research program on grain legumes and the International Year of Pulses

S. Sivasankar

CGIAR Research Program on Grain Legumes, ICRISAT, Telangana, India

No abstract has been submitted

Book of abstracts
ISBN 978-615-5270-39-0

Bean adapt: the genomics of adaptation during crop expansion of common bean

R. Papa[1], S.A. Jackson[2], P. Gepts[3], A. Graner[4], A.R. Fernie[5]

1 *Università Politecnica delle Marche, Ancona, Italy*
2 *University of Georgia, Athens, USA*
3 *University of California, Davis, USA*
4 *Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany*
5 *Max Planck Institute of Molecular Plant Physiology, Potsdam-Golm, Germany*

Here we present the preliminary results obtained during the project BEAN_ADAPT funded through the 2ndERA-CAPS call, ERA-NET for Coordinating Action in Plant Sciences. The main aim of our work was to dissect out the genetic basis and phenotypic consequences of the adaptation to new environments of the common bean (*Phaseolus vulgaris* L.), through the study of the introduction, from the centres of domestication in the Americas, and the expansion through Europe, as a recent and historically well-defined event of rapid adaptation.

The results of the resequencing of 220 common bean accessions (on average 10X coverage per accession), from Europe and from the centres of origin, will be presented, along with those obtained from genotyping a larger collection of more than 2000 accessions.

Moreover, the genomics analysis will be integrated by the results obtained by the phenotypic characterization of a subsets 500 genotypes under control conditions and in the field at two different latitudes (Northern Germany and Southern Italy).

The data are being analysed using population genomics approaches to highlight the history of crop expansion in the Old World and to dissect the role of selection for adaptation of *P. vulgaris* to the European agroecosystems.

How to refer your abstract:
R. Papa, S.A. Jackson, P. Gepts, A. Graner, A.R. Fernie (2017) Bean adapt: the genomics of adaptation during crop expansion of common bean; ICLGG 2017 - Book of abstracts, ICLGG2017/OL/20

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Gene identification in faba bean – to synteny and beyond

D.M. O'Sullivan[1], D. Angra[1], V. Tagkouli[1], K. Khamassi[2], W. El-Rodeny[3], M.K. Zeid[4]

[1] *School of Agriculture, Policy and Development, University of Reading, Reading, UK*
[2] *INRAT, Rue Hédi Karray, Ariana, Tunisia*
[3] *Agricultural Research Centre-Sakha, Food Legumes Section, Kafr-El-Sheikh, Egypt*
[4] *Alexandria University, El-Shatby, Alexandria, Egypt*

In this talk, I will outline progress in the further exploitation of the syntenic relationships established in earlier work [1] between *Vicia faba* and *Medicago truncatula* in pursuit of genes underlying a series of faba bean traits of interest.

The first example is *Dwarf1* (*Dwf1*) gene, a gibberellic acid-sensitive dwarfing gene, where synteny was used to identify a candidate gene, whose causative role was further confirmed by fine-mapping, allele re-sequencing and metabolite analysis.

The second example is the *VC* gene controlling a 10-fold reduction in the anti-nutritional factors vicine and convicine. The trait maps in a segment of *Vf* chr 1 which shows strong colinearity with Mt chr 2. Since only a handful of spp in the genus *Vicia* make these secondary metabolites, it is highly unlikely that the Medicago genome contains a strict orthologue of the *VC* gene. Therefore, the role of synteny in this case is to saturate the interval with markers which is being used as a basis for the ongoing positional cloning of the gene.

Ultimately, whilst synteny has been a useful framework to translate knowledge of gene function from model to crop species, traits such as *VC* and tolerance to the parasitic weed *Orobanche* challenge us to move beyond a dependency on generating marker coverage and causal gene hypotheses exclusively based on synteny. With this in mind, we have recently developed a 50K Axiom SNP genotyping array and will present validation of its use in re-cloning *dwf1*, in greatly narrowing the *vc* region and present plans for a major new initiative to map *Orobanche* tolerance.

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[1] Webb et al (2016) A SNP-based consensus genetic map for synteny-based trait targeting in faba bean (Vicia faba L.). Plant Biotechnology Journal 14:177-185

How to refer your abstract:
D.M. O'Sullivan, D. Angra, V. Tagkouli, K. Khamassi, W. El-Rodeny, M.K. Zeid (2017) Gene identification in faba bean – to synteny and beyond; ICLGG 2017 - Book of abstracts, ICLGG2017/OL/22

Book of abstracts
ISBN 978-615-5270-39-0

TrifoliGATE subterranean clover genomic resources: building a comprehensive user-friendly platform for future forage legume breeding

P. Kaur[1,2], C.-K.K. Chan[3], P.E. Bayer[3], D. Edwards[3], W. Erskine[1,2]

[1] *Centre for Plant Genetics and Breeding, School of Agriculture and Environment, The University of Western Australia, Crawley, Australia*
[2] *Institute of Agriculture, The University of Western Australia, Crawley, Australia*
[3] *School of Biological Sciences, The University of Western Australia, Crawley, Australia*

Subterranean clover (*Trifolium subterraneum L.)* is the predominant annual pasture legume in southern Australia (Nichols *et al*., 2012) and a newly established model species for genetic and genomic studies within the genus *Trifolium* (Kaur *et al*., 2017). A high quality reference genome (Tsub_Refv2.0) (Kaur *et al*., 2017) has been described for subterranean clover (based on cv. Daliak). The identification and annotation of expressed genes within the *T. subterraneum* genome assembly used high-throughput whole genome RNA sequencing analyses to predict a total of 32,333 transcripts for 31,272 protein-coding genes, with evidence for their expression across different tissue types. CEGMA analysis was conducted to test the quality of the genome assembly, which showed the presence of 240 complete (96.8%) and 247 (99.6%) partial genes present from the core set of 248 eukaryotic genes. A wide range of important traits are mapped onto the reference assembly using QTL and GWAS pipelines.

Trifoligate.info portal is a user-friendly platform for forage legume breeders to find genes and design markers readily for economically important traits with a view to breed future cultivars. This platform assists the integration of genomic and phenomic resources to identify loci governing traits allowing marker-assisted breeding, comparative mapping and identification of tissue-specific gene promoters for biotechnological improvement of forage legumes.

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How to refer your abstract:
P. Kaur, C.-K.K. Chan, P.E. Bayer, D. Edwards, W. Erskine (2017) TrifoliGATE subterranean clover genomic resources: building a comprehensive user-friendly platform for future forage legume breeding; ICLGG 2017 - Book of abstracts, ICLGG2017/OL/15

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

A collection of online resources for legume research

S. Hokin[1], J. Berendzen[1], C. Cameron[1], J.D. Campbell[2], E.K.S. Cannon[2], S.B. Cannon[2,3], A. Chan[4], A. Cleary[1,5], S. Dash[1], A.D. Farmer[1], D. Fernández-Baca[2], W. Huang[2], V. Krishnakumar[4], E. Lyons[6], C. Town[4], N.T. Weeks[2,3], A.P. Wilkey[2]

[1] *National Center for Genome Resources, Santa Fe, USA*
[2] *Iowa State University, Ames, USA*
[3] *USDA-ARS-CICGRU, Ames, USA*
[4] *J. Craig Venter Institute, Rockville, USA*
[5] *Montana State University, Bozeman, USA*
[6] *University of Arizona, Tucson, USA*

A diverse collection of online tools is available for legume research. This talk will describe several from the *Legume Information System* and *Legume Federation* projects (https://legumeinfo.org and https://legumefederation.org), including: (1) InterMine instances for common bean (*Phaseolus vulgaris*), chickpea (*Cicer arietinum* v. desi and kabuli), soybean (*Glycine max*), peanut (*Arachis duranensis* and *Arachis ipaensis*), cowpea (*Vigna unguiculata)*, and *Medicago truncatula*; (2) a geographic information system (GIS) viewer for visualizing germplasm collections globally against high-resolution maps; (3) genome browsers for more than a dozen sequenced legume genomes, including Medicago and Lotus; (4) sequence search tools for those genomes and gene sets, along with tools for visualizing sequence matches with respect to a genome or a gene; (5) gene family viewers for legume species; (6) a synteny viewer for exploring genome-wide and gene-focused synteny across the legumes; and (7) a new interactive genetic map viewer.

How to refer your abstract:
S. Hokin, J. Berendzen, C. Cameron, J.D. Campbell, E.K.S. Cannon, S.B. Cannon, A. Chan, A. Cleary, S. Dash, A.D. Farmer, D. Fernández-Baca, W. Huang, V. Krishnakumar, E. Lyons, C. Town, N.T. Weeks, A.P. Wilkey (2017) A collection of online resources for legume research; ICLGG 2017 - Book of abstracts, ICLGG2017/OL/78

ABSTRACTS OF POSTERS

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Approaches for enhancement of phosphorus use efficiency of chickpea (*Cicer arietinum* L.) under limiting phosphorus conditions

M. Lazali[1], J.J. Drevon[2]

[1] *Laboratoire de recherche ERP, Faculté des Sciences de la Nature et de la Vie et des Sciences de la Terre, Université de Khemis Miliana, Algeria*
[2] *Institut National de la Recherche Agronomique, UMR Ecologie Fonctionnelle & Biogéochimie des Sols et Agroécosystèmes, INRA-IRD-CIRAD-SupAgro, Place Pierre Viala, Montpellier, France*

Phosphorus (P) is a poorly bioavailable macronutrient that is essential for crop growth and yield. Overuse of P fertilizers results in low P use efficiency, has serious environmental consequences and accelerates the depletion of P mineral reserves [1]. It has become extremely challenging to improve P use efficiency while preserving global food supplies and maintaining environmental sustainability. Chickpea (*Cicer arietinum* L.) genotypes vary in their adaptation to low-P soils.

To investigate to what extent this variation may be related to P use efficiency for N_2 fixation, six genotypes of chickpea contrasting in P use efficiency for symbiotic N_2 fixation, namely FLIP 03-68C, FLIP 03-113C, FLIP 03-118C, FLIP 90-13C, FLIP 99-34C and FLIP 99-66C were studied in the field conditions during two growing seasons from 2015 to 2017.

At the flowering stage, the biomass of plants and nodules and their P contents was determined after measuring the quantity of N_2 fixed.

The results showed that low-P availability in the soil significantly affected plant growth, nodulation and SNF for all RILs though with highest extent for the genotypes FLIP 03-113C, FLIP 03-118C and FLIP 99-34C. Under low-P availability in the soil, the genotypes FLIP 03-68C, FLIP 90-13C and FLIP 99-66C showed highest efficiency in use of P for their symbiotic N_2 fixation than other genotypes. The genotypes with high P use efficiency showed greater efficiency in use of the rhizobial symbiosis than the genotypes with low P use efficiency for symbiotic N_2 fixation.

We concluded that P use efficiency for symbiotic N_2 fixation may be a useful functional trait that may contribute to the adaptation of N_2-fixing legumes to low-P soils.

Reference:

[1] Vance, CP. et al, Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource, New Phytologist, vol. 157, 2003, p. 423-447.

How to refer your abstract:
M. Lazali, J.J. Drevon (2017) Approaches for enhancement of phosphorus use efficiency of chickpea (Cicer arietinum L.) under limiting phosphorus conditions; ICLGG 2017 - Book of abstracts, ICLGG2017/P/01

Book of abstracts
ISBN 978-615-5270-39-0

Developing drought and heat stress tolerant chickpea genotypes

D. Yücel, M. Türkeri, D. Mart, N. Angın, V. Çatalkaya, C. Yücel

Eastern Mediterranean Agriculture Research Institute, Adana, Turkey

Chickpea (*Cicer arietinum* L.) with high protein content is a vital food, especially in under-developed and developing countries for the people who do not consume enough meat due to low income level. The objective of the proposed study to evaluate growing, yield and yield components of chickpea genotypes under Mediterranean condition so determine tolerance of chickpea genotypes against drought and heat stress. For this purpose, a total of 34 chickpea genotypes were used as material. The experiment were conducted according to factorial randomized complete block design with 3 reps at the Eastern Mediterranean Research Institute, Adana, TURKEY for 2014-15 growing season under three different growing conditions (Winter sowing, irrigated-late sowing and non-irrigated- late sowing). According to results of this experiment, flowering time, podding time, maturity time, plant height, height of first pod, seed yield and 100 seed weight were ranged between 94.22 to 85.00 days, 94.11 to 106.44 days, 198.56 to 214.44 days, 37.18 to 64.89 cm, 18.33 to 34.83 cm, 417.1 to 1746.4 kg/ha and 14.02 to 45.02 g, respectively. Among the chickpea genotypes, the Aksu, Arda, Çakır, F4 09 (X 05 TH 21-16189), FLIP 03-108 were least affected by drought and heat stress. Therefore, these genotypes can be used as sources of drought and heat tolerance in further breeding programmed for evolving the drought and heat tolerant genotypes in chickpea.

How to refer your abstract:
D. Yücel, M. Türkeri, D. Mart, N. Angın, V. Çatalkaya, C. Yücel (2017) Developing Drought and Heat Stress Tolerant Chickpea Genotypes; ICLGG 2017 - Book of abstracts, ICLGG2017/P/02

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Evaluation of drought stress responses in cowpea genotypes

M. Carvalho[1], T. Lino-Neto[2], J. Moutinho-Pereira[1], C. Correia[1], I. Castro[1,3], M. Matos[3,4], V. Carnide[1,3]

[1] *Centre for Research and Technology of Agro-Environment and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal*
[2] *Biosystems & Integrative Sciences Institute (BioISI), Plant Functional Biology Center (CBFP), University of Minho, Braga, Portugal*
[3] *Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal*
[4] *Biosystems & Integrative Sciences Institute (BioISI), Sciences Faculty, University of Lisbon, Lisbon, Portugal*

Drought is a critical constraint for agricultural production. Cowpea [*Vigna unguiculata* (L.) Walp.] is a grain legume native from Africa and a primary source of protein for millions of people of the developing world. This grain legume is pointed as one of the most tolerant to severe drought conditions, being considered as an ideal model to study the molecular mechanisms of drought tolerance. The main objective of this study was to evaluate and compare the tolerance level of the Portuguese cowpea genotypes Cp5051 and Vg50, submitted to three watering regimens (0%, 25% and 75% of field capacity, FC), during 15 days. Two genotypes, Bambey21 (highly susceptible) and CB46 (moderately susceptible), were used as controls.

Physiological, biochemical and molecular aspects were evaluated during stress imposition. Although photosynthetic activity (Fv/Fm, ETR) and gas exchange parameters did not reveal differences among genotypes, drought-stress related parameters (prolin and anthocyanin contents) and expression of six stress-related genes allowed to determine different tolerance levels. Vg50 genotype revealed the highest drought response while Bambey21 was the most susceptible genotype, as expected. The other two genotypes (Cp5051 and CB46) had an intermediate behavior. Oxidative stress parameters (MDA and H_2O_2 content) and expression of six related genes did not change, in general, among genotypes upon drought imposition.

Acknowledgement: *This study was funded by the EU-FP7 for Research, Technological Development and Demonstration under grant agreement no 613781, project EUROLEGUME.*

How to refer your abstract:
M. Carvalho, T. Lino-Neto, J. Moutinho-Pereira, C. Correia, I. Castro, M. Matos, V. Carnide (2017) Evaluation of drought stress responses in cowpea genotypes; ICLGG 2017 - Book of abstracts, ICLGG2017/P/03

Book of abstracts
ISBN 978-615-5270-39-0

PeaMUST (2012-2019) – Pea Multi-Stress adaptation and biological regulations for yield improvement and stability

J. Burstin[1], M.-L. Pilet-Nayel[2], C. Rameau[3], R. Thompson[1], F. Labalette[4], M. Leveugle[5], A. Remy[1], X. Pinochet[6]

1 *UMR Agroécologie, INRA, Dijon, France*
2 *UMR IGEPP, INRA, Le Rheu, France*
3 *UMR IJPB, INRA, Versailles, France*
4 *Terres Univia, Paris, France*
5 *Biogemma, Clermont-Ferrand, France*
6 *Terres Inovia, Thiverval Grignon, France*

PeaMUST (2012-2019) is a large French national project whose objective is to develop novel pea varieties and optimize plant-symbiotic interactions for stabilized seed yield and quality, in the context of climate change and pesticide reduction.

With the involvement of 28 partners from the public and private sectors, it will take advantage of NGS sequencing, genotyping and phenotyping technologies, to tackle the challenge of multiple stresses that penalize the performance of the pea crop.

How to refer your abstract:
J. Burstin, M.-L. Pilet-Nayel, C. Rameau, R. Thompson, F. Labalette, M. Leveugle, A. Remy, X. Pinochet (2017) PeaMUST (2012-2019) – Pea Multi-Stress adaptation and biological regulations for yield improvement and stability; ICLGG 2017 - Book of abstracts, ICLGG2017/P/04

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Global analysis and comparison of transcriptomic changes in *Medicago truncatula* and *Lotus japonicus* root nodules during drought stress

W. Czarnocka[1], I. Sańko-Sawczenko[1], B. Łotocka[1], H. Rekosz-Burlaga[2]

[1] *Department of Botany, Faculty of Agriculture and Biology, Warsaw University of Life Sciences, Warszawa, Poland*
[2] *Autonomous Department of Microbial Biology, Warsaw University of Life Sciences, Warszawa, Poland*

The symbiosis of fabaceans with nitrogen-fixing bacteria, collectively termed rhizobia, manifests itself in the formation of root nodules, in which bacteria are able to assimilate atmospheric nitrogen. Generally, fabaceans can form two types of root nodules: determinate, that are characterized by a spherical shape and definite nature of meristem, or indeterminate, that have cylindrical shape and persistent meristem.

The aim of our study was to compare metabolic processes and signaling pathways in root nodules of two fabacean model species, *Medicago truncatula*, forming indeterminate nodules, and *Lotus japonicus*, forming determinate nodules. Using next-generation RNA sequencing we analyzed and compared the transcriptomic changes in root nodules of both species at two different stages of drought stress.

Our results demonstrated that in *M. truncatula* after two and four days of drought stress, in comparison to well-watered plants, there was a difference in the expression level of 371 and 787 genes, respectively. In *L. japonicus*, after two and four days from the last watering, we found 122 and 1225 differentially expressed genes, respectively. Genes with significantly changed expression level encoded broad range of enzymes engaged in signal perception, phytohormones signaling, synthesis of osmoprotectants and secondary metabolites, cell wall remodeling and cell cycle.

Our study presents the first such broad comparison of transcriptomic changes in indeterminate *versus* determinate root nodules and reveals similarities and differences in their response to drought stress.

How to refer your abstract:
W. Czarnocka, I. Sańko-Sawczenko, B. Łotocka, H. Rekosz-Burlaga (2017) Global analysis and comparison of transcriptomic changes in Medicago truncatula and Lotus japonicus root nodules during drought stress; ICLGG 2017 - Book of abstracts, ICLGG2017/P/05

Book of abstracts
ISBN 978-615-5270-39-0

Marker assisted bred chickpea lines showed superior performance in multilocation testing in India

S. Samineni[1], R.K. Varshney[1], M. Thudi[1], V. Jayalakshmi[2], A. Vijayakumar[3], D.M. Mannur[4], P.M. Gaur[1,5]

[1] *International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana, India*
[2] *Regional Agricultural Research Station, Nandyal, Andhra Pradesh, India*
[3] *University of Agricultural Sciences (UAS), Dharwad, Karnataka, India*
[4] *Agricultural Research Station of UAS-Raichur, Gulbarga, Karnataka, India*
[5] *The UWA UWA Institute of Agriculture, University of Western Australia, Perth, Australia*

Chickpea (*Cicer arietinum* L.) is a cool season food legume largely grown in Asia and sub-Saharan Africa where terminal moisture stress is the major production constraint. Roots play a major role in extraction of moisture from deeper soil layers, and the efficient root system could moderate the moisture stress condition by meeting water demands from deeper soil layers. Phenotyping of root traits is a laborious and cumbersome procedure. Availability of large scale genomic resources made easy the selection for such complex traits in the breeding programs. A genomic region "QTL-hotspot" harbouring QTLs for several root traits was introgressed into popular chickpea cultivars JG 11, Bharati, JAKI 9218 and JG 16 in India. A set of 20 introgression lines (ILs) of JG 11 and 22 of Bharati were evaluated at three to four locations (Patancheru, Nandyal, Gulbarga and Dharwad) in southern India over two years (2012-13 to 2014-15). Several lines giving at least 10% higher yield than respective recurrent parents were identified. Further, some of these lines entered into All India co-ordinated Research Project (AICRP) on Chickpea for national level testing. These results demonstrate the practical application of molecular markers in improving the low heritable and complex traits in chickpea improvement.

How to refer your abstract:
S. Samineni, R.K. Varshney, M. Thudi, V. Jayalakshmi, A. Vijayakumar, D.M. Mannur, P.M. Gaur (2017) Marker assisted bred chickpea lines showed superior performance in multilocation testing in India; ICLGG 2017 - Book of abstracts, ICLGG2017/P/06

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Molecular diversity and quantitative trait loci related to drought tolerance in lentil (*Lens culinaris Medik., Fabaceae*)

O. Idrissi[1], S.M. Udupa[2], E. De Keyser[3], P. Van Damme[4,5], J. De Riek[3]

[1] *Institut National de la Recherche Agronomique du Maroc (INRA), Centre Régional de Settat, Settat, Morocco*
[2] *ICARDA-INRA Cooperative Research Project, International Center for Agricultural Research in the Dry Areas (ICARDA), Rabat, Morocco*
[3] *Institute for Agricultural and Fisheries Research (ILVO) Plant, Sciences Unit, Applied Genetics and Breeding, Melle, Belgium*
[4] *Department of Plant Production, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium*
[5] *Faculty of Tropical AgriSciences, Czech University of Life Sciences, Prague, Prague, Czech Republic*

Drought is one of the major abiotic stresses limiting lentil (*Lens culinaris* Medik.) productivity especially in rainfed agricultural systems. Rooting patterns are often associated with promising drought avoidance mechanisms targeted in breeding programs. Their molecular mapping aims to develop tools for efficient selection. Molecular genetic diversity analysis of landraces is a key step for enhanced use of these important genetic resources in developing adapted cultivars as well as for their better valorization for the benefits of local farmers. Lentil contributes to sustainable farming by biological fixation of nitrogen in soils and enhances nutrition thanks to its proteins and micronutrients-rich grains. Improvement of drought tolerance and targeted use of genetic resources will contribute to enhance lentil production to face increasing demand for staple food in the world.

In the absence of earlier molecular characterization, we assessed the genetic diversity of 51 Moroccan lentil landraces using simple sequence repeats (SSR) and amplified fragment length polymorphisms (AFLP). Nineteen SSRs yielded 213 alleles, whereas seven AFLP primer combinations gave 766 fragments of which 422 were polymorphic. Overall, we observed moderate to high genetic variation. We also differentiated several groups of landraces. Interestingly, one of these groups contained short-cycle landraces with high rapid vegetative growth. Landraces in that group were from the dryland location of Abda in west-central Morocco, where they were likely selected for adaptation to drought and heat stress over centuries. Another group contained landraces from highland areas that may have been selected for specific adaptation to cold stress. A third group contained one landrace from the Zear region, in north-western Morocco, known for its seed quality (especially short cooking time) and has been proposed in the national catalog of local products for a protected designation of origin (PDO) quality mark. Both molecular techniques evidenced that the latter landrace developed on its own some specific characteristics supporting the idea of PDO attribution. Genetic differentiation according to agro-environmental origins, cycle duration and early vegetative vigour was observed when combining genetic and agronomic information. Landraces from dry areas were differentiated from those of more favourable climatic condition areas and higher locations. Specific adaptation of these landraces to their respective agro-environments may be the reason of their genetic differentiation.

Keywords: *genetic differentiation, agro-environmental origins, ecophysiology, drought tolerance, breeding, marker-trait association, QTL, marker-assisted selection.*

How to refer your abstract:
O. Idrissi, S.M. Udupa, E. De Keyser, P. Van Damme, J. De Riek (2017) Molecular diversity and quantitative trait loci related to drought tolerance in lentil (Lens culinaris Medik., Fabaceae); ICLGG 2017 - Book of abstracts, ICLGG2017/P/07

Book of abstracts
ISBN 978-615-5270-39-0

Osmotic stress tolerance in the early vegetative stages of field pea at molecular level

G. Petrović[1], T. Živanović[2], R. Stikić[2], B. Vucelić-Radović[2], V. Đorđević[1], Z. Nikolić[1], J. Samardžić[3]

[1] *Institute of Field and Vegetable Crops, Novi Sad, Serbia*
[2] *Faculty of Agriculture, University of Belgrade, Belgrade, Serbia*
[3] *Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia*

Osmotic stress lead to creation of reactive oxygen species (ROS) in plants. Plants have developed antioxidant defense mechanisms in order to neutralise the effects of ROS. The aim of this research was to determine the molecular basis of osmotic stress tolerance in 7 field pea (*Pisum sativum* L.) varieties at the early seedling stage.

Osmotic stress of -0.1 MPa was stimulated using PEG 6000. Tap water was used as control. Activity of antioxidative enzymes: SOD, APx and GR was measured spectrophotometrically. Total RNA was isolated from stressed and non-stressed plant roots and shoots. The gene expression levels of genes encoding antioxidative enzymes were examined by semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) technique. *Arabidopsis* 18S rRNA was used as internal control.

Activity of antioxidant enzymes was changed in all pea varieties under osmotic stress compared to control. The mechanism of eliminating ROS in tolerant cultivar Trezor is based on the constantly increased activity of all three enzymes. Activity of enzymes was decreased in cultivar Junior, more sensitive to osmotic stress, as well as in the shoot of cultivar Javor in which sensitivity to osmotic stress of shoot is particularly strong. The molecular analysis can explain changes in antioxidant enzyme activity. The results show that antioxidative enzymes was up-regulated in tolerant cultivars (Trezor, Pionir, Mraz) increases the activity of antioxidant enzymes, in both shoot and root, i.e. down-regulated in more sensitive cultivars, which proved to be cultivars Junior and Javor.

How to refer your abstract:
G. Petrović, T. Živanović, R. Stikić, B. Vucelić-Radović, V. Đorđević, Z. Nikolić, J. Samardžić (2017) Osmotic stress tolerance in the early vegetative stages of field pea at molecular level; ICLGG 2017 - Book of abstracts, ICLGG2017/P/08

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

QTL identification for UV-B resistance traits in soybean using genotype-by-sequencing

M.Y. Yoon[1,2], M.Y. Kim[1,2], T. Lee[1], S.-H. Lee[1,2]

[1] *Department of Plant Science and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea*
[2] *Plant Genomics and Breeding Institute, Seoul National University, Seoul, Republic of Korea*

As one of the abiotic complex stressors, increased solar UV-B irradiation is induced by ozone depletion. Since the physiological damages and morphological changes are close to the reducing yields of soybean, it is important to investigate the responses in soybean under UV-B stress. Several studies tried to reveal quantitative trait loci (QTL) associated with resistance to supplementary UV-B treatment using soybean RIL population. However, QTLs and candidate genes responsible for UV-B stress resistance in soybean were still unclear. In the present study, we investigated phenotypic data of UV-B treated 174 F6 RIL population derived from Cheongja 3 and Buseok and we tried to construct genetic map by Genotype-by-sequencing. High degree of phenotypic variations was shown in response to UV-B irradiation. Frequency distribution of leaf damage degree for UV-B treatments were ranged between 10 and 100%. The mean range of damage leaf degree was 50.3%, paternal UV-B resistance Buseok showed 26.8% damage degree, and maternal UV-B sensitive Cheongja 3 exhibited 62.4 damage degrees (%).Total high quality 1,561 SNPs were obtained and used for construction of genetic map using Joinmap 4.1. The newly detected QTL in this study is *UVBR12-1* and the candidate genes on chr 12 need to be examined more as UV-B stress responsible genes. Our approach could be applied to breed high adaptable soybean under continuous climate changes in the future.

How to refer your abstract:
M.Y. Yoon, M.Y. Kim, T. Lee, S.-H. Lee (2017) QTL identification for UV-B resistance traits in soybean using genotype-by-sequencing; ICLGG 2017 - Book of abstracts, ICLGG2017/P/09

Book of abstracts
ISBN 978-615-5270-39-0

TEMPRANILLO as a good candidate gene for flowering time in chickpea

L. Ali[1], **P. Castro[1]**, J.Rubio[2], J.Gil[1], T. Millán[1]

[1] *Universidad de Córdoba, Dpto Genética, Campus de Rabanales, Córdoba, Spain*
[2] *Area de Genómica y Biotecnología, IFAPA, Alameda del Obispo, Córdoba, Spain*

Flowering time is a complex trait essential for crop adaptation to an environment. It is mainly affected by photoperiod and temperature. So, different genomic areas are expected to be involved on its control. In chickpea (*Cicer arietinum*), several QTLs (Quantitative Trait Loci) have been described for days to flowering. One of them (QTL_{DF1}) has been located on Linkage Group (LG) 4 using a recombinant inbred lines (RIL) population with JG62 as early flowering parental line [1].

In this study, we used information from *Medicago truncatula* chromosome 1 (Chr)1, which corresponds with chromosome 4 (Ca4) in *C. arietinum* [2]. Thirteen genes related to flowering time in *M. truncatula* Chr1 resulted homologous to 12 genes in *C. arietinum* Chr4 (Ca4). Besides, six STMS (sequence tagged microsatellite site) markers were selected within the interval of QTL_{DF1}. All those genes and markers were used for linkage analysis in the Recombinant Inbred Line Population RIP-1 derived from the cross Ca2156 (late flowering) x JG62 (early flowering). QTLs analysis under field conditions revealed that STMS GAA47 was the most associated with flowering time, explaining 25% of the total phenotypic variation. However, QTLs analysis under greenhouse conditions showed that STMS CaGM14822 and Indel (insertion/delection) 1 were the most associated with this trait (LOD=5.3, R^2= 27.1%). The gen *TEMPRANILLO (TEM)* located between GAA47 and CaGM14822 is an interesting candidate gene to be considered for QTL_{DF1}. This gene is a repressor, so it is in concordance with the dominance of late flowering in this population.

Acknowledgments *INIA RTA2013-00025, project co-financed by the European Union through the ERDF 2014-2020 "Programa Operativo de Crecimiento Inteligente" and Contract 77863. IJCI-2014-22301, project financed by the Spanish Ministerio de Economía y Competitividad through grant Juan de la Cierva-Incorporación*

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Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

The investigation of silicon effects on yield and growth of chickpea, under salinity stress

G.R. Zamani[1], J. Shabani[2], A. Izanloo[1]

[1] *University of Birjand, Iran*
[2] *University of Tehran, Iran*

Salinity is a main abiotic stress that limits growth and productivity of plants. Role of silicon (Si) nutrition in mitigating salt stress damages has gain an increasing attention in recent years. Chickpea is a sensitive crop to salinity and it is grown mainly in arid and semi-arid regions. This study was conducted to investigate protective effect of Si on chickpea landraces from Iran. A factorial experiment was conducted in a CRD with three replications. Salinity factor was at 4 levels (0, 3, 5, and 7 dS m^{-1}) and silicon factor was applied at 3 levels (0, 0.5 and 1 mM). Phenological, physiological and biochemical characteristics were studied to evaluate Si effects on chickpea yield and its components. Application of Si has no significant effect on phenological traits. Harvest index, percent of healthy pods and seed protein were not affected by any factors. Number of leaves and branches, total dry matter**,** percent of fertile branches and leaf carbohydrate content were deacresed by salinity stress, Howerver Si was able to mitigate the effect of salinity in the measured traits. The amount of leaf proline content increased by intensifying salinity stress, but using Si showed significantly deacrese in proline production. The result showed positive and highly significant correlations between grain yield and total dry matter, leaf number, and percent of fertile branches. While a negative and significant correlatin was observed between yield and phenological traits as well as leaf proline. It seems that Si can indirectly alleviate the indused damaging effects through increase in vegetative growth.

Keywords: *Chickpea, Salinity stress, Silicon, Factor analysis*

How to refer your abstract:
G.R. Zamani, J. Shabani, A. Izanloo (2017) The investigation of silicon effects on yield and growth of chickpea, under salinity stress; ICLGG 2017 - Book of abstracts, ICLGG2017/P/11

Book of abstracts
ISBN 978-615-5270-39-0

Genomic approaches to identifying bacterial and plant genes involved in pathogenicity and resistance to common bacterial blight in *Phaseolus vulgaris*

W. Xie, T. Smith, G. Perry, E.D. Castro, E. Morneau, **K.P. Pauls**

Department of Plant Agriculture, University of Guelph, Guelph, Ontario, Canada

Common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *phaseoli* and its fuscan variant *X. fuscans* subsp. *fuscans,* is a damaging disease of common bean throughout the world. Genetic studies have identified a locus associated with the molecular marker SU91 on bean chromosome 8 which confers very effective resistance to the disease that has been used in a number of breeding programs throughout the world. To examine the genes in the locus in more detail the genomic sequence of the resistant line OAC Rex [1]in the SU91 CBB resistance QTL was compared to the corresponding sequence of the susceptible genome reference line G19833 [2]and another reference genome Bat91 [3]. We also sequenced this region in the CBB resistant *Phaseolus acutifolius* accession (440795) that was used to breed OAC Rex. This comparison identified a reorganization of a sterol transport gene (Niemann Pick) and the occurrence of additional resistance (R) genes in the OAC Rex genome, which may be associated with resistance. To understand the diversity of the CBB pathogen, single colonies (lines), purified from each of four locally collected bacterial isolates, were tested for symptom development and 7 lines with differential aggressiveness were characterized by genome sequencing. Their genome sizes ranged from 5.32-5.36 Mbp and differences in candidate virulence factors were identified that may be related to differences in aggressiveness between the bacterial lines through interactions with the promoters of target genes in bean resistance genes in the SU91 QTL region. This information will facilitate breeding bean cultivars with durable CBB resistance.

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How to refer your abstract:
W. Xie, T. Smith, G. Perry, E.D. Castro, E. Morneau, K.P. Pauls (2017) Genomic approaches to identifying bacterial and plant genes involved in pathogenicity and resistance to common bacterial blight in Phaseolus vulgaris; ICLGG 2017 - Book of abstracts, ICLGG2017/P/12

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

The effect of the presence of symbiotic *Rhizobium* on the effectivity of the *Agrobacterium tumefaciens*-mediated transformation of *Phaseolus vulgaris*

K. Hnatuszko-Konka, A. Gerszberg, M. Walak

Department of Genetics, Plant Molecular Biology and Biotechnology, Faculty of Biology and Environmental Protection, University of Łódź, Łódź, Poland

Tissue culture and transformation of common bean (*Phaseolus vulgaris)* are repeatedly considered to be difficult. It is particularly troublesome as the lack of efficient and rapid procedures hampers the possibilities of its genetic improvement. The *Agrobacterium tumefaciens*-mediated transformation seems to remain a method of choice in the case of common bean. Very often, however, the protocols available in the literature are not easy to reproduce and the need for even genotype/cultivar-specific transformation protocols is mentioned.

In our research we focus on the optimization, and hence development of a repeatable procedure for regeneration and transformation of common bean. Here, we present a preliminary report on the influence of the presence of symbiotic *Rhizobium* on the effectivity of the *Agrobacterium tumefaciens*-mediated transformation of *Phaseolus vulgaris*.

The common bean explants (hypocotyls, cotyledonary nodes and epicotyls from the Złota Saxa cultivar) were succesfully transformed using two types of bacterial cultures: I – *Agrobacterium tumefaciens*, GV3101 strain, carrying pCAMBIA1305.1 plasmid and II – a mixture of *Agrobacterium tumefaciens*, GV3101 strain, and *Rhizobium phaseoli* bacteria (*both* species carrying pCAMBIA1305.1 plasmid). The level of transformation (preliminary evaluation: the number of shoots surviving on the selection medium and PCR analysis) was twice as high when the mixture was used. The results suggest a substantial role of the presence of symbiotic bacteria for the transformation effectivity.

How to refer your abstract:
K. Hnatuszko-Konka, A. Gerszberg, M. Walak (2017) The effect of the presence of symbiotic Rhizobium on the effectivity of the Agrobacterium tumefaciens-mediated transformation of Phaseolus vulgaris; ICLGG 2017 - Book of abstracts, ICLGG2017/P/13

Book of abstracts
ISBN 978-615-5270-39-0

Expanding genetic resources of *Vicia faba* – generation of a reference transcript set

M.J. Nadzieja, L. Escobar-Herrera, J. Stougaard, S.U. Andersen

Department of Molecular Biology and Genetics, Aarhus University, Denmark

In order to sustain a large livestock production European Union Countries import large quantities of protein, mainly soybean, from US and South America. Increasing local production of protein, improving sustainability and diversity is a challenge that could be addressed by genomics-based breeding of *Vicia faba* (faba bean)[1]. Improved faba bean cultivars would then be available as competitive protein crops on domestic farmlands.

Faba bean is a high-yielding, high protein and relatively disease resistant crop legume. However, genetic resources available remain scarce. 13 GB diploid genome, occupied mostly by repetitive sequences, poses difficulties extracting important genetic information impairing possibilities of precise genomic-based breeding[2]. In order to expand available genetic resources, we aimed at generating a reference transcript set of a highly inbred line Hedin/2. We have collected tissue samples, including leaves, stems, flowers, pods, and seeds from field grown plants and extracted high quality RNA. mRNA was sequenced using Illumina standard protocol. Also, we sequenced bulk mRNA with strand-specific Illumina and PacBio for improved transcript assemblies. The tissues will be further analysed using mass spectrometry for specific metabolite signatures.

The obtained data will be used for generation of *V. faba* gene expression atlas. We will couple this information with metabolite data in order to investigate gene-to-metabolite correlations. The samples collection will be further expanded by *in vitro*/greenhouse obtained material such as roots, nodules and cotyledons.

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Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Comparative genome-wide-association mapping identifies common loci controlling root system architecture and resistance to *Aphanomyces euteiches* in pea

A. Desgroux[1,2], V. Baudais[2], V. Aubert[1], G. Le Roy[2], H. de Larambergue[1], H. Miteul[2], G. Aubert[1], G. Boutet[2], G. Duc[1], A. Baranger[2,3], J. Burstin[1], M. Manzanares-Dauleux[2], M.-L. Pilet-Nayel [2,3*], **V. Bourion**[1*]

[1] *Agroécologie, INRA, AgroSup Dijon, Univ. Bourgogne Franche-Comté, Dijon, France*
[2] *INRA, UMR 1349 IGEPP, Institut de Génétique et Protection des Plantes, Le Rheu, France*
[3] *PISOM, UMT INRA/Terre Inovia, Le Rheu, France*

** Contributed equally to the work*

Plant architecture can contribute to decrease plant susceptibility to pathogens by favoring mechanisms leading to infection escape or increased tolerance. Combining genetic resistance with architectural traits that can negatively impact disease development is thus a strategy of great interest to reduce epidemics. Until now, most strategies exploiting plant architecture have focused on the aerial parts of plants. Few studies have been done on the identification of root system architecture (RSA) traits limiting root disease development and even less on their use in breeding.

Aphanomyces euteiches, a soil-borne pathogen infecting roots, is a major limiting factor of pea crop yield. Consistent quantitative trait loci controlling partial resistance were identified in this species [1,2], and other studies reported its RSA as highly variable and under polygenic control [3]. However, the contribution of RSA to resistance to *A. euteiches* has not yet been explored in pea.

To identify common loci controlling RSA and resistance to *A. euteiches*, we performed a genome wide association (GWA) study using a collection of 266 pea lines contrasted for root architecture or Aphanomyces root rot resistance. The study used recent 14,157 Single Nucleotide Polymorphism resources developed in pea [4,5]. The collection was phenotyped in controlled conditions and using image analysis. The GWA study identified precise genomic regions controlling RSA and common loci associated with resistance to *A. euteiches*, opening prospects in mining RSA loci in breeding to limit Aphanomyces root rot severity on pea.

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Book of abstracts
ISBN 978-615-5270-39-0

Identification of new faba bean (*Vicia faba* L.) lines tolerant to *Orobanche* in the Southern Spain

C.I. González-Verdejo[1], I. Casimiro-Soriguer[1], J.A. López-Ráez[2], F. Maalouf[3], **A.M. Torres**[1]

[1] *IFAPA Centro Alameda del Obispo, Genomics and Biotechnology, Córdoba, Spain*
[2] *Estación Experimental del Zaidín (CSIC), Soil Microbiology and Symbiotic Systems, Granada, Spain*
[3] *International Center for Agricultural Reseach in Dry Areas, ICARDA-Terbol Station, Bekaa Valley, Lebanon*

One of the most limiting factors for faba bean production in the Mediterranean Basin is the parasitic plant **Orobanche crenata**. This parasite is very difficult to control being necessary the development of new methods, including tolerant crop varieties.

Parasicitc plants have evolved to ensure that germination takes place only in response to signals from a nearby suitable host root. The most important and effective germination signals are the phytohormones **strigolactones (SLs)** [1, 2].

Resistance at early stages of the parasite development is the most effective way of control, so that, lines showing lower SLs production are of most interest. Nevertheless, an important issue to take into account is the fact that SLs have also a role in plant architecture [2], which could influence crop production. For this reason, our objective is to develop **faba bean varieties** showing a reasonable equilibrium between tolerance-production.

With this aim, a set of field tolerant lines procedents of ICARDA "Faba Bean International Orobanche Nursery" (FBION) were evaluated under Southern Spain field conditions. Seven of the most tolerant lines were considered to be good candidates for a more in-depth analysis. The evaluation also included contrasted parental lines from two RILs populations segregating for O. crenata resistance, as well as, the susceptible cv. Prothabon and two resistant lines from Tunisia as controls [3]. In order to pinpoint the resistant stage of these lines, rhizotrom experiments and germination bioassays were carried out. Preliminary results show that line Sel. F7/8990/05 is one of the most tolerant, inducing 4-fold less O. crenata germination than the control. In addition, this line showed lower number of tubercles and a good yield, being a promising candidate.

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Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Identifying pathogen variability and virulence of *Uromyces viciae-fabae* on common cultivated legumes in Australia

U. Ijaz[1,2], **K.N. Adhikari**[1], H.S. Bariana[2], U.K. Bansal[2]

[1] *I.A. Watson Grains Research Centre, School of life and environmental Sciences, The University of Sydney, Narrabri, Australia*
[2] *Plant Breeding Institute, School of life and environmental Sciences, The University of Sydney, Cobbitty, Australia*

Uromyces vicia-fabae, commonly known as faba bean rust, is a biotrophic fungi causing rust in faba bean (*Vicia faba*), pea (*Pisum sativum*), lentil (*Lense culinaris*) and common vetch (*Vicia sativa*) [1]. This pathogen is widespread across all agricultural regions worldwide and occupies a complex host range because of existence of huge diversity within pathogen populations [2]. Rust is the most important disease of faba bean in northern New South Wales and southern Queensland region of Australia, but little information is available on its pathogen variability as currently there are no host differentials. This study was aimed to identify faba bean lines with differential pathogenecity that can be used to characterise the pathogen diversity and find out the interaction of Australian *U. Viciae-fabae* isolates with common cultivated grain legumes – chickpea, lentil, pea, lupin and mungbean.

Ten faba bean rust isolates collected from popular crop growing regions of New South Wales, Queensland and South Australia were purified and multiplied from a single spore culture. Each isolate was inoculated separately on forty faba bean and ten chickpea, lentil, pea, lupin and mungbean genotypes under glasshouse conditions. Among faba bean genotypes, we identified PBA Warda, IX486/7-6, IX552Rb-2-4, IX114#15033, IX585c-1-11, IX474/4-3, IX524Rb-2-1, Doza##14916, Doz#12034, Ac1655, Ac1227#14908 and Ac1257#14904 that can distinguish pathogen isolates. Nine physiological pathogen races were identified through this differential set and pathotypes were named according to the binary pathotype nomenclature [3]. No sporulation occurred on chickpea, lentil, lupin and mungbean except peas. Although, sporulation occurred on pea genotypes, it was a resistant reaction as lesions did not develop further. Necrotic reactions (resistant) were visible on infected chickpea, lentil and lupin leaves. Therefore, it was concluded that faba bean and pea were host species and others as non-host species for *U. Viciae-fabae* pathotypes in Australia.

The spore germination and development was observed under microscopy using UV light on non-host resistance displayed by chickpea, lentil, lupin and mungbean. Similar to host species, haustoria development started within 24 h of inoculation in chickpea and lupin. However, in lentil and mungeban, the haustoria development was significantly delayed and only about 10% of spores developed haustoria. Moreover, after haustoria development, hypersensitive cell death was visible in lentil. Sporulation did not occur in rust infected leaves of chickpea and lupin and leaves died after 21 days just before the onset of uredia development. Thus, it was concluded that chickpea, lupin, lentil and mungbean were non host showing post haustorial resistance against Australian *U. Viciae-fabae* isolates.

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U. Ijaz, K.N. Adhikari, H.S. Bariana, U.K. Bansal (2017) Identifying pathogen variability and virulence of Uromyces viciae-fabae on common cultivated legumes in Australia; ICLGG 2017 - Book of abstracts, ICLGG2017/P/17

Book of abstracts
ISBN 978-615-5270-39-0

Mining wild-chickpea (*Cicer reticulatum and C. echinospermum*) for adaptive traits to Australian growing conditions

L.G. Kamphuis[1,2], C. Grimes[1], F.L. Kamphuis[1], R. Syme[1], M. Pazos-Navarro[3], J. Berger[2], R. Lee[1], J. Croser[3]

[1] *Centre for Crop and Disease Management, Curtin University, Bentley, Australia*
[2] *CSIRO Agriculture & Food, Floreat, Australia*
[3] *The UWA School of Agriculture and Environment, Centre for Plant Genetics and Breeding, University of Western Australia, Crawley, Australia*

Chickpeas (*Cicer arietinum*) are used in rotational cropping systems worldwide, but production is contrained by a number of biotic and abiotic stresses. Cultivated chickpea has an inheretly low genetic diversity and a recent international effort to collect wild chickpea germplasm from a range of eco-geographic regions spanning the distribution of wild chickpeas (both *C. reticulatum* and *C. echinospermum*) has allowed the chickpea community to broaden the genetic diversity and identify germplasm with adaptive traits suitable to local growng conditions. This project aims to broaden the genetic base of Australian chickpea cultivars through the introgression of wild chickpea material (*C. reticulatum* and *C. echinospermum*) with adaptive traits to Australian growing conditions. A total of 24 crosses between wild chickpea accessions and the Australian cultivar PBA Hat Trick were generated and the F_2 populations are being evaluated for growth habit, flowering time and shattering in the field. The wild chickpea germplasm has also been screened for its response to Ascochyta blight infection, a major constraint in chickpea production in Australia, and range of response were observed. An overview of the population development and Ascochyta disease screening will be presented.

How to refer your abstract:
L.G. Kamphuis, C. Grimes, F.L. Kamphuis, R. Syme, M. Pazos-Navarro, J. Berger, R. Lee, J. Croser (2017) Mining wild-chickpea (Cicer reticulatum and C. echinospermum) for adaptive traits to Australian growing conditions; ICLGG 2017 - Book of abstracts, ICLGG2017/P/18

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Multi-environment QTL analyses for Ascochyta blight resistance in a recombinant inbred population of chickpea (*Cicer arientinum* L.)

A. Hamwieh[1], I. Mohammad[2], S. Ahmed[1], S. Kebabeh[1], A.M. Alsamman[3], T. Istanbuli[1]

[1] *International Center for Agricultural Research in the Dry Areas (ICARDA), Beirut, Lebanon*
[2] *International Maize and Wheat Improvement Center (CIMMYT) Pakistan Office, Islamabad, Pakistan*
[3] *Agricultural Genetic Engineer Research Institute (AGERI), Giza, Egypt*

Chickpea (*Cicer arietinum* L.) crop occupies the third position among grain legumes cultivated around the world. Ascochyta blight (AB) caused by *Ascochyta rabiei* (Pass.) Labr. is one of the most destructive foliar diseases that can cause complete loss of the crop in many chickpea growing regions around the world. A recombinant inbred line (RIL) population, comprising 165 lines derived from FLIP98-1065 (R) x ILC1929 (S) have been evaluated in 6 environments across three years (2008-2011) and three locations in Syria (Tel Hadya "TH", Lattakia "Lat" and the greenhouse). Field screening was conducted using alpha lattice design with two replications. The greenhouse experiments was conducted against AB pathotype II. ANOVA analysis indicated significant differences between the RILs and environments. A total of 1398 (134 SSR, 652 DArTseq and 612 SNP) markers have been produced to develop a genetic map. This study produced a high-resolution map (1244 markers spanning 2503 cM on eight linkage groups). Three major conserved QTLs conferring AB resistance in chickpea, two QTLs on Chromosome 2 (LG2-A and LG2-B) and one on LG4 explaining maximum 18.5%, 11.1% and 25% of the total variation respectively. In total, 18 predicted genes were located in the LG4, and 9 and 10 predicted genes were located in the LG2-A and LG-B respectively. This study presents a first set of SNP markers in chickpea located within genes, which could be applied as MAS for AB resistance in chickpea breeding programs.

How to refer your abstract:
A. Hamwieh, I. Mohammad, S. Ahmed, S. Kebabeh, A.M. Alsamman, T. Istanbuli (2017) Multi-environment QTL analyses for Ascochyta blight resistance in a recombinant inbred population of chickpea (Cicer arientinum L.); ICLGG 2017 - Book of abstracts, ICLGG2017/P/19

Book of abstracts
ISBN 978-615-5270-39-0

New SNP associated with common bacterial blight resistance in dry edible bean breeding lines

K.J. Simons[1], R.S. Lamppa[1], P.E. McClean[2], J.M. Osorno[2], **J.S. Pasche**[1]

[1] *Department of Plant Pathology, North Dakota State University, Fargo, USA*
[2] *Department of Plant Sciences, North Dakota State University, Fargo, USA*

Common bacterial blight (CBB) of dry bean is of worldwide importance causing up to 50% yield loss through loss of photosynthetic area and poor harvested seed quality. The most effective method for controlling *Xanthomonas axonopodis pv. phaseoli* (*Xap*) infection is through the use of resistant cultivars. Host resistance is controlled by minor and major QTL identified across all eleven chromosomes of *Phaseolus vulgaris*. QTL markers, particularly SU91 and SAP6, have been used intensively for marker assisted selection (MAS). A preliminary study of breeding lines from the NDSU dry bean breeding program belonging to seven market classes indicated 25% of the lines exhibited a resistant reaction to *Xap* and 20% of those lines did not contain either SU91 or SAP6. The preliminary study was expanded to include over 500 breeding lines which underwent both phenotypic (greenhouse screening) and genotypic analysis via reduced complexity sequencing, and association mapping. Phenotypic results were similar to the preliminary study, with 25% of the breeding lines exhibiting a resistant reaction on the primary and trifoliate leaves. The reduced complexity sequencing and subsequent filtering produced over 9,000 SNP for use in association mapping. Preliminary association results identified two previously described QTL for CBB resistance found on chromosomes Pv5 and Pv11. The SNP underlying these QTL will provide targets for developing new markers for use in MAS.

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Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Identification of a candidate gene for double podding in chickpea

C. Caballo Linares[1], A. Berbel[2], R. Ortega[3], M.J. Domenech[2], T. Millán[4], J. Gil[4], F. Madueño[2], J. Rubio[1]

[1] *Área de Genómica y Biotecnología, IFAPA, Córdoba, Spain*
[2] *Instituto de Biología Molecular y Celular de Plantas, CSIC-UPV, Valencia, Spain*
[3] *School of Biological Sciences, University of Tasmania, Hobart, Australia*
[4] *Dept. Genética, Universidad de Córdoba, Campus Rabanales, Córdoba, Spain*

The basic type of inflorescence in legumes is the compound inflorescence, where flowers appear in secondary inflorescences. Chickpea normally have one flower per node but mutants with two or more flowers have been reported. We know very little about the genes that control the activity of the meristem in chickpea (*Cicer arietinum*), and determine the number of flowers by penduncle. In previous studies our group located a 92.6 kb region related with the character simple/double pod[1], inside this area we have 7 genes, one of them, RAX2, is the mayor candidate controlling the type of inflorescence. In order to find differences between simple and double pod genotypes, we use bioinformatics tools to compare resequencing of JG62 (double pod) against CDC-Frontier[2] (simple pod). This allowed us to identify a deletion around 44kb in JG62 including RAX2 and two other genes, one of them with a partial deletion.

Functional characterization of these genes by SEM microscopy, *in situ* hybridization and q-PCR is being carried out. The next step will be the use of VIGs (Virus-induced gene silencing) to test the responsible gene of the character.

We hope that the identification and characterization of the gene implicated in the character simple/double pod will allow us to better understand the genetic control of the development of the compound inflorescence of legumes.

Acknowledgements: *This work has been supported by the INIA project contract RTA2013-00025, co-financed by the European Union through the ERDF 2014-2020. Caballo C. acknowledges her Ph.D. fellowship INIA-CCAA.*

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Book of abstracts
ISBN 978-615-5270-39-0

Legume response to varied light quality and genetic control of flowering induction

M. Pazos-Navarro[1], F. Ribalta[1], R.G. Bennett[1], P. Kaur[1,2], J. Croser[1]

[1] *The UWA School of Agriculture and Environment, Centre for Plant Genetics and Breeding, University of Western Australia, Perth, Australia*
[2] *Personalised Medicine Centre for Children (PMCC), Telethon Kids Institute, Perth, Australia*

Light quality, quantity and duration are critical parameters for flowering induction. In previous studies, we have elucidated the effect of light quality on early flowering onset in cool-season grain and pasture legume species [1 and 2]. Through these studies, we developed predictive models for time to flowering under optimised light quality parameters. The red to far red ratio and the amount of photons in the far red region of the spectrum play a key role in accelerating floral onset. In Arabidopsis, significant progress has been made toward understanding the role light quality plays on floral initiation pathways. However, in legumes, questions remain about the genetic mechanisms involved in this response. An improved understanding of the gene networks underlying flowering induction in response to light quality requires better characterisation of the transcriptome.

We relate our phenotyping observation of floral behaviour under varied light spectra with the network involved in flowering responses to light quality by characterising the transcriptional activity in Trifolium.

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M. Pazos-Navarro, F. Ribalta, R.G. Bennett, P. Kaur, J. Croser (2017) Legume response to varied light quality and genetic control of flowering induction; ICLGG 2017 - Book of abstracts, ICLGG2017/P/22

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Heterosis in relation to genetic divergence and hybridity in chickpea (*Cicer arietinum* L.) under rice based cropping system

P.L. Johnson, R.N. Sharma, H.C. Nanda

Department of Genetics and Plant Breeding, Indira Gandhi Krishi Vishwavidyalaya, Chhattisgarh, India

Chickpea (*Cicer arietinum* L.) is one of the most important pulse crops of India. It behaves differently when grown under varying rice based cropping system. In present study these have been defined on the basis duration of rice varieties *viz*. after the harvest of early rice (E_1), after harvest of medium duration rice (E_2) and after harvest of long duration rice (E3). Though heterosis breeding is utilized to achieve substantial enhancement in yield and quality aspect of crop plants, in case of self pollinated crops include chickpea, heterosis cannot be exploited by the way of heterotic crosses due to biological infeasibility but cross combination showing heterotic vigour can be utilized as the source population for driving superior progenies.

The experimental material comprised of 21 f_1s developed through Line X Tester crossing between 7 lines and 3 testers. The testers were selected for early maturity. Whereas, line were the agronomically well adopted varieties of Chickpea. Whereas, parents were selected during 2014-15. These f_1s along with parents were evaluated during, *rabi* 2015-16. under three rice based cropping systems at Research Cum Instructional farm, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.) India.

Result of the genetic divergense study for selection of parents revealed that the D^2 value between parents ranged from 341.10 to 4814.93, the crosse JG 315 x ICCV 96029 had the highest parental diversity (4814.93). It exhibited positive heterobeltiosis for the days to 50 % flowering, days to maturity, secondary branches plant$^{-1,}$ pods plant, biological yield and seed yield plant^{-1} .whereas, it showed negative heterobeltiosis for 100 seed weight. The genetic purity of cross Vaibhav x JG 97 and JG 11 x JG 97 for SSR 21 markers was done to determine the hybridity banding pattern of f_1s and their parental lines which indicated that pure hybrid showed two bands similar to their parental lines off types did not showed similar to banding pattern of parental lines. Implications of these findings have been discussed in the paper.

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Book of abstracts
ISBN 978-615-5270-39-0

Identification of QTLs associated with number of branches in soybean

S. Shim[1], **J. Ha**[1,2], M.Y. Kim[1,2], S.-H. Lee[1,2]

[1] *Department of Plant Science and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea*
[2] *Plant Genomics and Breeding Institute, Seoul National University, Seoul, Republic of Korea*

The number of branches is one of the major yield components, directly affecting the number of total pods per plant. A number of genetic studies for branching in soybean have been reported. The QTL regions previously reported, however, still embrace a great of seeming genes owing to low level of resolution of markers flanking the QTLs. In this study, QTLs conferring branching were identified based on the high-density genetic map. Additionally, correlation of branching with total pod number was also investigated. Although there were previously reported QTLs for branch number and total pod number on the same chromosomes we identified the QTLs, we narrowed down the QTL regions from 0.7 Mb to 0.1 Mb at least, from 26 Mb to 0.5 Mb at most so that we could identify highly promising candidate genes. The BRANCHED1 (BRC1) gene, which encodes TEOSINTE-BRANCHED1/CYCLOIDEA/PCF (TCP) transcription factor, and genes which regulate developmental growth associated with auxin signaling were identified as candidate genes for branching. This study will help breeders improve soybean yield using marker assisted selection (MAS) of branch number and will facilitate identification of the causative genes for the traits in the near future.

How to refer your abstract:
S Shim, J. Ha, M.Y. Kim, S.-H. Lee (2017) Identification of QTLs associated with number of branches in soybean; ICLGG 2017 - Book of abstracts, ICLGG2017/P/24

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Investigation on inflorescence architecture of mungbean associated with synchronous maturity in pods

E.S. Lee[1], M.Y. Kim[1,2], J. Ha[1,2], **M.Y. Yoon**[1,2]. H.-J. Jang[1,2], S.-H. Lee[1,2]

[1] *Department of Plant Science and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea*
[2] *Plant Genomics and Breeding Institute, Seoul National University, Seoul, Republic of Korea*

Mungbean (*Vigna radiate*) is a legume crop that has a short life-cycle and has an ability to self-fertilize. Both the production and consumption of mungbean have increased steadily, but one of challenges faced by farmers is the inability to harvest all the produce at the same time because of non-synchronous maturity due to indeterminate growth. Here, this study describes the general inflorescence architecture of mungbean and associates inflorescence architecture traits with synchrony in pods. Typically, mungbean has a compound raceme inflorescence architecture consisting of main (primary) branch and secondary branches that make flowers. However, there is an exceptional genotype named 'Binh khe D.X.' that has a simple raceme inflorescence architecture and produces flowers directly from the main branch. In this study, plants were harvested either once or several times for measuring the degree of synchrony in pod maturity. Our results suggest that the difference in synchronous and non-synchronous pod maturity was caused by indeterminate characters such as branches and peduncles. Our description of the general inflorescence architecture and evaluation method for synchrony in pod maturity could be used to improve the development of mungbean breeding in the future.

How to refer your abstract:
E.S. Lee, M.Y. Kim, J. Ha, M.Y. Yoon. H.-J. Jang, S.-H. Lee (2017) Investigation on inflorescence architecture of mungbean associated with synchronous maturity in pods; ICLGG 2017 - Book of abstracts, ICLGG2017/P/25

Book of abstracts
ISBN 978-615-5270-39-0

Phytosulfokine-alpha, an enhancer of *in vitro* regeneration competence in recalcitrant legumes

S.J. Ochatt, C. Conreux, G. Despierre, J.B. Magnin-Robert, B. Raffiot

Agroécologie, AgroSup Dijon, INRA, Univ. Bourgogne Franche-Comté, Dijon, France

Oligopeptides have been recognized as signalling molecules playing an important role in plant cell growth and development. Phytosulfokine-alpha (PSK), a plant-specific disulfated pentapeptide, is involved at nanomolar concentrations in initial steps of cellular dedifferentiation, proliferation, and re-differentiation, with a biological function similar to that of plant hormones. On the other hand, legume crops are generally known for their recalcitrance to *in vitro* regeneration approaches, which has restrained the exploitation of biotechnological tools for their genetic improvement.

Against this background, we added PSK at concentrations of 10^{-10} to 10^{-6} M, to semisolid MS-based culture media previously shown to permit some regeneration responses with a number of genotypes of pea (*Pisum sativum*), *Medicago truncatula* and also the highly recalcitrant faba bean (*Vicia faba*).

Callus, cell suspensions and embryo-derived explants of barrel medic R108, pea cvs Frisson and Cameor and a zero vicin, zero tannin faba bean genotype were tested and their embryogenic and organogenic regeneration competence was assessed. PSK had a strong and significant enhancing effect on the regeneration competence of all genotypes, producing somatic embryos and organs that yielded regenerated plants of both pea and *M. truncatula*, and with a major organogenic effect leading to plant regeneration with faba bean where somatic embryos, although produced, failed to convert into viable plants. This is the first report on the use of PSK with legume species.

How to refer your abstract:
S.J. Ochatt, C. Conreux, G. Despierre, J.B. Magnin-Robert, B. Raffiot (2017) Phytosulfokine-alpha, an enhancer of in vitro regeneration competence in recalcitrant legumes; ICLGG 2017 - Book of abstracts, ICLGG2017/P/26

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

RNA-seq analysis uncovers common bean genes involved in pod maturation and dehiscence

C. Gómez-Martín[1,2,*], A. González[3,*], C.R. Lebrón[1,2], C. Capel[4], F.J. Yuste-Lisbona[4], M. Hackenberg[1,2], J.L. Oliver[1,2], M. Santalla[3], **R. Lozano**[4]

[1] *Department of Genetics, Faculty of Science, University of Granada, Granada, Spain*
[2] *Laboratory of Bioinformatics, Centro de Investigación Biomédica, Granada, Spain*
[3] *Grupo de Biología de Agrosistemas, Misión Biológica de Galicia-CSIC, Pontevedra, Spain*
[4] *Centro de Investigación en Biotecnología Agroalimentaria (CIAIMBITAL), Universidad de Almeria, Almeria, Spain*

** Both authors contributed equally to this work*

Fruit maturation and dehiscence are important developmental processes in legume species as they influence fruit quality and crop productivity (Tang et al., 2013). Despite that key regulators of fruit development have been characterized in the model species *Arabidopsis thaliana*, the genetic basis of fruit maturation and dehiscence remains unknown in agricultural species like common bean (Li and Olsen, 2016). Recent advances in genetic, molecular and physiological research, mostly brought about by the implementation of high-throughput and next generation sequencing (NGS) technologies have begun to decipher the genetic networks regulating fruit development. With the aim to unravel the transcriptomic changes associated to pod maturation and dehiscence, we used a powerful RNA-seq bioinformatics protocol to compared immature and mature pods of two accessions, which also differed in deshiscence and fiber content. RNA-seq short reads were aligned to the most recent update of the *Phaseolus vulgaris* reference genome, then computing the fold changes in assembled transcript expression among different developmental stages. Using *Phytozome* gene annotation for common bean, a significant number of differentially expressed genes (DEGs) were associated with pod maturation in both accessions, which were mainly involved in transmembrane transporter activity, carbohydrate metabolism and photosynthesis. Furthermore, among DEGs between dehiscent and indehiscent fruits highlights genes related to oxidation-reduction and lipid metabolic processes. This work provides comprehensive insights into pod maturation and dehiscence, identifying gene expression profiles with major roles on these developmental processes.

Acknowledgements: *This work was financially supported by the Ministerio de Economía y Competitividad (AGL2014-51809-R and AGL2015-64991-C3-1-R) and UE-FEDER Program.*

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Book of abstracts
ISBN 978-615-5270-39-0

An examination of QTL architecture underlying pod shattering resistance in common bean

A.M. González[1], F. Yuste-Lisbona[2], A. Fernández-Lozano[2], C. Capel[2], O. Muñoz[1], A.P. Rodiño[1], R. Lozano[2], **M. Santalla**[1]

[1] *Grupo de Biología de Agrosistemas, Misión Biológica de Galicia-CSIC, Pontevedra, Spain*
[2] *Departamento de Biología y Geología (Genética), Centro de Investigación en Biotecnología Agroalimentaria (BITAL), Universidad de Almería, Almería, Spain*

The domesticated common bean (*Phaseolus vulgaris*) originated in the Mesoamerican and Andean regions independently. Similar to other legume crops, the reduction of pod shattering represents a key domestication syndrome in the domesticated common bean. Understanding the genetic variation of common bean in relation to pod shattering is important for elucidating the underlying genetic mechanisms of parallel evolution (Don and Wang, 2015) and also because this will provide breeders with key tools to improve this trait, thus reducing yield loss (Santalla et al., 2004). Most of these studies have been conducted in cereals (Lin et al., 2012), while in legumes, the identification of pod-shattering genes lag far behind those of the cereal crops (Li and Olsen, 2016). In this study, we identified quantitative trait loci (QTLs) controlling pod fiber in the ventral suture (string) and pod shattering in a recombinant inbred line (RIL) population derived from a cross between a cultivated common bean and wild nuña bean. $F_{2:7}$ flines were grown under short-day conditions and pod string and pod shattering were analysed at several developmental stages. Correlation analysis showed positive relationship among pod string and shattering. Multi-environment QTL mapping revealed that a major QTL on linkage group 2 (LG02) controlled pod string and co-localized to pod shattering. In addition, minor QTLs related to pod string and pod shattering were identified on LGs01, 03, 04 and 07. Genes underlying the pleiotropic QTL regions could be potential targets for improving shattering resistance performance through marker assisted selection. These QTLs not only provide useful information for isolating candidate genes involved in pod development but also mean potential targets for improving pod shattering resistance by marker assisted selection.

Acknowledgements: *This work was financially supported by the Ministerio de Economía y Competitividad (AGL2014-51809-R and AGL2015-64991-C3-1-R) and UE-FEDER Program.*

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Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Development of an interspecific linkage map and identification of genomic regions controlling agronomic traits in lentil

L.E. Sáenz de Miera, C. Polanco, P. García, F. Vaquero, F.J. Vences, A.I. González, **M. Pérez de la Vega**

Área de Genética, Fac. Biología, Universidad de León, León, Spain

Interspecific crosses allow the introgression of interesting alleles from wild species. Here we present a preliminary interspecific genetic map constructed using a population of 50 RIL advanced to F_7 from a cross between *Lens culinaris* subsp *culinaris* cv. Alpo and the wild relative *L. odemensis* (ILL235). DiscoSnp++ software was used to identify 28,414 SNPs and short indels from expressed paired-end reads obtained using the RNAseq procedure and an Illumina HiSeq 2500 system. The markers were located in the draft v. 1.2 lentil Redberry genome using MagicBlast searches and identified 11,216 different segregating annotated genes. Non-distorted segregation ($p>0.01$) was observed only for 2,972 markers that were used to construct both physical and linkage maps. A linkage map containing 29 groups was obtained using MSTmap software with LOD 6 and 15 cM distance threshold. The correlation between linkage groups and draft genome chromosomes suggest the existence of chromosome rearrangements between both lentil species.

The analysis of phenotypic agronomic traits revealed in the linkage group LG01 a single genomic region controlling seed coat pattern and flower colour, another region controlling flowering date, and a third region related to total stem number; two close regions in LG03 controlling stem colour and another two regions related to seed size; a region in LG05 for tendril presence and other three at lower level significance in LG01 for this trait.

How to refer your abstract:
L.E. Sáenz de Miera, C. Polanco, P. García, F. Vaquero, F.J. Vences, A.I. González, M. Pérez de la Vega (2017) Development of an interspecific linkage map and identification of genomic regions controlling agronomic traits in lentil; ICLGG 2017 - Book of abstracts, ICLGG2017/P/29

Book of abstracts
ISBN 978-615-5270-39-0

DNA barcoding studies on two endemic species of Astragalus L. from Turkey using sequences of nrDNA ITS and cpDNA trnL intron and the trnL-trnF IGS

Ö. Çetin[1], M. Çelik[2], T. Özcan[3]

[1] *Department of Biotechnology, Selçuk University, Konya, Turkey*
[2] *Advanced Technology Research and Application Center, Selçuk University, Konya, Turkey*
[3] *Department of Biology Education, Balıkesir University, Balıkesir, Turkey*

The flowering plant family Fabaceae comprises approximately 750 genera and 19.500 species.The large genus *Astragalus* L. comprises about 3000 taxa in the world and is represented by 480 taxa in Turkey. Two endemic species of the genus *Astragalus*, *Astragalus vulnerariae* DC. and *A. ovalis* Boiss. & Balansa, have been included in the present analysis. Total DNA was extracted from silica-gels dried leaves using the Plant Dneasy Extraction Kit. The trnL intron and the trnL-trnF IGS regions of the chloroplast genome and nuclear ribosomal ITS DNA region of two species were amplified using the universal primers. Amplified DNA fragments were separated by electrophoresis in a 1.5% agarose gel at 70 V in TBE buffer. The PCR products were sent to sequencing company. The amplified fragments were sequenced using the same primers used for amplification. DNA sequences were checked and edited using Sequencher 5.4, and aligned using CLUSTAL X. Our main purpose to evaluate the potential utility of trnL intron and the trnL-trnF IGS regions and nuclear ITS region for identifying and discriminating *Astragalus* species based on a representative samples. The sequences data for two species are ranged from 740 to 950 base pairs (bp) for trnL-F and 710-720 base pairs for ITS region. Comparison of the DNA sequences between two species showed that *A. ovalis* has 11 units single-nucleotide difference, one 2-nucleotide, one 4-nucleotide and three 6-nucleotide insertions (duplications), and one 6-nucleotide deletion at the trnL intron and trnL-trnF IGS region. Analyses of the ITS region of nrDNA showed that *A. ovalis* has 32 units single-nucleotide difference and one 8-nucleotide deletions.

How to refer your abstract:
Ö. Çetin, M. Çelik, T. Özcan (2017) DNA barcoding studies on two endemic species of Astragalus L. from Turkey using sequences of nrDNA ITS and cpDNA trnL intron and the trnL-trnF IGS; ICLGG 2017 - Book of abstracts, ICLGG2017/P/30

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

DNA barcoding study on Lotononis genistoides (Fenzl) *Benth*

M. Çelik[1], Ö. Çetin[2]

[1] *Advanced Technology Research and Application Center, Selçuk University, Konya, Turkey*
[2] *Department of Biotechnology, Selçuk University, Konya, Turkey*

Fabaceae is represented about 770 genera and 19.500 species in the world. It comprises approximately 72 genera and 1218 taxa in Turkey. *Lotononis* (DC.) Eckl. & Zeyh. is a large genus in the tribe Crotalarieae and represented about 150 species in the world. The tribe Crotalarieae is represented only one species, *Lotononis genistoides* (Fenzl) Benth., in Turkey. In our study, two DNA regions, internal transcribed spacer (ITS) region of nuclear genome and the *trn*L intron and the *trn*L-*trn*F IGS regions of chloroplast genome, were evaluated as potential DNA barcodes for *Lotononis*. Total DNA was obtained from 20–30 mg leaf parts from *Lotononis genistoides*. Qiagen miniprep kit was used for the DNA isolation with some modifications. Two different regions from nuclear and chloroplast genome were amplified using ITS5a (forward)- ITS4 (reverse) and *trn*L-c (forward)- *trn*F-f (reverse) primers respectively. The amplified fragments were sequenced using the same primers used for amplification. ITS1+5.8S rDNA+ITS2 and *trn*L-F sequences were aligned and compared via Bioedit programme. ITS and *trn*L-F sequences of *Lotononis genistoides* was obtained in our study. According to our sequence data, entire ITS region is around 690 bp in length and the cpDNA trnL intron and trnL-F IGS region is around 704 bp in length. The ,average G+C content of the ITS and trnL-F regions was 60.29% and 27.36%, respectively. These sequences were compared with other *Lotononis* sequences from NCBI. The main purpose of this study is to determine the minor base changing of *Lotononis genistoides*.

How to refer your abstract:
M. Çelik, Ö. Çetin (2017) DNA barcoding study on Lotononis genistoides (Fenzl) Benth; ICLGG 2017 - Book of abstracts, ICLGG2017/P/31

Book of abstracts
ISBN 978-615-5270-39-0

Ecological and evolutionary genetics of wild *Cicer species*

F. Javadi, H. Yamaguchi

Kyushu University, Japan

The wild species are adapted to a wide range of habitats and possess considerable variability for most biotic and abiotic traits. Crop wild relatives (CWR) provide a wide range of valuable attributes for plant breeding and have enabled rapid advances in ecological and evolutionary genetics. Nevertheless, distantly related wild species are given less priority, however some of CWR growing in harsher environments should be collected and characterized because they might possess useful characteristics beneficial for crop improvement. Here we address this need by inferring phylogenetic relationship, ecological and seed traits of thirty wild relatives of chickpea (*Cicer arietinum*). Our results reveal four robust geographically distinct lineages within the genus *Cicer*. Phylogenetically related species from a geographical region and habitat showed characteristic feature of the seed coat texture. Wild *Cicer* species have high level of diversity and should be considered for conservation to help mitigate loss of genetic diversity.

How to refer your abstract:
F. Javadi (2017) Ecological and evolutionary genetics of wild Cicer species; ICLGG 2017 - Book of abstracts, ICLGG2017/P/32

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Finalizing the *Tnt1* mutant population in *Medicago truncatula*

X. Cheng, L. Sun, K.S. Mysore, **J. Wen**

Noble Research Institute LLC, Ardmore, USA

Over the last 14 years, we have generated a near-saturated insertional mutant population in *Medicago truncatula* using the tobacco retrotransposon, *Tnt1*. Meanwhile, we organized annual forward genetics screening workshops from 2005 to 2017 with participants from the legume community. Using thermal asymetric interlaced PCR (TAIL-PCR)[1] and subsequent Sanger or Illumina Miseq sequencing platforms[2], we recovered nearly 400,000 *Tnt1* flanking sequence tags (FSTs) from all of the 21,700 regenerated *Tnt1* insertion lines. Photographs of visible phenotypes and BLAST-searchable FSTs are accessible to all users through a web-based database. In addition, we pooled genomic DNA from all the 21,700 lines for PCR-based reverse-genetic screening and recovered at least one *Tnt1* insertion line for at least 85% of 2,100 screened genes[3]. Over the last ten years, we have distributed more than 9,000 mutant lines to about 200 research groups in 27 countries. The coverage and the range and diversity of mutant phenotypes obtained to date suggest that the *Tnt1* mutant population in *M. truncatula* is a great resource for dissecting the function of most genes in legume biology. About ten years ago, we performed an analysis on *Tnt1* insertion site preference and distribution frequency in the Medicago genome based on 2461 FSTs[4]. Now we have more than 390,000 FSTs and we performed a similar analysis to validate the previous conclusions. The analysis results will be presented.

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Book of abstracts
ISBN 978-615-5270-39-0

Flow cytometry measurements contribute to *Pisum* taxonomy

S.J. Ochatt[1], P. Smýkal[2], E. Patat-Ochatt[1], C. Conreux[1]

[1] *Agroécologie, AgroSup Dijon, INRA, Univ. Bourgogne Franche-Comté, Dijon, France*
[2] *Department of Botany, Faculty of Sciences, Palacky University in Olomouc, Olomouc, Czech Republic*

Pea (*Pisum sativum* L.) has been widely used in early hybridization studies, as model for experimental morphology and physiology, and was Mendel's model species to untangle the laws of inheritance, which puts it at the foundation of modern genetics. Its large genome size (4775 Mbp as assessed by Feulgen method in 1976) slowed down progress in pea genomics compared with other plant species, but the recent availability of genome sequences of various legume species now permits genome wide comparison and allows to identify genes underlying agronomically important traits by combining candidate gene and synteny approaches. The efficient use of existing genomic resources is a key to success in these goals and several types of molecular marker sets as well as both transcriptome and proteome datasets exist. Despite this impressive background, and the fact that *P. sativum* is one of the most frequently used internal standards for flow cytometry studies with other species, there is still a need to further clarify the taxonomy within species of the genus *Pisum*. Thus, we have analysed by flow cytometry 42 accessions from a range of geographic origins and belonging to two wild species: *P. sativum* subsp. *elatius (e.g.* including former *P. elatius* and *P. humile/syriacum)*, *P. fulvum* and cultivated: *P. abyssinicum, P. sativum* as well as some primitive *P. sativum* cultigens (such as subsp. *transcaucassicum, asiaticum*), where some of them had been identified differently or tentatively in the past based on botanical characteristics. In these studies, all materials were analysed simultaneously with *Medicago truncatula* as the internal standard, and with various fluorochromes (DAPI, Propidium Iodide, Chromomycine A3) to assess the relative nuclear DNA content, genome size and AT/GC ratio. For some of the species studied this is the first report on these traits.

How to refer your abstract:
S.J. Ochatt, P. Smýkal, E. Patat-Ochatt, C. Conreux (2017) Flow cytometry measurements contribute to Pisum taxonomy; ICLGG 2017 - Book of abstracts, ICLGG2017/P/34

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Genetic diversity assessement of some Moroccan lentil landraces using electrophoresis (SDS-PAGE) of seed storage proteins

F.Z. El Hamzaoui[2], **N. Benbrahim**[1], F. Gaboun[1], M. Taghouti[1]

[1] *National Institute for Agricultural Research (INRA), Regional Center for Agricultural Research in Rabat, Morocco*
[2] *Sciences Faculty of Kenitra, Morocco*

Lentil is among the main food legumes in Morocco that has been cultivated to satisfy nutritional, socioeconomically and ecological needs. Farmers use mainly landraces because of their specific adaptability in environment constraints. These landraces showed an appreciable genetic diversity according to morphological traits. They could contain genes of interest related to their adaptation ability to biotic and abiotic stresses. However, these landraces are threatened because of novel varieties, economic development and climate change. The objective of this study is to optimize genetic resources management in plant breeding program. Thus, genetic polymorphism of nine landraces of lentil collected from different sites of the country (*Lens culinaris* Med.) was assessed using seed storage proteins electrophoresis (SDS-PAGE). Genetic diversity within and among samples was based on 23 reproducibly scored bands identified in the zones of highest variation of protein profile around 97 kDa and from 65 to 30 kDa. The variation within populations was expressed according to genetic parameters and Shannon–Weaver Index whiles distribution of variation and differential connectivity among populations were examined using PhiPT parameter. Results show that Moroccans' lentil landraces exhibit considerable genetic diversity. Polymorphism rate was more important between landraces (P=62%) ranging from 6% to 70% than within landraces (P=38%) supposing a regional differentiation under natural and human pressures. Zaer lentil landrace was the larger pool genetic according to alleles frequency (Na= 1.580), Shannon-weaver's Index (0.423) and the expected heterozygosity (0.293). These local genetic cores might be useful in selecting genotypes according to the objectives of breeding programs.

Keywords: *Lentil, Genetic variability, seed storage proteins electrophoresis (SDS-PAGE), Genetic parameters.*

How to refer your abstract:
F.Z. El Hamzaoui, N. Benbrahim, F. Gaboun, M. Taghouti (2017) Genetic diversity assessement of some Moroccan lentil landraces using electrophoresis (SDS-PAGE) of seed storage proteins; ICLGG 2017 - Book of abstracts, ICLGG2017/P/35

Book of abstracts
ISBN 978-615-5270-39-0

Genetic relationship of *Vigna unguiculata* spp. accessions based on cpSSR markers

E. Monteiro[1], I. Castro[1,2], **M. Carvalho**[2], V. Carnide[1,2]

[1] *Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal*
[2] *Centre for Research and Technology of Agro-Environment and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal*

The genus *Vigna* contains more than 80 agricultural important species, including black gram (*V. mungo*) that is an important summer pulse crop in many South Asian countries and cowpea (*V. unguiculata*) that is native from Africa and contains 11 subspecies. All cultivated cowpeas are grouped under *V. unguiculata* spp. *unguiculata* and are sub-divided into four cultigroups, being Unguiculata and Sesquipedalis the most important. *Vigna unguiculata* chloroplast genome is 152,415 bp in length. Conserved gene order, low mutation rate and maternal inheritance make the cpDNA an excellent tool to investigate species origin, domestication events and phylogeography.

The main objective of this study was to differentiate *Vigna unguiculata* subspecies using 10 chloroplast microsatellite (cpSSR) primer pairs. A total of 47 accessions, 34 from Europe countries and 13 from African, Asian and American countries, including specimens of *V. unguiculata* ssp. *unguiculata*, *sesquipedalis*, *alba, pubescens*, *tenuis* and *V. mungo* (outgroup), were used. This set of primers allowed detecting a total of four different haplotypes, two of them exclusive of the ssp. *alba* and ssp. *pubescens* and one of the species *mungo*. In the dendrogram, it was possible to visualize that the cultigroups Unguiculata and Sesquipedalis did not separate from each other, neither from the *ssp. tenuis*. It was also shown that *V. mungo* forms a perfectly individualized cluster.

Acknowledgment: *This study was funded by the EU-FP7 for Research, Technological Development and Demonstration under grant agreement no 613781, project EUROLEGUME.*

How to refer your abstract:
E. Monteiro, I. Castro, M. Carvalho, V. Carnide (2017) Genetic relationship of Vigna unguiculata spp. accessions based on cpSSR markers; ICLGG 2017 - Book of abstracts, ICLGG2017/P/36

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Genome wide association study to identify SNPs associated with folate profile in pea

A.B. Jha, H. Zhang, K.K. Gali, R.W. Purves, B. Tar'an, A. Vandenberg, T.D. Warkentin

Crop Development Centre, Department of Plant Sciences, University of Saskatchewan, Saskatoon, Canada

Folates, water-soluble B vitamins, are cofactors in various metabolic pathways and therefore important for human health. Pulse crops are known to be rich in folates. Our previous pilot study had indicated a relatively low concentration of total folate in pea compared to chickpea, lentil, and common bean [1]. To explore a wider range of variation, a pea genome wide association study panel of 177 accessions consisting of cultivars and landraces from North America, western and eastern Europe, and Australia were developed at the Crop Development Centre, University of Saskatchewan. Initially, 87 diverse accessions were selected for evaluation of folate profile based on origin and variation in measured phenotypic traits arising from multi-year trials to capture diversity among accessions. Overall, eight different monoglutamate folates were quantified using ultra-performance liquid chromatography coupled with mass spectrometry. Results indicated a wide range of variation in concentration of total folate (14 to 55 µg/100 g). The 5-methyltetrahydrofolate and 5-formyltetrahydrofolate were the two most abundant folates. The accessions were genotyped using genotyping by sequencing. These data will be utilized for a genome wide association study to identify SNPs associated with folate profile.

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How to refer your abstract:
A.B. Jha, H. Zhang, K.K. Gali, R.W. Purves, B. Tar'an, A. Vandenberg, T.D. Warkentin (2017) Genome wide association study to identify SNPs associated with folate profile in pea; ICLGG 2017 - Book of abstracts, ICLGG2017/P/37

Book of abstracts
ISBN 978-615-5270-39-0

Genome-wide identification and expression analysis of auxin response factor gene family in *Cicer arietinum*

J.V. Die, J. Gil, T. Millán

Department of Genetics, University of Cordoba, Cordoba, Spain

The plant hormone auxin (indole-3-acetic acid) is a key regulator of virtually every aspect of plant growth and development. Auxin response factor proteins (ARFs) represent the core of auxin signalling. Genome wide analysis identified 24 *ARF* genes in the chickpea genome that comprises ~0.09% of the total annotated genes. The *ARF* genes are not evenly distributed across the chickpea genome. Two chromosomes (chr1 with 6 *ARF* genes, and chr6 with 7 *ARF* genes) contained more than 56% of the mapped *ARFs*, whereas chromosomes 3 and 1 contained 1 gene each (4%). The *ARF* genes located on the same chromosome belong to different classes according to phylogenetic analysis. Two major molecular mechanisms underlay the diversification of ARF proteins. First, diversification by expansion. Our results suggest that segmental duplications, but not tandem duplications, might have contributed to the expansion of the *ARF* gene family. Next, structural diversification that comprises both, domain rearrangements and alternative splicing. Computational survey of the alternative transcripts revealed that at least half of the gene family members display alternative splicing.

In this work, we will provide comprehensive information on the genomic structures, chromosomal locations, sequences homology, cis-regulatory elements and evolutionary duplication history of the 24 *ARF* genes in *C. arietinum*. Finally, *ARF* gene expression patterns during development/abiotic/biotic stress will be discussed.

Acknowledgments *INIA RTA2013-00025, project co-financed by the European Union through the ERDF 2014-2020 "ProgramaOperativo de CrecimientoInteligente" and Contract 77863.*

Keywords: *ARF, bioinformatics, gene expression, qPCR*

How to refer your abstract:
J.V. Die, J. Gil, T. Millán (2017) Genome-wide identification and expression analysis of auxin response factor gene family in Cicer arietinum; ICLGG 2017 - Book of abstracts, ICLGG2017/P/38

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Genomics advances for enhancing genetic gains in pigeonpea

R.K. Saxena, C.V. Sameerkumar, K.B. Saxena, R.K. Varshney

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India

Genomics advances in pigonpea are leading to a new revolution in breeding, as they facilitate the study of the genotype and its relationship with the phenotype. Availability of draft genome and re-sequencing data in pigeonpea coupled with Next Generation Sequencing (NGS) technologies are producing a vast array of information. This allows discovering new genes and regulatory sequences and provides large collections of molecular markers linked to desired traits. For instance, re-sequencing of >400 pigeonpea genomes has provided millions of genome-wide markers amenable for high-throughput genotyping platforms and information on genes related to photoperiod, days to flowering, 100 seed weight etc. Simultaneously this data has also provided genes/genomics segments under selection pressure during domestication and modern breeding. In parallel, Genotyping by Sequencing approach has been used to construct a number of high density genetic maps for different populations segregating for important traits such as fusarium wilt, sterility mosaic disease, flowering, plant growth habit, seed protein content, fertility restoration, yield contributing traits etc. This also allows the identification of markers linked to genes and quantitative trait loci. Further, above mentioned genomics advances in pigeonpea are being used in developing improved lines, which in turn enhancing the genetic gains.

How to refer your abstract:
R.K. Saxena, C.V. Sameerkumar, K.B. Saxena, R.K. Varshney (2017) Genomics advances for enhancing genetic gains in pigeonpea; ICLGG 2017 - Book of abstracts, ICLGG2017/P/39

Book of abstracts
ISBN 978-615-5270-39-0

Identification of genes involved in the alkaloid biosynthesis pathway in narrow-leafed lupin (*Lupinus* angustifolius L.) on the basis of transcriptome sequencing

M. Kroc, G. Koczyk, K. Kamel, O. Fedorowicz-Strońska, W. Święcicki

Institute of Plant Genetics, Polish Academy of Sciences, Poznan, Poland

Lupin seeds are a valuable protein source in animal feed, provided that the alkaloids level is strictly reduced. Quinolizidine alkaloids (QA) are serious antinutritional factors due to their toxicity as well as bitter taste. Although a few individual genes associated with QA biosynthesis have already been identified in lupins, its molecular mechanism is still poorly understood.

To address this issue RNA sequencing was performed for two low alkaloid (sweet) and two high alkaloid (bitter) genotypes of narrow-leafed lupin (100PE, HiSeq1500). The short reads of a bitter line P27255 have been *de novo* assembled, by multiple methods, resulting in a high-quality consensus transcriptome rivaling the currently available legume genomes, in terms of ortholog completeness. On the basis of RNA-seq results we described a comprehensive landscape of differentially expressed genes (DEGs) serving as a source of candidate genes selection. The accuracy of RNA-seq data assembly was confirmed on a subset of DEGs by means of qRT-PCR. Genetic mapping of selected DEGs (key biosynthetic enzymes and implicated transcription factors) revealed candidate genes position in relation to the main alkaloid locus *Iucundus*. Our findings substantially contribute to unraveling factors affecting QA biosynthesis and accumulation in narrow-leafed lupin.

***Acknowledgement**: Ministry of Agriculture and Rural Development (Governmental Multiannual Project, Resolution of the Council of Ministers No. 222/2015).*

How to refer your abstract:
M. Kroc, G. Koczyk, K. Kamel, O. Fedorowicz-Strońska, W. Święcicki (2017) Identification of genes involved in the alkaloid biosynthesis pathway in narrow-leafed lupin (Lupinus angustifolius L.) on the basis of transcriptome sequencing; ICLGG 2017 - Book of abstracts, ICLGG2017/P/40

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Identification of QTL and qualitative trait genes for agronomic traits in adzuki bean

Y. Li[1*], K. Yang[1*], W. Yang, L. Chu[1], C. Chen[2], B. Zhao[1], Y. Li[1], J. Jian[2], T.Wang[1], **P. Wan**[1]

[1] *College of Plant Science and Technology, Key Laboratory of New Technology in Agricultural Application, Beijing University of Agriculture, Beijing, China*
[2] *Beijing Genomics Institute -Shenzhen, Shenzhen, China*

** These authors contributed equally to this work.*

The adzuki bean (*Vigna angularis*) is an important grain legume. Fine mapping of quantitative trait loci (QTL) and qualitative trait genes plays an important role in gene cloning, molecular-marker-assisted selection (MAS), and trait improvement. However, the genetic control of agronomic traits in the adzuki bean remains poorly understood. Single-nucleotide polymorphisms (SNPs) are invaluable in the construction of high-density genetic maps. We mapped 26 agronomic QTLs and five qualitative trait genes related to pigmentation using 1,571 polymorphic SNP markers from the adzuki bean genome via restriction-site-associated DNA sequencing of 150 members of an F_2 population derived from a cross between cultivated and wild adzuki beans. We mapped 11 QTLs for flowering time and pod maturity on chromosomes 4, 7, and 10. Six 100-seed weight (SD100WT) QTLs were detected. Two major flowering time QTLs on chromosome 4, firstly *VaFld4.1* (PEVs 71.3%), were co-segregated with SNP markers s690-144110, and *VaFld4.2* (PEVs 67.6%) had a 0.974 cM genetic distance with the SNP marker *s165-116310*. Three QTLs for seed number per pod (*Snp3.1*, *Snp3.2* and *Snp4.1*) were mapped on chromosomes 3 and 4. One QTL *VaSdt4.1* of seed thickness (SDT) and three QTLs for branch number on the main stem were detected on chromosome 4. QTLs for maximum leaf width (LFMW) and stem internode length were mapped to chromosomes 2 and 9, respectively. Trait genes controlling the colour of the seed coat, pod, stem and flower were mapped to chromosomes 3 and 1. Three candidate genes, *VaAGL*, *VaPhyE*, and *VaAP2*, were identified for flowering time and pod maturity. *VaAGL* encodes an agamous-like MADS-box protein of 379 amino acids. *VaPhyE* encodes a phytochrome E protein of 1,121 amino acids. Four phytochrome genes (*VaPhyA1*, *VaPhyA2*, *VaPhyB*, and *VaPhyE*) were identified in the adzuki bean genome. We found candidate genes *VaAP2/ERF.81* and *VaAP2/ERF.82* of SD100WT, *VaAP2-s4* of SDT, and *VaAP2/ERF.86* of LFMW. We detected candidate gene *VaUGT*, which was related to black seed coat colour. These mapped QTL and qualitative trait genes provide information helpful for future adzuki bean candidate gene cloning and MAS breeding to improve cultivars with desirable growth periods, yields, and seed coat colour types.

Keywords: *Adzuki bean (Vigna angularis), agronomic trait, QTL, qualitative trait, SNP marker, candidate gene*

How to refer your abstract:
Y. Li, K. Yang, W. Yang, L. Chu, C. Chen, B. Zhao, Y. Li, J. Jian, T.Wang, P. Wan (2017) Identification of QTL and qualitative trait genes for agronomic traits in adzuki bean; ICLGG 2017 - Book of abstracts, ICLGG2017/P/41

Book of abstracts
ISBN 978-615-5270-39-0

Identification of the translocation breakpoint between chromosome 4 and 8 in the genomes of *Medicago truncatula* A17 and A20

Z. Szabó[1], M. Balogh[1], K. Miró[1], F. Debelle[3], D.R. Cook[4], T.H.N. Ellis, Gy.B. Kiss[1,2], **P. Kaló**[1]

[1] *NARIC, Agricultural Biotechnology Institute, Gödöllő, Hungary*
[2] *AMBIS Biotechnology and Research Ltd, Szeged, Hungary*
[3] *INRA, Toulouse, France*
[4] *University of California Davis, Davis, California, USA*
[5] *School of Biological Sciences, University of Auckland, Auckland, New Zealand*

Extensive genomic resources have been developed for *Medicago truncatula* in the last two decades that made this species one of the model legumes. A previous genetic analysis of the F2 mapping population derived from the cross of *Medicago truncatula* A17 and A20 detected an unusual linkage phenomenon between markers located in the lower arms of chromosome 4 and 8 (Choi et al. 2004). It was found that some markers displaying high degree of genetic linkage showed cruciform-like linkage and no linear genetic map could be constructed based on the detected recombination events between these markers. The map position of these markers were resolved by positioning them to either linkage group 4 or 8 using the mapping population of *M. sativa*. A subsequent study indicated that accession A17 of *M. truncatula* carries a translocation between chromosome 4 and 8 that resulted in reduced viability of F1 pollens (Kamphuis et al., 2007) when crossing with other *M. truncatula* accessions:.

In order to pinpoint the breakpoint of the translocated chromosomal segments in A17, a study was initiated using further genetic mapping of another segregating population of *M. truncatula* combined with the systematic sequencing of BAC clones using recently developed genetic markers around the breakpoint of the chromosomal rearrangement. We are going to present how the identification of the translocation breakpoint has been progressed.

How to refer your abstract:
Z. Szabó, M. Balogh, K. Miró, F. Debelle, D.R. Cook, T.H.N. Ellis, Gy.B. Kiss, P. Kaló (2017) Identification of the translocation breakpoint between chromosome 4 and 8 in the genomes of Medicago truncatula A17 and A20; ICLGG 2017 - Book of abstracts, ICLGG2017/P/42

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Improvement of mungbean reference genome assembly and QTL identification for synchronous pod maturity

H. Jeong[1], J. Ha[1,2], D. Satyawan[1], **H.-J. Jang**[1,2], S.-H. Lee[1,2]

[1] *Department of Plant Science and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea*
[2] *Plant Genomics and Breeding Institute, Seoul National University, Seoul, Republic of Korea*

Mungbean (*Vigna radiata* (L.)) is a great source of carbohydrate and dietary protein in South, East and Southeast Asia. However, uneven pod maturity of mungbean leads to low harvest index and high labor. In this study, we improved genome assembly of cultivated mungbean (*V. radiata* var. radiata VC1973A) by Pacbio sequencing platform. In total, 557 scaffolds were assembled with N50 length of 5.2 Mb. Total bases of the scaffolds were 475 Mb covering 87.5% of the estimated mungbean genome size. To anchor the scaffolds to 11 pseudochromosomes, we constructed high resolution genetic map by whole genome re-sequencing of 187 Recombinant Inbred Lines (RILs). In addition, quantitative trait loci (QTL) analysis for synchronous maturity of pods was conducted by using single nucleotide polymorphism (SNP) markers used for genetic map construction. To evaluate synchronous maturity of pods, we phenotyped 187 RILs. Two QTLs for synchronous maturity were detected on chromosome 4 and 7 with LOD scores 2 or higher. The improved genome assembly of mungbean will facilitate genome research and future breeding program. Furthermore, newly identified QTLs can help breeders improve elite cultivars with synchronous maturity leading shorter harvesting time and higher yield.

How to refer your abstract:
H. Jeong, J. Ha, D. Satyawan, H.-J. Jang, S.-H. Lee (2017) Improvement of mungbean reference genome assembly and QTL identification for synchronous pod maturity; ICLGG 2017 - Book of abstracts, ICLGG2017/P/43

Book of abstracts
ISBN 978-615-5270-39-0

The first step for adaptation: Width and distribution of the first flowering and podding dates in wild chickpeas

A. Aydogan[1], J. Berger[2], A. Kahraman[3], C. Toker[4], B. Aydin[3], B. Bukun[5], E.J. von Wettberg[6], R.V. Penmetsa[7], A. Greenspan[7], S. Nuzhdin[8], D.R. Cook[7]

[1] *Central Research Institute for Field Crops (CRIFC), Ankara, Turkey*
[2] *CSIRO Agriculture and Food, Wembley, Australia*
[3] *Department of Field Crops, Harran University, Turkey*
[4] *Department of Field Crops, Akdeniz University, Turkey*
[5] *Dicle University, Diyarbakir, Turkey*
[6] *Florida International University, Miami, USA*
[7] *University of California Davis, Davis, USA*
[8] *USC University of Southern California, USA*

The current agronomic challenge is the generally lower yield in grain legumes compared to cereals, together with the difference between their role as a diet staple for certain populations and the geographical locations where they are grown (Foyer et al, 2016). The timing of flowering and podding, and in particular the degree to which it is responsive to the environment, is a key factor in the adaptation of a given species to various eco-geographic locations and agricultural practices. Wild relatives are potential genetic resources for crop improvement (Harlan, 1976). The annual wild *Cicer* species are becoming increasingly important to the cultigen (*Cicer arietinum* L.) as a source of genetic diversity, and resistance to both biotic and abiotic stresses (Berger et al, 2003). Genotypes which are consistent with the environmental factors of flowering and podding were effective in increasing chickpea yield.

244 C. Reticulatum, 42 C. Echinosporum collected from Anatolia and 2 checks (Azkan and Gökçe) were planted with two replications in Atumn and spring in 2016/17 season. Lines and checks were evaluated terms of first flowering and podding dates. The aim of this study is to compare wild chickpea lines the first flowering and podding dates, to relate their daily environmental temperature requirements and so as to determine the optimal temperature for the adaptation.

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Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Wild relatives of domesticated pea in the Mediterranean Region and the Fertile Crescent will respond to global climate change

P. Smýkal[1], I. Hradilová[1], O. Trněný[2], J. Brus[3], A. Rathore[4], M. Bariotakis[5], R.R. Das[4], C.J. Coyne[6], S. Pirintsos[5]

[1] *Department of Botany, Palacký University in Olomouc, Czech Republic*
[2] *Agricultural Research, Ltd. Troubsko, Czech Republic*
[3] *Department of Geoinformatics, Palacký University in Olomouc, Czech Republic*
[4] *ICRISAT, Hyderabad, India*
[5] *Department of Biology and Botanical Garden, University of Crete, Heraklion, Greece*
[6] *USDA-ARS, Washington State University, Pullman, USA*

There is growing interest in conservation and utilization of crop wild relatives (CWR) in international food security policy and research. Pea belongs to the ancient set of cultivated plants of the Near East domestication centre and is still an important crop today. Genetic diversity was revealed by phylogenetic markers (*trn*SG, ITS) and genome-wide DArT seq assay, applied to comprehensive set of wild pea samples. Potential climatic niches were modelled using Maxent and potential spatial patterns were investigated and projected in the past (Last Glacial Maximum), current and future climatic conditions. Niche diversity was investigated with the use of Shannon's index of diversity. *P. fulvum* was identified as a clear-cut species, while the diversity of wild *P. sativum* subsp. *elatius* was structured into 6 clusters. Several *P. fulvum* accessions showed disagreement between ITS, DArT seq and chloroplast sequences which might be explained by hybridization. We explored macroecological patterns of wild pea in the Mediterranean Basin and the Fertile Crescent in relation to the past, current and future climate suitability. The genetic diversity of wild pea may be driven by Miocene-Pliocene events, while the species diversity centers may reflect Pleisto-Holocene climatic changes. Most of the haplotypes are predicted to vanish from their current distribution areas in Europe by the 2070, while the rest of them are expected to present a southward shift, setting the urgent need to revise the hitherto conservation priorities.

Acknowledgements: *This was supported by the Grant Agency of the Czech Republic, 14-11782S and 16-21053S projects.*

How to refer your abstract:
P. Smýkal, I. Hradilová, O. Trněný, J. Brus, A. Rathore, M. Bariotakis, R.R. Das, C.J. Coyne, S. Pirintsos (2017) Wild relatives of domesticated pea in the Mediterranean Region and the Fertile Crescent will respond to global climate change; ICLGG 2017 - Book of abstracts, ICLGG2017/P/45

Book of abstracts
ISBN 978-615-5270-39-0

Implementation of reverse genetics tools for improvement of pea cultivation in Poland

F.A. Bakro, S. Blicharz, R. Malinowski

Institute of Plant Genetics of the Polish Academy of Sciences, Plant Integrative Department, Poznan, Poland

Cultivated pea is one of the oldest domesticated crops and it has been improving through the breeding strategies worldwide. In Polish climates, the growth of pea is affected by many factors, mainly the rainfall during the pod-filling stage that causes heavy lodging of the plants which eventually develops various diseases and yield loss [1].

In order to improve the Polish pea cultivation and enhance the genetic diversity, we started developing a TILLING population in *Pisum sativum L*, variety '*Walor*' starting with 10000 seeds and using 20mM of Ethyl methane sulfonate EMS mutagen.

M1 and M2 generation were raised during (2016-2017) in the field. The early morphological characterization of M1 and M2 has been referred to the efficiency of EMS where different mutations were characterized for instance, Chlorosis mutations, dwarf plant, compact phenotypes, long-short internodes, tendril-less and 'afila' phenotype beside to Trifoliate phenotypes.

Taking the advantage of the reverse genetics tools for the next step, we are planning to apply the High resolution melting (HRM) combined with the Next Generation Sequencing to screen some genes involved in controlling of the meristem differentiation, lodge resistance, stem thickness and cell wall developing.

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How to refer your abstract:
F.A. Bakro, S. Blicharz, R. Malinowski (2017) Implementation of reverse genetics tools for improvement of pea cultivation in Poland; ICLGG 2017 - Book of abstracts, ICLGG2017/P/46

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

A series of fortunate events: unlocking flowering time variation in narrow-leafed lupin through an allelic series of mutation events at a major flowering time gene, *LanFTc1*

C.M. Taylor[1], L.G. Kamphuis[2,3], J.D. Berger[2], J. Clements[4], W.A. Cowling[1,5,6], M.N. Nelson[5,6,7]

[1] *School of Biological Sciences, The University of Western Australia, Perth, Australia*
[2] *Centre for Environment and Life Sciences, CSIRO Agriculture and Food, Floreat, Australia*
[3] *Centre for Crop and Disease Management, Curtin University, Bentley, Australia*
[4] *Department of Agriculture and Food Western Australia, Government of Western Australia, South Perth, Australia*
[5] *School of Agriculture and Environment, The University of Western Australia, Perth, Australia*
[6] *The UWA Institute of Agriculture, The University of Western Australia, Perth, Australia*
[7] *Natural Capital and Plant Health, Royal Botanic Gardens, Kew, Ardingly, UK*

Manipulation of phenology is critical for adaptating cropping species to their respective growing environments. In narrow-leafed lupin (*Lupinus angustifolius* L.), the selection of an early flowering mutant (*Ku*) was important for the successful adaptation of this legume grain crop to warm, short-season winter environments in Australian agricultural systems [1]. Additionally, early phenology has been valuable in adapting spring-sown narrow-leafed lupin to Europe [2], ensuring completion of grain fill before the onset of cool, autumn conditions [1].

Recently, *Ku* was identified as *LanFTc1*, an *FT* homologue [3]. A 1.4 kb deletion in the 5' upstream regulatory region of *LanFTc1* was associated with the loss of vernalization requirement and constitutively high gene expression in non-vernalized domestic lines, leading to early flowering.

Using a combination of targeted amplicon sequencing and a comprehensive whole-genome re-sequencing data set created for 42 wild and domestic narrow-leafed lupin accessions, two new deletions within the 5' upstream regulatory promoter region of *LanFTc1* were identified. These two new alleles include a 1.2 kb deletion in a wild type from Israel and a ~5 kb deletion in a domestic breeding line from Belarus.

To determine whether variations in promoter deletions in the 5' regulatory region of *LanFTc1* are associated with modified vernalization responsiveness and flowering time phenotype, the three different deletions were investigated and the findings will be presented.

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Book of abstracts
ISBN 978-615-5270-39-0

Plant and pathogen genomics: Towards building resilience into narrow-leafed lupin crops

S. Mousavi-Derazmahalleh[1], P.E. Bayer[2] , D. Edwards[2,3], W. Erskine[1,3], J.K. Hane[4], M.N. Nelson[1,3,5]

[1] *UWA School of Agriculture and Environment, The University of Western Australia, Perth, Australia*
[2] *School of Biological Sciences, The University of Western Australia, Perth, Australia*
[3] *The UWA Institute of Agriculture, The University of Western Australia, Perth, Australia*
[4] *CCDM Bioinformatics, Centre for Crop Disease Management, Curtin University, Perth, Australia*
[5] *Natural Capital and Plant Health, Royal Botanic Gardens Kew, West Sussex, UK*

Narrow-leafed lupin (*Lupinus angustifolius* L.) is an emerging crop in agricultural systems due to its adaptation to infertile, acidic soils. This make it valuable in crop rotations, raising soil fertility and providing a disease break. However, several limitations prevent broader adoption, including its narrow genetic base [1] and its susceptibility to various pathogens [2].

We applied genome-wide DArTseq genotyping to understand the impact of domestication on genome-wide diversity in narrow-leafed lupin. We also developed whole-genome sequence resources for seven foliar fungal pathogens of lupin (i.e. *C. lupini*, *D. toxica*, *P. setosa*, *B. cinerea*, *S. botryosum* and two isolates of *S. sclerotiorum*), as a cornerstone towards understanding the genomics of plant-pathogen interactions in this species.

Our research identified the Iberian Peninsula as the likely geographic origin of this legume species and as the source of the founder populations that gave rise to domesticated forms of narrow-leafed lupin. Domestication increased genome-wide linkage disequilibrium, including regions near previously mapped domestication genes [3]. A genome-wide association analysis identified several genomic regions associated with domestication traits. To elucidate the interaction between lupin evolution and host-pathogen interaction, seven pathogens of lupin have also been assembled using PacBio and/or Illumina sequencing reads, functionally annotated and disease-causing effector proteins were predicted.

Taken together, these results shed light on crop improvement and disease management.

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Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Crosstalk between photoperiod and vernalization pathways - insight into genes involved in flowering induction in the narrow-leafed lupin

S. Rychel[1], M. Książkiewicz[1], B. Wolko[1], M.N. Nelson [2]

[1] *Department of Genomics, Institute of Plant Genetics, Polish Academy of Sciences, Poznań, Poland*
[2] *Natural Capital and Plant Health, Royal Botanic Gardens Kew, London, UK*

The general aim of the study is to determine the interference between **photoperiod** and **vernalization** pathways on regulation of flowering induction in the narrow-leafed lupin, *Lupinus angustifolius* L. The survey is based on the differential expression profiling in response to short vs long day length and presence or absence of prolonged exposition to low temperature during seed germination (~21 days).

In a model species *Arabidopsis thaliana* (L.) Heynh. the *FKF1*, *CO* and ***FLOWERING LOCUS T*** (*FT*) genes play key role in response to day length, whereas the *SVP* and *VIN3* address the vernalization. In theory, the promoter and intronic regions of *FT* carry all the elements that are necessary to alter *FT* expression in response to these cues. In legumes, numerous whole-genome duplication events increased the number of *FT* homologs, which can be grouped into three subclades *FTa*, *FTb* and *FTc*. In the model legume, *M. truncatula*, vernalization responsiveness is maintained by a gene from the *FTa* subclade, whereas *FTb* is considered to be involved in the photoperiod pathway. In *L. angustifolius*, the whole *FTb* subclade has been lost during evolution and the promoter region of *FTc* homolog has become a key regulation node gathering signals from photoperiod and vernalization. This is the only species which has a large deletion in this region, encompassing many putative binding sites for transcription factors. **The results will shed light on the complex machinery regulating flowering induction in plants.**

Acknowledgement: *The National Science Centre, project PRELUDIUM 8, no. 2014/15/N/NZ9/03919.*

How to refer your abstract:
S. Rychel, M. Książkiewicz, B. Wolko, M.N. Nelson (2017) Crosstalk between photoperiod and vernalization pathways - insight into genes involved in flowering induction in the narrow-leafed lupin; ICLGG 2017 - Book of abstracts, ICLGG2017/P/49

Book of abstracts
ISBN 978-615-5270-39-0

Development of chickpea Near Isogenic Lines for QTL_{DF1} linked to flowering time

J. Rubio[1], L. Ali[2], **T. Millán**[2], J. Gil[2]

[1] *Area de Genómica y Biotecnología, IFAPA, Alameda del Obispo, Córdoba, Spain*
[2] *Universidad de Córdoba, Dpto. Genética, Campus de Rabanales, Córdoba, Spain*

Flowering time is an agronomic trait that has effect on crop adaptation at different environments. In chickpea, QTLs (Quantitative Trait Loci) (QTL_{DF1}) were detected on linkage group (LG) 4 using a recombinant inbred lines (RIL) population with JG62 as early flowering parental line [1].

This study was conducted to develop pairs of near-isogenic lines (NILs) for QTL_{DF1} associated with flowering time in chickpea. NILs have the advantage that only a small target region of the genome is segregating; consequently, are useful genetic stocks to perform expression profiles and to look for candidate genes related to this trait. Pair of NILs were produced taking advantage of residual heterocigosity in $F_{6:7}$ RILs derived from JG62 (early flowering) x ILC72 (late flowering). RILs showing segregation for flowering time were selected and individual plants showing extreme values in days to flowering (early or late) over three generations were harvested. Along the process, segregation for early/late flowering in progenies from individual plants with late flowering phenotype were found. Whereas, progenies derived from early flowering always showed early phenotype. That means that late flowering is dominant regard to early flowering in plant material where phenotypic variation is mostly controlled by QTL_{DF1} in LG4. The pair of NILs (early and late) derived from RIL82 showed a difference of 60 days under short day conditions in the greenhouse. This pair of NILs will be validated under field conditions during the next season.

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Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Development of the common bean core collection referring to the Central and South Eastern European germplasm

B. Pipan, A. Sedlar, J. Šuštar-Vozlič, V. Meglič

Crop Science Deaprtment, Agricultural Institute of Slovenia, Ljubljana, Slovenia

Common bean is a major and the most important grain legume for direct human consumption in the world. Thousands of landraces, old and modern cultivars are maintained in gene banks across the European continent.

According to basic multi-crop passport descriptors and seed characteristics, we selected over 800 accessions with distinct genotypes, covering diverse environments from different parts of the European continent following the line East to West and East to South. Altogether, we managed to obtain 782 accessions from 9 gene banks and 12 different geographic origins. To evaluate their genetic diversity, we applied 33 species-specific SSR markers covering all linkage groups among *P. vulgaris* genome. **Four strategies** were used to construct core collection combining different parameters of genetic variability with the selection of the most, the least and proportionally equally arranged significant representatives of each specific genetic cluster regarding to Bayesian approach. Out of the entire collection, 63 highly diverse accessions were selected for core collection on the basis of their genetic structure, without the geographic origin preference. Additionally, we selected 14 representatives as standard genotypes for their specific agronomically important traits.

Final core collection represents valuable source of important traits and (multiple) alleles with potential to carry biotic and abiotic stress resistance, having a great value for common bean breeding programmes.

How to refer your abstract:
B. Pipan, A. Sedlar, J. Šuštar-Vozlič, V. Meglič (2017) Development of the common bean core collection referring to the Central and South Eastrn European germplasm; ICLGG 2017 - Book of abstracts, ICLGG2017/P/51

Book of abstracts
ISBN 978-615-5270-39-0

Exploring the potential of genomic prediction in NS soybean breeding programs: preliminary results

M. Ceran, V. Djordjevic, S. Balesevic-Tubic, J. Miladinovic, K. Petrovic, S. Mikic, Z. Miladinov

Institute of Field and Vegetable Crops, Novi Sad, Serbia

Soybean breeding is mostly focused on the enhancement of yield potential and yield stability. Advances in next-generation sequencing technologies offer new and practical approaches, such as genomic selection, that can help in the crop breeding for the improvement of the rate of genetic gain for yield [1].

This study aimed to evaluate the efficiency of genomic assisted selection for soybean breeding at Institute of Field and Vegetable Crops (IFVC) in Serbia. Population of 230 soybean lines originating from IFVC breeding programs was used to train the prediction models. Genotyping-by-sequencing [2] was performed on the training set producing initially more than 85000 SNPs. Yield from this population has been recorded in field trials from 2014-2016. Obtained data sets were combined to develop prediction models considering different statistical methods (rrBLUP, Bayes A, Bayes B, Bayes CPi, Random Forest, SVM). Estimation of models accuracy for predicting breeding value was conducted by cross-validation. Moreover, an independent set of 29 elite soybean varieties was used for the external validation. Pre-existing data for this set, originated from trials conducted from 2010-2016 at 14 different locations in Serbia, were used.

Preliminary results indicate that prediction accuracy of genomic selection is 0.48-0.64. External validation showed variable ability of developed models to predict phenotypic performance in different years. Obtained results are aimed to be implemented in soybean breeding programs to enhance genetic gain and increase the efficiency of creating new varieties.

Acknowledgement: *The project Regional cooperation in overcoming negative effects of global climate changes on soybean production through selective soybean breeding of Deutsche Gesellschaft für Internationale Zusammenarbeit GmbH (GIZ)*

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Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Genomic approaches to identify candidate genes controlling pod dehiscence in chickpea and faba bean

D. Aguilar[1], T. Millán[2], J. Gil[2], J. Rubio[1], **A.M. Torres**[1]

[1] *IFAPA, Centro Alameda del Obispo, Área Mejora y Biotecnología, Córdoba, Spain*
[2] *Dept. of Genetics, University of Córdoba, Córdoba, Spain*

Pod dehiscence is the principal cause of yield loss in legume crops. To identify candidate genes related with dehiscence, two approaches were followed: (a) QTL analysis of dehiscence data from Recombinant Inbred Lines (RILs) coming from an interespecific cross between ILC72 (*Cicer arietinum* L.) x Cr5-10 (*C. reticulatum*) [1], and (b) bibliographic research of genes associated to dehiscence in other legume crops. Dehiscence was measured by the number of dehiscent pods in each RIL in two repeats. The analysis revealed one QTL located in chromosome VI. In soybean, *Pdh1* encodes a dirigent (DIR)-like protein which is related with lignin biosynthesis and dehiscence [2]. BLAST analysis showed that *Pdh1* was also present in the *Cicer* genome, specifically in Chr VI. In addition, a SNP was found when comparing the nucleotide sequences of the parental lines. This SNP mapped in Ca-ChrVI and after the QTL analysis explained a 20% of the trait variation. To validate these results, a new interspecific cross between ICCL81001 (*C. arietinum*) x Cr5-9 (*C. reticulatum*) is pending to be analysed. Moreover, a comparative mapping of the trait is being performed in faba bean (*Vicia faba* L.) and will be used to found new candidate genes. Apart of the validation in a different species, the approach may facilitate future marker-assisted strategies to efficiently select for resistance to pod dehiscence in several legume species.

***Acknowledgement**: This work was supported by the INIA project contract RTA2013-00025, co-financed by the European Union through the ERDF 2014-2020 "Programa Operativo de Crecimiento Inteligente"*

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Book of abstracts
ISBN 978-615-5270-39-0

Genomics tools for the improvement of horsegram (*Macrotyloma uniflorum*): an orphan legume

R.K. Chahota[1], T.R. Sharma[1], Sachiko Isobe[2], Hideiki Mirayaba[2]

[1] *Department of Agricultural Biotechnology, CSK HPKV Palampur HP, India*
[2] *Kazusa DNA Research Laboratory, Chiba, Japan*

Horsegram (*Macrotyloma uniflorum*) an orphan legume crop but provides protein supplement for large vegetarian populace of Indian sub-continent and also grown as a fodder crop in some other semi-arid regions of the world. A self-pollinated diploid plant (2n=20) has been originated in Africa. Due to lack the genomic information and the absence of the linkage map; systematic molecular breeding could not be initiated. Therefore the present study was initiated with the objectives to develop genomic resource in this resource poor legume. The study was started with the identification of 97 translational SSRs markers from the related well-characterized model legumes. NCBI's databases of horsegram were explored to develop 63EST SSRs and 27IPLs. Transcriptome analysis of two horsegram lines helped us to develope 3410 genic SSRs (Bhardwaj etal 2013). Hiseq Illumina sequencing data were used to mine genomic SSRs and developed the 5456 novel genomic SSRs. The newly developed genic and genomic SSR were employed to determine the genetic diversity and population structure of Indian germplasm, which reveled the presence of two gene pools in this crop (Vikas et al 2015a, 2015b). This resulted into the development of core set of 125 lines, which is being used in genome wide association studies. The RIL mapping population of 190 individual was analysed using SSRs and framework linkage map was constructed and important QTLs of various traits were indentified. A popular cultivar HPK4 was used for whole genome sequencing project which enabled us to identify 36105 genes in this crop.

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Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Large scale SNP mining and validation in *Vicia faba*

D. Angra, D.M. O'Sullivan

School of Agriculture, Policy and Development, University of Reading, Reading, UK

Faba bean (*Vicia faba* L.) is a prominent high yielding grain legume which is a rich source of protein for large populations. In the present study, we aimed to exploit burgeoning transcriptome datasets from multiple genotypes to systematically discover and validate SNP variants to provide a powerful tool for germplasm characterization and genetic mapping and breeding applications. In pursuit of this overall goal, we first carried out an assembly of existing transcriptome datasets available in the public domain with a view to large scale SNP mining. 4,115,283 454-based reads with an average read length of 474 bp and 214,442,800 Illumina GA-based reads with an average read length of 100 bp from nine distinct genetic backgrounds were assembled. A total of 260,259 isotigs were generated by the *de novo* Trinity assembler with a median contig length of 444 bp. This assembly referred to as VfTRAC (Vicia faba transcriptome assembly) was used as a reference to which the genotype-specific transcriptome assemblies were aligned individually using BWA-MEM, followed by SNP calling using Varscan. This resulted in identification of 206,363 SNPs, which overlap and greatly extend SNP lists reported by (1-3). Our second aim in this study was to produce a high density, validated and publicly available 50K Axiom (Affymetrix) SNP array for faba bean which could be readily used in a number of genetic mapping and breeding applications. We will give an update on the current status of the 50K array production (expected July 2017) and illustrate the utility an underpinning SNP database of this magnitude and quality could have in a typical case by showing its application in targeting a far higher density of SNP coverage to a genetic interval harbouring an important seed quality trait – namely, a recessive allele of the *VC* gene conferring a substantial reduction in the anti-nutritional factor vicine-convicine.

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D. Angra, D.M. O'Sullivan (2017) Large scale SNP mining and validation in Vicia faba; ICLGG 2017 - Book of abstracts, ICLGG2017/P/55

Book of abstracts
ISBN 978-615-5270-39-0

Non coding RNAs: key actors of root developmental plasticity in *Medicago truncatula*

H. Proust[1,2], V. Sanchez Garcia de la Torre[3], T. Blein[1], J. Moreau[1], S. Lageix[1], C. Hartmann[1,2], C. Sorin[1,2], M. Crespi[1], **C. Lelandais-Brière**[1,2]

[1] *Institute of Plant Sciences Paris Saclay IPS2, CNRS, INRA, Université Paris-Sud, Université Evry, Université Paris-Saclay, Orsay, France*
[2] *Institute of Plant Sciences Paris-Saclay IPS2, Paris Diderot, Sorbonne Paris-Cité, Orsay, France*
[3] *Institute of Agricultural Sciences, ICA-CSIC, Madrid, Spain*

The development of the root system presents a large plasticity in response to soil environment. These last years, small non coding RNAs emerged as negative regulators of gene expression and key players in the control of root development and adaptation to the environment. In *Medicago truncatula*, we previously investigated small RNA diversity in roots under biotic and abiotic interactions [1] and studied several miRNAs in root development and endosymbioses [2, 3, 4].

Recently, we analyzed sRNA populations during nodule organogenesis and the crucial roles of ribonucleases III in their dynamics. We also focused on small RNAs involved in responses to cadmium (Cd), an heavy metal which strongly impacts root growth and represents an increasing problem in soils. In addition to miRNAs and their targets, siRNA-regulated genes were identified, suggesting complex regulations of Cd responses by small RNAs. We studied a novel Cd-responsive isoform of the legume-specific miR1509. This miRNA mediates the cleavage of long non coding RNAs (*lnc1* to *lnc4*), leading to the production of secondary phased siRNAs that may regulate metal response genes in *trans*. In Cd-treated roots of the A17 reference and a known Cd sensitive genotypes, miR1509c levels increased while *lnc2* RNA levels decreased. Inactivation of miR1509c or ectopic expression of *lnc2* reduced root growth sensitivity to Cd and a similar phenotype was observed in an *rdr6* mutant impaired in secondary siRNA biogenesis [5]. The link between miR1509/*lnc2*, secondary siRNAs and root growth in response to metals in legumes will be discussed.

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Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Renaissance of pigeonpea breeding: via hybrid pigeonpea technology

V. Chanda

International Crop Reserach Institute for the Semi-Arid Tropics, India

Enhancing pigeonpea productivity bears importance for small and marginal rainfed farming community of globe owing to nutritive value of the crop and its role in various agro ecologies. Varietal improvement programmes aimed towards breeding and development of high yielding varieties with resistance to major diseases like fusarium wilt and sterility mosaic resulted in the release of more than 120 varieties since 1950. The profits to the rainfed growers of the crop are not economical because of low yields which is directing the farmers to shift towards other crops which are not as sustainable as pigeonpea. Floral biology and mode of pollination in pigeonpea created an opportunity for the researchers to explore the possibility of development of hybrid technology. Availability of stable male sterile sources from the wild relatives and complete fertility restoration from the cultivated gene pool ushered the crop in to the field of hybrid breeding. The efforts of ICRISAT in collaboration with ICAR led to evolution of stable and standardized hybrid technology which is translated in to farmers' fields by the release of three hybrids from Madhya Pradesh, Odisha and Telangana states of India. The private sector is also expanding its roots in commercializing of pigeonpea hybrids. There is substantial increase in yield and net income by the cultivation of the hybrids in rainfed and irrigated ecosystems which demands for expansion of area under hybrids to break the yield stagnation in the crop.

How to refer your abstract:
V. Chanda (2017) Renaissance of pigeonpea breeding: via hybrid pigeonpea technology; ICLGG 2017 - Book of abstracts, ICLGG2017/P/57

Book of abstracts
ISBN 978-615-5270-39-0

SNP genotyping of putative candidate genes involved in broomrape and *Ascochyta fabae* resistance in faba bean (*Vicia faba* L.)

N. Gutierrez[1,2], I. Casimiro-Soriguer[1], A.M. Torres[1]

[1] *Área Genómica y Biotecnología, IFAPA-Centro Alameda del Obispo, Córdoba, Spain*
[2] *ZAYINTEC, edificio PITA - Universidad de Almería, Almería, Spain.*

Major priorities in faba bean breeding include resistance/tolerance to broomrape (*Orobanche crenata* Forsk.) and *Ascochyta fabae* Speg. Marker association analysis for efficient selection requires saturated maps with high resolution in the QTL intervals in order to identify allele specific markers linked to the trait of interest. To reduce confidence intervals in QTLs previously described for both traits [1, 2, 3, 4], 46 putative genes related to stress and defense responses (23 WRKY, 22 resistance protein and 1 RGA), derived from transcriptome analyses in this crop after *A. fabae* infection (5), were selected. The SNPs were genotyped by MassArray iPLEX in the 29HxVf136 RIL population which varies for ascochyta blight and broomrape resistance. To build a more saturated map and refine the position of the QTLs, we considered the most recent linkage maps and the assessments for *A. fabae* [1, 4] and broomrape [2] resistance reported in this population. Only 23 markers (13 WRKY and 10 resistance protein), fitting the expected 1:1 ratio could be combined with the previous data set and the linkage analysis involved 330 markers. The final map consisted of 292 loci distributed on 22 LGs and spanning 3124.98 cM. New SNPs markers were linked to *O. crenata* and *A. fabae* resistance QTLs. Thus *Oc7* (chr. VI), associated with broomrape resistance, was saturated with six markers, one of which (WRKY) was within the QTL interval. Besides, *Af3* (chr. III) related with Ascochyta resistance was saturated with four markers, two of which (a WRKY and a R protein) were located within the QTL interval. Besides refining the position of target regions, this approach has potential to identify other relevant candidate gene(s) determining disease resistance in this crop.

Acknowledgements. *Research funded by the Ministerio de Innovación y Ciencia (MICINN) grant RTA2013-00025 co-financed by the EU through the ERDF 2014-2020 "Programa Operativo de Crecimiento Inteligente" and the EU Programme (FP7/ 2007-2013) under the grant agreement n°FP7-613551, LEGATO project. N. Gutierrez acknowledges financial support from the Spanish Ministerio de Economia y Competitividad ('Torres Quevedo' program).*

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How to refer your abstract:
N. Gutierrez, I. Casimiro-Soriguer, A.M. Torres (2017) SNP genotyping of putative candidate genes involved in broomrape and Ascochyta fabae resistance in faba bean (Vicia faba L.); ICLGG 2017 - Book of abstracts, ICLGG2017/P/58

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Sweetening the deal for narrow-leafed lupin (*Lupinus angustifolius* L.): genomic research to manage quinolizidine alkaloid accumulation in the grain

K.M. Frick[1,2,3], R.C. Foley[2], L.G. Kamphuis[2,3,4], K.H.M. Siddique[3], G. Garg[2], K.B. Singh[2,3,4]

[1] *University of Western Australia, School of Agriculture and Environment, Crawley, Australia*
[2] *CSIRO Agriculture and Food, Floreat, Australia*
[3] *UWA Institute of Agriculture, Crawley, Australia*
[4] *Centre for Crop and Disease Management, Curtin University, Bentley, Australia*

Narrow-leafed lupin (NLL) is a grain legume crop that has gained attention as a human health food. The grain is high in protein (30-40%) and fibre (30%), however one factor complicating its acceptability into the health food market is the accumulation of quinolizidine alkaloids (QAs) in the grain. Levels of these toxic and bitter secondary metabolites must remain below 200 mg/kg for food purposes in many lupin-producing countries, although levels can vary considerably, often exceeding this limit. Currently, mechanisms of both QA production and environmental influence on this are poorly understood [1]. This project makes use of available genomic [2] and transcriptomic data [3] for NLL [4] to further elucidate the QA biosynthetic pathway, by identifying candidate genes associated with QA biosynthesis, the regulation of the biosynthetic pathway, and the transport of QAs from vegetative tissue to the grain. We investigate the expression patterns of QA biosynthetic genes in tissues of lupin species, the changes in this expression in NLL under stress conditions, and how QA production is affected by this. We also conduct research to identify the mutation responsible for the low-alkaloid locus *iucundus* - a recessive mutation bred into all modern NLL cultivars. This research provides a better understanding of the QA biosynthetic pathway and identifies environmental factors which can increase grain QA levels. This serves to assist lupin breeders and growers to produce a higher value NLL crop with low grain QA levels that is suitable for human consumption.

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How to refer your abstract:
K.M. Frick, R.C. Foley, L.G. Kamphuis, K.H.M. Siddique, G. Garg, K.B. Singh (2017) Sweetening the deal for narrow-leafed lupin (Lupinus angustifolius L.): genomic research to manage quinolizidine alkaloid accumulation in the grain; ICLGG 2017 - Book of abstracts, ICLGG2017/P/59

Book of abstracts
ISBN 978-615-5270-39-0

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

L. Záblatzká[1], M. Nelson[2], G. Aubert[3], M.C. Le Paslier[4], M. Hýbl[5], **P. Smýkal**[1]

[1] *Department of Botany, Palacký University in Olomouc, Olomouc, Czech Republic*
[2] *Millenium Seed Bank, Kew, RBG, UK*
[3] *INRA, Dijon, France*
[4] *INRA, Evry, France*
[5] *Crop Research Institute, The Centre of the Region Haná for Biotechnological and Agricultural Research, Olomouc, Czech Republic*

Plant evolution under domestication has altered numerous traits, introducing domestication bottleneck resulting in high degree of relatedness, leading to narrower genetic base of cultivated germplasm, prone to pests and diseases. We report development of the chromosome segment substitution lines (CSSL) of wild pea (*Pisum fulvum* WL2140) in the cultivated pea (*P. sativum* subsp. *sativum* cv. Terno) genetic background. The 145 lines were genotyped by genome wide DARTseq technology. All together 1,880 sequence based marker could be placed into respective linkage groups using synteny to *Medicago* and 50 lines were also genotyped by 13.2k Pea Illumina SNP assay. Once pea genome is available it is expected that larger number could be positioned. Fifty lines were selected for field trials, recording morphological traits and agronomic parameters (14 traits). There was substantial transgression in most of the traits, including seed weight, flowering time and maturity compare to cv. Terno control. Harvested seeds were analyzed for total protein, soluble proanthocyanidins, raffinose-verbascose-stachyose, raffinose family oligosaccharides, total starch, phytate-total phosphorus and galactomannan contents.

Establishment of such permanent introgression library will allow phenotypic characterization of unlimited number of target traits, which will provide means for QTL and gene identification and subsequent incorporation into desired commercial genotypes.

Acknowledgement: *This work received funding from the European Community's Seventh Framework Programme (FP7/ 2007-2013) under the grant agreement n°FP7-613551, LEGATO project.*

How to refer your abstract:
L. Záblatzká, M. Nelson, G. Aubert, M.C. Le Paslier, M. Hýbl, P. Smýkal (2017) The genomic and phenotypic evaluation of chromosome segment substitution lines of wild pea (P. fulvum) to widen the genetic diversity of pea crop; ICLGG 2017 - Book of abstracts, ICLGG2017/P/60

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

The International Mungbean Improvement Network – mobilizing the mungbean genetic diversity as a source for new traits for crop improvement

R. Schafleitner[1], R.M. Nair[2], C. Douglas[3], T. Shwe[4], A.K.M.M. Alam[5], A. Pratap[6], S. Gupta[6], E. Huttner[7]

[1] *The World Vegetable Center, Shanhua, Tainan, Taiwan*
[2] *The World Vegetable Center, South Asia, ICRISAT Campus, Patancheru, Hyderabad, Telangana, India*
[3] *Agri-Science Queensland, Department of Agriculture and Fisheries, Hermitage Research Facility, Warwick, Australia*
[4] *Myanmar Department of Agricultural Research, Yezin, Nay Pyi Taw, Myanmar*
[5] *Pulses Research Centre, Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh*
[6] *Indian Institute of Pulses Research (IIPR), Kanpur, Kalyanpur, Kanpur, Uttar Pradesh, India*
[7] *Crop Improvement and Management, Australian Centre for International Agricultural Research (ACIAR), Bruce, Australian Capital Territory, Australia*

Mungbean (*Vigna radiata*) is an important food and cash crop in the rice based farming systems of South and Southeast Asia. Short crop duration, low input requirement, high global demand and the capacity to improve soils through nitrogen fixation make mungbean an ideal rotation crop for smallholder farmers. Lack of investment in mungbean variety improvement led to a narrow genetic base of the crop. Consequently current mungbean varieties lack key traits to cope with emerging pests and diseases. A minicore set of 296 accessions derived from the World Vegetable Center germplasm collection was produced to improve the access to new traits for breeders. The International Mungbean Improvement Network distributed the set collection to Australia, Bangladesh, India and Myanmarfor multi-location field testing. The first trials showed large variation in phenology (days to 50% flowering and days to maturity), plant height, yield and quality parameters. In addition, accessions with tolerance to abiotic stress such as heat and saline soils, and, most importantly, new sources for resistance to *Mungbean yellow mosaic disease* and dry root rot (*Macrophomina phaseolina*) were identified. The minicore set was densely genotyped with single nucleotide polymorphic markers. A genome-wide association study identified candidate loci for *Mungbean yellow mosaic* disease resistance on chromosomes 6 and 7 and on unmapped scaffold sequences. Validation of these loci and mapping of additional traits is ongoing to identify the genetic loci conditioning breeder-desired traits to facilitate crop improvement.

How to refer your abstract:
R. Schafleitner, R.M. Nair, C. Douglas, T. Shwe, A.K.M.M. Alam, A. Pratap, S. Gupta, E. Huttner (2017) The International Mungbean Improvement Network – mobilizing the mungbean genetic diversity as a source for new traits for crop improvement; ICLGG 2017 - Book of abstracts, ICLGG2017/P/61

Book of abstracts
ISBN 978-615-5270-39-0

Towards a localization of the "vc-"gene which is responsible for low vicine and convicine content in seeds of faba bean (*Vicia faba* L.) and towards a low vicine and convicine winter faba bean cultivar

R. Tacke, W. Link

DNPW, Georg-August-Univ. Göttingen

Faba beans (*Vicia faba* L.) are appreciated as domestic protein sources due to their high seed protein content of app. 30%. Moreover, they are used as an alternative crop to improve soil fertility and break rotation circles of pests and diseases. However, faba beans are still cultivated at low hectarage in Germany, which is in part due to antinutritive compounds in their seed, such as vicine and convicine. Taking faba beans for feeding, these compounds can cause oxidative stress and can reduce the performance of animals such as laying hens and broilers. Therefore, breeding of faba beans with low contents of these components is a topical issue. To facilitate such breeding efforts, the development of highly informative DNA-markers, closely linked to the vicine and convicine locus (or loci) and in high linkage disequilibrium with its or their alleles will be aimed at in this study, including a fine-mapping of the chromosomal region of such gene(s). The currently existing genetic and genomic knowledge in this research area should pave the way to success. The results of these studies will be directly used for pre-breeding an autumn-sown faba bean cultivar for Germany, with low vicine and convicine content. Strategies and first findings will be reported. This project is part of the BLE/BMEL-funded 'Abo-Vici' consortium.

How to refer your abstract:
R. Tacke, W. Link (2017) Towards a localization of the "vc-"gene which is responsible for low vicine and convicine content in seeds of faba bean (Vicia faba L.) and towards a low vicine and convicine winter faba bean cultivar; ICLGG 2017 - Book of abstracts, ICLGG2017/P/62

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Untapping the potential of genome wide variations discovered through resequencing of germplasm lines for chickpea improvement

M. Thudi[1], R.K. Varshney[1,2]

[1] *International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Telangana, India*
[2] *School of Agriculture & Environment, and Institute of Agriculture, The University of Western Australia (UWA), Crawley, Australia*

Chickpea (*Cicer arietinum* L.) is an important food legume grown in arid and semi-arid regions of the world, but productivity is <1 ton ha^{-1} due to several biotic and abiotic stresses. In order to harness the untapped genetic potential, understand the impact of breeding on genetic diversity and gain insight into temporal trends in diversity we re-sequenced ~1500 chickpea germplasm lines using whole genome re-sequencing approach at 5X to 13X coverage. As a result, genome-wide variations like single nucleotide polymorphisms (>4 million), copy number variations (~0.5 million) and structural variations (~0.6 million) were identified. The variations identified in 35 parental lines of 16 mapping populations are a valuable resource in genetic research and helpful in locating genes/genomic regions responsible for economically important traits. Re-sequencing of a 129 released varieties provided opportunities to inspect the genetic and genomic changes reflecting the history of breeding and the selected loci may provide targets for crop improvement. We also report enhanced diversity in both desi and kabuli varieties as a result of recent chickpea breeding efforts. In addition, sequence data analysis of chickpea reference set (300 genotypes) provided insight into population structure, genetic diversity, gene loss, domestication and selection sweeps. Re-sequencing of multi-parent advanced generation intercross (1138 lines) enabled fine mapping and marker-trait associations for drought tolerance related traits that can be deployed to accelerate efforts for chickpea improvement.

How to refer your abstract:
M. Thudi, R.K. Varshney (2017) Untapping the potential of genome wide variations discovered through resequencing of germplasm lines for chickpea improvement; ICLGG 2017 - Book of abstracts, ICLGG2017/P/63

Book of abstracts
ISBN 978-615-5270-39-0

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

R. Thompson[1], L. Lebeigle[1], J. Marais[1], V. Vernoud[1], C. Le Signor[1], M.-A. Lacaille-Dubois[2]

[1] *INRA UMR1347 Agroécologie Dijon, France*
[2] *2Dept. Medicine, Univ. Burgundy Franche-Comté, France*

The use of pulses as ingredients for the production of food products rich in plant proteins is increasing. However, protein fractions prepared from pea or faba beans contain significant amounts of saponins, glycosylated triterpenes which can impart a bitter taste to the final food product. In addition, saponins have also been described to be involved in plant responses to biotic and abiotic stresses[1]. In this study, we identified and characterized the genes involved in saponin biosynthesis during pea seed development[2], and optimized a saponin extraction protocol to follow the biosynthesis of these compounds during the development of pea and faba bean seeds. The identification of mutants affecting the function of key genes of the saponin biosynthetic pathway is currently underway in pea[3]. This study is funded under the LEG'UP FUI (Unique Interministerial Fund) project (AAP No. 18).

Keywords: *protein, flavour, food*

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Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Comparative transcriptomic, anatomical and metabolic analysis of wild pea seed coat in relation to dormancy

O. Trněný[1], I. Hradilová[2], M. Válková[3], M. Cechová[3], A. Janská[4], N. Krezdorn[5], B. Rotter[5], P. Winter[5], A. Soukup[4], P. Bednář[3], P. Smýkal[2]

[1] *Agricultural Research, Ltd. Troubsko, Czech Republic*
[2] *Department of Botany, Palacký University in Olomouc, Czech Republic*
[3] *Regional Centre of Advanced Technologies and Materials, Department of Analytical Chemistry, Palacký University in Olomouc, Czech Republic*
[4] *Department of Experimental Plant Biology, Charles University, Prague, Czech Republic*
[5] *GenXPro, Frankfurt, Germany*

Seed germination is one of the two key traits which have been selected to facilitate cultivation and harvesting of crops. The objective of this study was to analyze the anatomical structure of seed coat and pod, identify metabolic compounds associated with water-impermeable seed coat and expression profiling. Comparative analyses were carried out on wild dormant *Pisum elatius* (JI64, VIR320) and cultivated *P. sativum* non-dormant (JI92, Cameor). Considerable differences were found in the texture of testa surface, length of macrosclereids and seed coat thickness. Histochemical and biochemical analyses indicated genotype related variation in composition and heterogeneity of seed coat cell walls within macrosclereids. Liquid chromatography – electrospray ionization/mass spectrometry and Laser desorption/ionization–mass spectrometry of separated seed coats revealed significantly higher contents of proanthocyanidins (dimer and trimer of gallocatechin), quercetin and myricetin rhamnosides and hydroxylated fatty acids in dormant compared to non-dormant genotypes. High throughput RNA sequencing of three developmental stages resulted in identification of differentially expressed genes between dormant and non-dormant seeds. The expression of selected genes was studied by qRT-PCR [1]. This integrated analysis of the seed coat in wild and cultivated pea provides new insight as well as raises new questions associated with domestication and seed dormancy.

Acknowledgements: *Study was mostly supported by the Grant Agency of the Czech Republic, 14-11782S and EU Legato (n°FP7-613551) projects and partially by institutional funding on the long-term conceptual development of Agricultural Research, Ltd. organisation.*

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Book of abstracts
ISBN 978-615-5270-39-0

Gene expression and localization of narrow-leafed lupin seed proteins evidence the functional interplay between conglutin protein families driving seed germination

E. Lima-Cabello, P. Robles-Bolívar*, J.D. Alché, **J.C. Jimenez-Lopez**

Department of Biochemistry, Cell and Molecular Biology of Plants; Estacion Experimental del Zaidin; Spanish National Research Council (CSIC), Granada, Spain

**Current address: MoBioFood Research Group, Department of Biochemistry and Biotechnology; Universitat Rovira i Virgili, Tarragona, Spain*

Lupinus angustifolius or narrow-leafed lupin (NLL) is characterized by multiple agricultural benefits. As an example, we have recently characterized the protective function of seed β-conglutin proteins against necrotrophic pathogens attack [1]. It is also noteworthy the multiple nutraceutical properties of seed compounds, i.e. conglutin proteins β1, β3, and β6, fighting type 2 diabetes throughout the modulation of the insulin molecular signalling pathway [2]. These properties may be attributed to their particular structural features [3].

Currently, no information is available at molecular and cellular level about the functional interplay between members of the NLL seed conglutin families. In the present work, we have studied different stages of the NLL seed germination by means of microscopy and RT-qPCR approaches to analyse the potential roles of conglutin protein families (α, β, γ, δ) in seed germination and cotyledon tissues differentiation.

The study outcomes evidence for the implications of particular isoforms of conglutin β, γ and δ proteins as key players in functional and metabolic changes, and regulatory networking underlying morphogenesis and developmental processes closely-related to the seed germination capability. Such processes are taking place after dormancy breaking and during the initial germination stages.

Acknowledgement: *EU Marie Curie grant PIOF-GA-2011-301550; ERDF co-funded grants RYC-2014-16536, CVI-7487, BFU2016-77243-P and RTC20154181-2 (MINECO).*

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E. Lima-Cabello, P. Robles-Bolívar, J.D. Alché, J.C. Jimenez-Lopez (2017) Gene expression and localization of narrow-leafed lupin seed proteins evidence the functional interplay between conglutin protein families driving seed germination; ICLGG 2017 - Book of abstracts, ICLGG2017/P/67

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Variation in seed coat colour and phytochemicals in *Lablab purpureus* in Australia

A.T. James, **A. Yang**

CSIRO Agriculture and Food, Brisbane, Australia

Legumes are playing an increasingly important role in sustainable agriculture and food production. They are an integral part of cropping systems in many parts of the world and provide an important source for proteins and micro nutrients for many populations. *Lablab purpureus* possess a number of qualities that can be used successfully under various conditions. In Australia, *L. purpureus* is a dual-purpose legume which can be grown as a pulse crop for human consumption or as a fodder crop for grazing and conservation in tropical environments in summer. We examined a total of 40 lines including 6 commercial varieties available in Australia for their phenotypic and chemical properties. They were grown under the same conditions with four plants per line and seeds were harvested at maturity. At harvest, the pods were flat or round, ranged from 3.5 - 10 cm in length, appeared smooth or wrinkled and had the shape of linear, oblong or scimitar. The seed colour ranged from white (e.g. Chinese White), cream, light brown, dark brown to black (e.g. Highworth). The seed size also varied greatly, from 10.6 to 49.3 g for 100 seed weight while the moisture content was similar at an average of 10.4%. The phytochemicals (total phenolic content, total flavonoid content and condensed tannin content) in the seeds were mostly in the seed coat with the cotyledon containing very small amount. These phytochemicals correlated strongly and positively with each other as well with the radical scavenging activity and the colour of the seed coat.

How to refer your abstract:
A.T. James, A. Yang (2017) Variation in seed coat colour and phytochemicals in Lablab purpureus in Australia; ICLGG 2017 - Book of abstracts, ICLGG2017/P/68

Book of abstracts
ISBN 978-615-5270-39-0

Wild pea (*Pisum sativum* subsp. *elatius*) and *Medicago truncatula* seed dormancy as adaptation to environment

I. Hradilová[1], J. Brus[2], V. Pechanec[2], M. Duchoslav[1], M. Hýbl[3], **P. Smýkal**[1]

[1] *Department of Botany, Palacký University in Olomouc, Czech Republic*
[2] *Department of Geoinformatics, Palacký University in Olomouc, Czech Republic*
[3] *Crop Research Institute, The Centre of the Region Haná for Biotechnological and Agricultural Research, Olomouc, Czech Republic*

The timing of transition from seed to seedling is important developmental transition determining the survival of individual, populations and species. Thus seed germination is vital for plant adaptation and acts in the context of natural selection. Little is known about clues which release the physical dormancy. Temperature and soil moisture oscilations could be the major players. Wild pea (*Pisum sativum* subsp. *elatius*) and *Medicago truncatula* offer excellent models to study seed dormancy in ecological context, due to wide range of species geographical distribution and existing variation in dormancy levels. We ask following questions: Is there an association between seed dormancy variation and geography along distributional range? If so, which ecological factors (climate, soil conditions, local habitat conditions) as adaptation drivers are correlated with geographical distribution of dormancy? 55 geographically different wild pea and 142 *Medicago* lines were scored at fluctuating and constant temperatures regimes. Significant differences were found. Two main groups were found, one with highly dormant seeds and second including samples responsive to higher temperature. Selected bioclimatic, topographic and humidity conditions were extracted based on geographical coordinates and subjected to Principal Component analysis. Main gradient is correlated with altitude and is related to the temperature variation (BIO 2, 4, 7) and mean temperature (BIO 1, 6, 11). The results of seed dormancy behaviour will be discussed in light of adaptation.

Acknowledgement: *This is supported by the Grant Agency of the Czech Republic, 16-21053S project.*

How to refer your abstract:
I. Hradilová, J. Brus, V. Pechanec, M. Duchoslav, M. Hýbl, P. Smýkal (2017) Wild pea (Pisum sativum subsp. elatius) and Medicago truncatula seed dormancy as adaptation to environment; ICLGG 2017 - Book of abstracts, ICLGG2017/P/69

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Genetics and genomics of the Nod factor-independent *Aeschynomene evenia* to shed light on the evolution of the *Rhizobium*-legume symbiosis

L. Brottier[1], L. Lamy[1,2], J. Quilbe[1], A. Delteil[1], J. Fardoux[1], C. Chaintreuil[1], P. Leleux[1,2], C. Klopp[2], F. Cartieaux[1], E. Giraud[1], **J.F. Arrighi**[1]

[1] *IRD, Laboratoire des Symbioses Tropicales et Méditerranéennes (LSTM), Campus International de Baillarguet, Montpellier, France*
[2] *INRA, Plateforme GenoToul Bioinfo, INRA Auzeville, Castanet-Tolosan, France*

Aeschynomene evenia has emerged as a new model legume for the deciphering of an alternative symbiotic process that is independent of Nod factors (NF) [1,2]. First insights on the molecular symbiotic mechanisms in *A.evenia* were obtained from transcriptomic analysis and reverse genetics revealing that some symbiotic determinants identified in conventional legumes appear to be recruited but that several key genes involved in bacterial recognition, symbiotic infection and nodule functioning were found to be not expressed [3,4]. Forward genetics and genomics are now expected to allow the identification of the specific molecular determinants of the NF-independent process. For this purpose, an EMS-mutagenized population was generated from a 9.000 seeds treatment in *A. evenia* and its screening allowed the isolation of 300 mutants altered in nodulation (42% Nod^-, 56% Fix^-, 2% Nod^{++}). Characterization of 30 independent Nod^- mutant lines revealed new and original symbiotic traits. In parallel to the mutant strategy, we performed PacBio sequencing that provided a ~78x sequencing coverage and resulted in a scaffold assembly that represented 92% of the *A. evenia* genome. The forthcoming completion of this reference genome sequence will facilitate the identification of symbiotic genes by performing deep re-sequencing at gene or whole genome-level of the nodulation mutants selected. A comparative genetic analysis of *A. evenia* with other diploid but NF-dependent *Aeschynomene* species is also being set up to further illuminate the evolution of the symbiotic genes within this legume group [5].

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Book of abstracts
ISBN 978-615-5270-39-0

Expression of *PIN* genes in root nodules of fabacean model plants

I. Sańko-Sawczenko, B. Łotocka, W. Czarnocka

Department of Botany, Faculty of Agriculture and Biology, Warsaw University of Life Sciences, Warsaw, Poland

Fabaceans can form either determinate-type root nodules, which exhibit temporary meristematic activity or indeterminate-type root nodules with persistent meristematic zone. Auxin distribution can vary depending on the nodule type. Therefore, our study aimed to compare the expression level and tissue specificity of genes encoding auxin efflux carriers – PIN-formed proteins (PINs) in *Medicago truncatula*, and *Lotus japonicus*, forming indeterminate- or determinate-type root nodules, respectively.

Our previous results revealed that *PINs* encoding orthologs of *Arabidopsis thaliana* ER-localized PIN5 subclade were especially highly expressed in *M. truncatula* root nodules. Based on those data, *MtPIN4*, *MtPIN9*, *MtPIN11* were selected for further analysis and their expression was analyzed using GUS reporter system. Meanwhile, our study on determinate-type root nodules enabled to identify *PIN* sequences in *L. japonicus*. In order to indicate which of them play a potential role in the development of root nodules, we compared their expression levels in developing and mature nodules.

Our results further support the statement that auxins, along with their polar transporters, play pivotal role in root nodules development regardless of their type. Moreover, we postulate that intracellular auxin homeostasis, dependent on ER-localized PINs, is essential for nodulation in both tested model fabacean species.

Acknowledgements: *This work was supported by the 505-10-011100-P00133-99 project operating within the Internal grants for young scientists and PhD students at Warsaw University of Life Sciences.*

How to refer your abstract:
I. Sańko-Sawczenko, B. Łotocka, W. Czarnocka (2017) Expression of PIN genes in root nodules of fabacean model plants; ICLGG 2017 - Book of abstracts, ICLGG2017/P/71

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Host genetic control of symbiotic specificity in the legume-rhizobial interactions

H. Zhu

University of Kentucky, USA

Establishment of a successful symbiotic relationship between legumes and rhizobia requires that the rhizobium can nodulate and invade the host plant and subsequently differentiate and persist in nodule cells to fix nitrogen. However, incompatibility between the interacting partners frequently occurs during the process of symbiotic development, leading to a failure of nodulation (Nod^-) or formation of nodules that are defective in nitrogen fixation (Nod^+/Fix^-). Genetic control of symbiotic specificity is complex, involving both host and bacterial genes. Whereas nodulation specificity (i.e., Nod^+ vs. Nod^-) appears to be mostly controlled by single host genes, nitrogen fixation specificity (i.e., Fix^+ vs. Fix^-) or natural variation in nitrogen fixation efficiency between different plant-bacteria combinations is quantitatively inherited. Understanding the molecular mechanisms underlying symbiotic compatibility is important for development of tools to enhance the potential of nitrogen-fixing symbiosis in sustainable agriculture. We will report our recent progress in the study of symbiotic specificity in soybeans and Medicago.

How to refer your abstract:
H. Zhu (2017) Host genetic control of symbiotic specificity in the legume-rhizobial interactions; ICLGG 2017 - Book of abstracts, ICLGG2017/P/72

Book of abstracts
ISBN 978-615-5270-39-0

Investigating the role of ethylene in the sanctioning response of leguminous hosts to ineffective rhizobial partners

S.L. Rowe[1], M.L. Friesen[2]

[1] *Department of Plant Biology, Michigan State University, East Lansing, USA*
[2] *Departments of Plant Pathology and Soil and Microbial Sciences, Washington State University, Pullman, USA*

Nitrogen is essential for plant growth, but much of the nitrogen on earth is in a form that is unavailable to plants. In order to access this nitrogen, leguminous plants form a symbiotic relationship with rhizobia, where the bacteria fix nitrogen for the plant in exchange for photosynthetically produced carbon in root nodules. As with any cooperative relationship, there is the potential for "cheating" where some rhizobial strains will form root nodules but fail to fix nitrogen. It has been observed that hosts are able to restrict the growth of these ineffective partners but the mechanisms driving this are unknown. Reactive oxygen species (ROS) are a group of small, chemically reactive molecules containing oxygen. ROS are inevitable byproducts of oxygen metabolism and are used in cellular signaling and homeostasis. In the legume rhizobia symbiosis, ROS have been identified as important regulatory mechanisms in the nodule initiation and development processes. The role of ROS during nodule organogenesis remains unknown. Preliminary evidence indicates that H2O2 is differentially expressed in root systems exposed to ineffective partners in comparison to roots exposed to effective partners. My work aims to understand the molecular mechanisms that regulate the growth of ineffective partners. My current work focuses on understanding what role ROS plays in this process. This information will shed light on how ROS regulates the symbiotic relationship and control mechanisms of this relationship and enable us to make further predictions and test potential regulatory mechanisms.

How to refer your abstract:
S.L. Rowe, M.L. Friesen (2017) Investigating the role of ethylene in the sanctioning response of leguminous hosts to ineffective rhizobial partners; ICLGG 2017 - Book of abstracts, ICLGG2017/P/73

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Pan-genome assembly of population haplotypes provides a comprehensive solution to common obstacles in modern breeding

A. Feinstein

NRGene, Ness-Ziona, Israel

Much of the World's food production, in both livestock and crops, relies on modern breeding programmes which increasingly apply advanced genomic tools to achieve genetic gains, cost reduction, and development of new varieties and breeds. The increased usage of "genomic selection" and other approaches utilizing genetic markers is an important part of this change. However, often the use of genetic markers is limited due to the lack of high quality genome assemblies and genetic maps, or reliance on a non-ideal reference genome sequence.

NRGene's technology offers a comprehensive solution to analyzing a wide variety of food-producing organisms and detecting the most useful set of genomic markers to use.

NRGene's proprietary de-novo assembly software (DeNovoMAGIC) uses a unique pan-genome creation technology which creates a haplotype database that captures the full scope of the genomic diversity within a population. The haplotype database consists of very dense dominant sub-sequences distinctly related to a haplotype at any given position, which are used instead of SNP markers. On this basis, any low coverage genotype method (e.g. GBS or SNP array) can be used to impute back the haplotype of any given sample. The haplotype database is a "live system" that can be updated to account for new introduced germplasm in a time and cost-effective manner.

The above approach and its application to realistic breeding scenarios will be demonstrated in maize while detailing the cost benefit of the approach for different breeding entities.

How to refer your abstract:
A. Feinstein (2017) Pan-genome assembly of population haplotypes provides a comprehensive solution to common obstacles in modern breeding; ICLGG 2017 - Book of abstracts, ICLGG2017/P/74

Book of abstracts
ISBN 978-615-5270-39-0

Transcriptomic profiling of genes involved in epicatechin biosynthesis in soybean

J. Ha[1,2], **M. Kim**[3], M.Y. Kim[1,2], M.Y. Yoon[1], T. Lee[1], Y.-H. Lee[1], Y.-G. Kang[3], J.S. Park[3], J.H. Lee[3], S.-H. Lee[1,2]

[1] *Department of Plant Science and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea*
[2] *Plant Genomics and Breeding Institute, Seoul National University, Seoul, Republic of Korea*
[3] *Applied Technology & Research Division, R&D Center, AmorePacific Corporation, Yongin-si, Gyeonggi-do, Republic of Korea*

Proanthocyanidins are oligomeric or polymeric end products of flavonoid metabolism starting from central phenylpropanoid pathway. Because the presence of proanthocyanidins in crop species has double-sided effects depending on their agricultural or commercial usage, regulation of proanthocyanidins has become a target of breeding and genetic modification. Epicatechin and catechin are the building blocks of proanthocyanidins, of which epicatechin is more common extension unit and starter unit in the plant species. Soybean seeds have been utilized as ingredients for healthful human diet and cosmetic formulas because they are rich in flavonoid metabolites including isoflavonoids, anthocyanins and proanthocyanidins. Although it has been shown that there are large variations in proanthocyanidin contents among soybean genotypes, genetic regulatory mechanisms in biosynthesis of proanthocyanidins in soybean still remain unclear. We evaluated interspecific and intraspecific variability in flavonoid component contents using 43 cultivated, landrace and wild soybeans. Transcriptomic profiling of genes for enzymes involved in flavonoid biosynthesis was conducted using three soybean genotypes, Hwangkeum (cultivated), IT109098 (landrace) and IT182932 (wild) at two developmental stages of R5 and R7. Among three genotypes, more genes were differentially expressed in seed sample at R5 stage, compared to leaf sample and R7 stage. Among 367 genes in flavonoid biosynthetic pathway, 153 genes were identified as differentially expressed genes (DEGs). Different homologous genes for anthocyanidin synthase (ANS), 3-O-glucosyltransferase (3GT) and anthocyanidin reductase (ANR), the enzymes that derive end products of flavonoid metabolism, were detected as DEGs between wild and cultivated soybeans and between landrace and cultivated soybeans. Our results provide primary insights on underlying genetic variations for proanthocyanidin biosynthesis between cultivated, landrace and wild soybeans.

How to refer your abstract:
J. Ha, M. Kim, M.Y. Kim, M.Y. Yoon, T. Lee, Y.-H. Lee, Y.-G. Kang, J.S. Park, J.H. Lee, S.-H. Lee (2017) Transcriptomic profiling of genes involved in epicatechin biosynthesis in soybean; ICLGG 2017 - Book of abstracts, ICLGG2017/P/75

Book of abstracts
ISBN 978-615-5270-39-0

ICLGG 2017
18-22 September 2017
Siófok, Hungary

Dissection of genetic architectures of soybean protein, oil, and amino acids

S. Lee[1,3], K. Van[2], M. Sung[3], L. McHale[2], R. Nelson[4], J. LaMantia[5], R. Mian[6]

[1] *Chungnam National University, Daejeon, South Korea*
[2] *The Ohio State University, Columbus, USA*
[3] *North Carolina State University, Raleigh, USA*
[4] *Soybean/Maize Germplasm, Pathology, and Genetics Research Unit, USDA-ARS, Urbana, USA*
[5] *Corn, Soybean and Wheat Quality Research Unit, USDA-ARS, Wooster, USA*
[6] *Soybean Nitrogen Fixation Unit, USDA-ARS, Raleigh, USA*

Soybean [*Glycine max* (L.) Merr.] is an economically important crop due to its various use in animal feeding, food, and industrial raw materials. Genome-wide association mapping is a promising analytic approach to dissect complex traits by using historical recombinant events. The objectives of this study were to evaluate selected 621 soybean accessions in maturity groups I to IV for protein, oil, and amino acid contents and to analyze statistical association between the trait values and genotypic data to identify useful alleles for the seed composition traits. During 2014 and 2015, the 621 accessions were grown in four locations in Ohio, North Carolina, and Illinois (total 5 environments), and seed composition trait data was obtained from the seeds harvested from each environment via near-infrared spectrometer analysis. Using the trait data and SoySNP50K data of the 621 accessions, genome-wide association analyses were conducted. This study detected 8 and 11 genomic regions significantly affecting protein and oil profiles. Two to five loci were identified for each amino acid. The most significant QTL for protein/oil was on Gm20, also reversely interacting with each other. Four QTL for protein had increased protein, while had little or no effects on oil. These QTL will be important to increase protein content without reduced oil content in soybean seed. The extent of individual allele effect was small for most of the SNP loci. Nine QTL for the amino acids are newly reported in this study, though individual QTL effect was small as in protein/oil contents.

How to refer your abstract:
S. Lee, K. Van, M. Sung, L. McHale, R. Nelson, J. LaMantia, R. Mian (2017) Dissection of genetic architectures of soybean protein, oil, and amino acids; ICLGG 2017 - Book of abstracts, ICLGG2017/P/76