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

Marion Prudent, Christophe Salon, Sylvie Girodet, Christian Jeudy, Nadia Rossin, Karen Boucherot, Françoise Jacquin, Gregoire Aubert, Stephanie Pateyron, Annick Moing, et al.

BOOK OF ABSTRACTS

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ABSTRACTS OF TALKS

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Physiological limits to legume genetic improvement

T.R. Sinclair

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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.

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

L. Moreau, **A. Charcosset**

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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.

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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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Exploiting systematic mutagenesis to identify targets for gene editing

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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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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]

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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.

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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.

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Lentil genomes: weird and wonderful wildlings

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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.

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A reference genome sequence of cowpea

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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.

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

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Narrow-leafed lupin (NLL) is the main grain legume grown in Australia and forms an important part of sustainable farming systems, reducing the need for nitrogenous fertilizer, providing valuable disease breaks and boosting cereal yields.

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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The *Lathyrus sativus* genome project

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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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Genetic diversity and strategies for seed quality enhancement in pea

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

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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.

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Nodule-specific plant peptides control intracellular accommodation of symbiotic bacteria

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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.

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Comparative genetic analysis of flowering time adaptation in legumes

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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.

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

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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*.

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

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

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

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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.

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

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

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

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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.

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

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

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

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

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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.

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

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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.

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

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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.

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