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Legume models

Tools and resources available

Ongoing Research

Special issue on *Model Legumes*

EDITORIAL

Why study "Model" Legumes? Hopefully, the following collection of short reports will go some way towards answering this question, and provide the reader with some enjoyment at the same time.

We would like to take this opportunity to thank all the colleagues who have made this issue possible: the article contributors, and the associated research teams, who've consecrated their valuable time; the AEP President Diego Rubiales, who has re-launched the Grain legumes magazine and who proposed this topic; Dominique Millot and Alexsandar Mikic, for their respective roles in producing the printed version. We sincerely hope you will enjoy reading this issue.

Richard THOMPSON

Nicolas RISPAIL

Managing Editors of GLM53

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Carte blanche

to...

Ulrike Mathesius

Model legume issue

Legumes are one of the most diverse families of flowering plants. They display a fascinating array of beneficial microbial interactions, nodulation types, plant architecture, and climatic adaptations.

The decision to concentrate research on initially two, and now an increasing number of model legumes has been essential for the establishment of extensive genetic and genomic resources, which have accelerated the discovery of critical genes in symbiotic pathways and stress response. Identification of key receptor and signal transduction genes, together with a vast array of gene, protein and metabolite changes characterized during symbiosis have seen fast progress on our understanding of how rhizobial and mycorrhizal symbionts communicate with their legume hosts. This knowledge is now also being translated to actinorhizal plants and their symbionts. In addition, receptors and regulators for response to abiotic stresses like salt stress are being unraveled. Model legumes have also contributed much to our knowledge of plant-pathogen interactions, including those with pathogenic fungi, nematodes and aphids. In particular, the large germplasm of Medicago truncatula cultivars has enabled identification of several pathogen resistance genes.

Legumes provide nutritious and protein rich food for a large part of the world's population. Their ability to form nitrogen fixing and mycorrhizal symbioses under a large range of climates means that different species of legumes can be utilized in most areas of the world as crops and pastures. While research tools are increasingly becoming available for crop legumes like soybean, bean and pea, our understanding of nodulation processes and abiotic stress responses in most native legumes of the world is underexplored compared to model legumes. As we are facing challenges of growing more food under less predictable climatic conditions, it is imperative, especially for farmers in developing countries, to translate our knowledge from model legumes to other crop and native legumes that grow in arid and low nutrient environments. Comparative genomics, combined with the increased power of high-throughput sequencing and the knowledge of local farmers and breeders around the world could enable real progress in improving crop legumes, so that the diversity of legumes can be fully utilized.

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Medicago truncatula as a model legume

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In the mid-1980s, a network of French (INRA and CNRS) researchers interested in the plant-rhizobium symbiosis began to search for a model system to facilitate genetic studies of this interaction. As Arabidopsis could not be used, the network turned to the genus *Medicago* (*Leguminosae*)(1) and screened a collection of annual *Medicago* species for several characters including genome size and regenerability in tissue culture, and decided to focus on an autogamous species with a relatively small genome size, *Medicago truncatula*. The first plant transformation experiments were carried out using an *Agrobacterium rhizogenes* strain and transgenic plants were obtained. Subsequently, efficient *A. tumefaciens* transformation protocols have been published for *M. truncatula*. *M. truncatula* was also shown to be efficiently nodulated by *Rhizobium meliloti*, in contrast to many *Medicago* species. The potential of *M. truncatula* as a model legume was published in 1990 (2). *M. truncatula* has a small diploid genome of *ca.* 5 x 10^8 base pairs, is self-fertile and produces large quantities of seed thus facilitating genetic analysis. After the 1990 paper, *M. truncatula* was one of two model legumes, the other being *Lotus japonicus*, which were rapidly adopted. Suitable genotypes (principally A17 Jemalong (J5), and R-108) were selected, and a series of genetics and genomics resources was developed (Table 1). In parallel, the suitability of *M. truncatula* as a subject for numerous pathogen and symbiont systems and abiotic stress studies was evaluated (3). Many groups have exploited for this and other objectives large collections of accessions of *M. truncatula* and other annual *Medicago* species held in Montpellier (France) (http://www1.montpellier.inra.fr/BRC-MTR/, curator Jean-Marie Prosperi) and at SARDA, Adelaide (Australia) (http://ressources.ciheam.org/om/pdf/c45/00600172.pdf).

M. truncatula is closely related to other members of the Galegoid family of legumes, which include important crops such as pea, faba bean, chickpea, pigeonpea and lentil. The extensive genomic synteny within this group therefore allows for the rapid transfer of knowledge acquired in *M. truncatula* to these species.

In 2003, an international genomic sequencing consortium was launched to sequence the gene-rich regions of the *M. truncatula* genome. This project, which is nearing completion, has enabled the production of comprehensive genomics tools; mutant platforms described elsewhere in this issue, a transcription factor platform for qRT-PCR analyses (4), and proteomics reference maps (http://195.220.91.17/legumbase/medicago-seed-proteome/).

As part of the sequencing project, a physical map of the genome has been constructed, and a set of anchor markers identified which allow rapid mapping of unplaced markers. These markers are invaluable for defining genomic regions of interest and the sequences they harbour.

As a sequel, a HAP Mapping project is underway (http://www.nsf.gov/awardsearch/showAward.do?AwardNumber=0820005) that will permit high-density SNP mapping and trait mapping by association genetics within the *M. truncatula* germplasm collection.

Over the past 20 years, genomic resource development has been increasingly ambitious. Whilst in 1998 a collection of 900 ESTs (~transcripts) was reported, in 2008, incorporating genomic sequence information, a 55,000 EST collection was used to create an Affymetrix chip (5). The resulting transcriptomic data has been gathered in the *M. truncatula* Gene Expression Atlas, (http://bioinfo.noble.org/gene-atlas/v2/), a public database regrouping expression data for 55,000 genes obtained for more than 50 different conditions.

In 2007, a practical *Medicago* Handbook, combining methods and basic biology sections, was conceived and put online (http://www.noble.org/MedicagoHandbook/)

See also (http://www.medicago.org/) for access to public *Medicago* genomics resources including genomic sequence releases. Specific queries can also be sent to the Bionet Newsgroup for *Medicago* (mailto:medicago@net.bio.net). ■

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(4) Kakar, K. *et al.* (2008). Plant Meth. 4, 18.
(5) Benedito, V. A. *et al.* (2008). Plant J. *55*, 504-513

Table 1. Development of genomics resources for *Medicago truncatula*

Resource	Date	Publication
MUTANT COLLECTIONS		
Gamma-ray mutant collection	1995	Sagan, M. *et al.*, Plant Sci. 111, 63-71.
EMS mutant collection	2000	Penmetsa, R.V. and Cook, D.R., Plant Physiol. 123, 1387-1397.
T-DNA insertion mutants	2002	Scholte, M. *et al.*, Mol. Breeding 10, 203-215.
Tnt1 transposon insertions	2003 2008	d'Erfurth, I. *et al.*, Plant J. 34, 95-106. Tadege, M. *et al.*, Plant J 54, 335-347.
Fast neutron deletion collection	2009	Rogers, C. *et al.*, Plant Physiol. 151, 1077-1086.
TILLING mutant collection	2007 2009	Javot, H. *et al.*, Proc. Natl. Acad. Sci. USA 104, 1720-1725. Le Signor, C. *et al.*, Plant Biotech. J. 7, 430-441.
GENE EXPRESSION		
Proteome reference maps (suspension cell culture)	2005	Lei, Z. *et al.*, Mol. Cell. Proteomics 4, 1812-1825.
Proteome reference maps (seeds)	2003, 2007	Gallardo, K. *et al.*, Plant Physiol. 133, 664-682. Gallardo, K. *et al.*, Mol. Cell. Proteomics 6, 2165-2179.
Transcriptomics : Gene expression atlas	2008	Benedito, V.A. *et al.*, Plant J. 55, 504-513.
GENOME SEQUENCING		
BAC library	1999	Nam, Y.W. *et al.*, Theor. Appl. Genet. 98, 638-646.
Genetic linkage map	2002	Thoquet, P. *et al.*, BMC Plant Biol. 2, 1.
A sequence-based genetic map of *Medicago truncatula*	2004	Choi, H.K. *et al.*, Genetics 166, 1463-1502.
Sequencing the genespaces of *Medicago truncatula* and *Lotus japonicus*	2005	Young, N.D. *et al.*, Plant Physiol. 137, 1174-1181.
Three sequenced legume genomes and many crop species: rich opportunities for translational genomics	2009	Cannon, S.B. *et al.*, Plant Physiol. 151, 970-977.

Lotus japonicus, a model for the legume family

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Lotus japonicus is a close relative of Birds-foot trefoil and the cultivated *L. corniculatus*, *L. glaber*, *L. subbiflorus* and *L. uliginosus*. As the name implies it originates from Asia and is widespread on the Japanese islands, where the common name is Miyakogusa. Like for example pea, clover and alfalfa it belongs to the papilionoid clade of the legume family. Compared to other legumes, L. *japonicus* has biological features that are desirable in an experimental organism (Table 1) and was therefore chosen as model plant (2) (Fig. 1A). All *Lotus* species studied form nitrogen fixing root nodules of the determinate type, as do soybean and common bean. Interestingly, they can be divided in two groups, those that nodulate effectively with *Mesorhizobium* strains and ineffectively with *Bradyrhizobia*, and those responding opposite.

Lotus - a pasture and fodder crop

The main *Lotus* species with high forage value are *L. corniculatus*, *L. glaber*, *L. subbiflorus* and *L. uliginosus*. The four species grow in natural or cultivated pastures and their areas of distribution vary according to soil and climatic conditions. The adoption of *Lotus* species for pasture-crop rotation contributes to the sustainable agriculture in marginal areas (Fig.1B and C). Compared to other forage legumes, *Lotus* species have a number of advantages that make them successful. First, characteristics such as growth under low phosphorus availability, adaptability to acid soils and saline soils, growth under frequent water deficit or flooding have determined their adaptation to adverse ecological conditions. Second, tannin content preventing bloating in grazing ruminant animals is important. However, these cultivated species are not amenable for genetic studies: they are cross-pollinated, self-incompatible, and some are tetraploid.

Lotus research perspectives

A substantial part of the *L. japonicus* genome corresponding to ~ 90 % of the gene space has been sequenced, large data sets of *Lotus* expressed sequences tags (ESTs) are available in public databases and high throughput transcriptome, proteome and metabolome studies have been performed. Resources for genetic and genomic research in *L. japonicus* have been developed, and an integrated genetic map of *Lotus* has been assembled. A high degree of colinearity and a substantial synteny exist towards the genomes of other legumes such as pea (4), common bean and peanut (3). To transfer discoveries to crops unfit for genetic analysis a bioinformatics based legume anchor marker design was developed (1). *Lotus* has over the last decade contributed the discovery of novel genes involved in diverse biological processes. Several research groups investigate the basis of secondary metabolite production and anthocyanin and proanthocyanin pathways involved in disease and insect interactions. Other focus areas are flower and leaf development, seed formation and quality, rhizobial and mycorrhizal symbioses, abiotic stress responses, as well as hormone and long range signalling between root and shoot tissues. ■

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***Lotus japonicus* characteristics**
True diploid
Small genome
Genomic colinearity to different legume crops
Genetic variation in mutants, ecotypes and diploid relatives
Self-fertile
Short generation time
Ample seed set
Large flowers
Manual crossing
Perennial plant
Regrowth from stem base
Propagation from nodal cuttings
Agrobacterium transformable
Regeneration of transgenic plants
Symbiosis with rhizobia and mycorrhiza
Tannin and secondary metabolites

Table 1. Model plant characterstics of *Lotus japonicus*

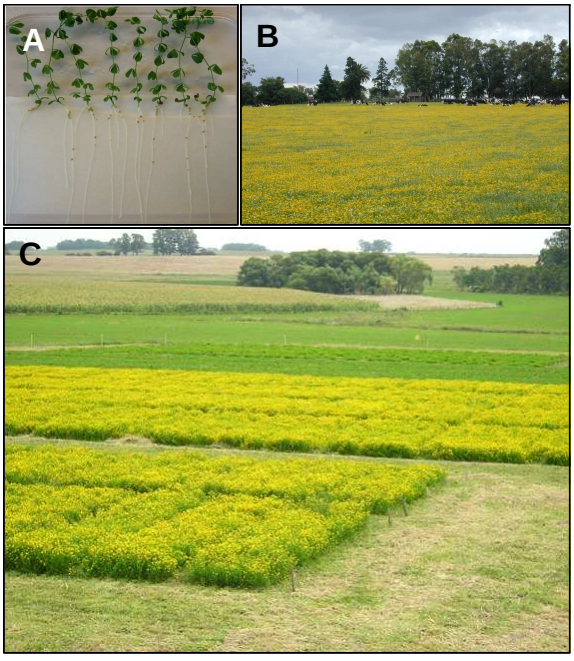

Figure 1 : Picture of the model legume *Lotus Japonicus* and its close cultivated relative *Lotus corniculatus*. A Nodulated *Lotus Japonicus* plant grown in a Petri dish. B, *Lotus corniculatus* in pasture. C, *Lotus corniculatus* in experimental plot (Photos B and C by Monica Rebuffo)

Soybean as a model legume

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Yes, soybean is a model legume again! Not so long ago, soybean (*Glycine max*) and pea (*Pisum sativum*) were the model species for legume physiology. They provided tremendous insight into legume development that could be extrapolated to other less-researched legumes such as beans, peanuts, alfalfa and the other 18,000 legume species. Soybean has always been an important research crop, being the most economically significant legume in the world. However, in recent years some limitations at the molecular level had left soybean somewhat on the backburner of legume research in favor of the more molecularly-friendly *Medicago truncatula* and *Lotus japonicus*.

So how and why did the transition of soybean from important research crop plant to 'model legume' occur? For one, the soybean genome has now been completely sequenced and is readily available (http://www.phytozome.net/soybean) (7). This has completely eliminated many of the difficulties previously caused by the large size of the soybean genome. As a result, gene discovery in soybean is more efficient and feasible than ever. Furthermore, when used in concert with next generation transcriptomic technologies (e.g., 454, Illumina and SOLiD platforms), the soybean genome provides a powerful high-throughput and non-targeted approach to gene expression studies.

Together with the complete genome sequences of *Medicago truncatula* (http://www.medicago.org/genome) and *Lotus japonicus* (http://www.kazusa.or.jp/lotus), the soybean genome also provides an excellent resource for comparative legume genomics (1). Other legume genomes, such as that of the legume tree, *Pongamia pinnata*, are also likely to be fully sequenced in the near future, further benefiting this area of research.

Historically, genetic research using soybean was complicated by an ancestral genome duplication event that resulted in there being two copies of most genes. However, this too has been overcome by the sequencing of the soybean genome, and thus genetic investigations using soybean are no longer disadvantageous. Instead, studies into sequence divergence and functional redundancy of the duplicated soybean genes will no doubt be highly informative in regards to gene and genomic evolution. The recently assembled soybean genome and the chromosomal 'clock' that elegantly illustrates the position of genes and segmental duplications in the genome will also benefit such studies (7).

Soybean also has a strong agronomic research base and has a large germplasm including robust mutant (e.g., 2) and TILLING populations (e.g., 3, PM Gresshoff and J Batley, unpublished). It is also readily amenable to *Agrobacterium rhizogenes*-mediated transformation required for over-expression and RNA interference studies (e.g., 6). Functional genomics approaches, such as Virus-Induced Gene Silencing (VIGS), are also well established in soybean and have proven to be instrumental in identifying the function of candidate genes (e.g., 8).

Compared with the much smaller *M. truncatula* and *L. japonicus*, the relatively large size of soybean is also advantageous, enabling large quantities of tissue to be harvested for transcriptomic, metabolomic and proteomic studies. It also makes soybean ideal for most nodule, root, shoot seed and embryo investigations, in addition to grafting (4) and xylem sap analysis experiments (5).

Overall, soybean has long been an important crop plant to study developmentally. As technology has advanced, so too has its use as a model system for molecular and genomic research. Welcome back soybean! ■

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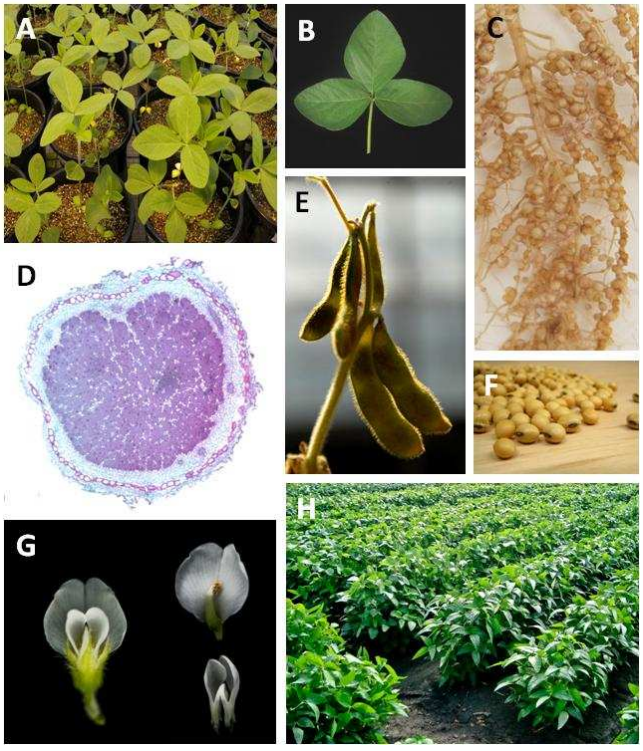

Figure 1 : Photographs of soybean plants and parts. A) Growing seedlings; B) a mature trifoliate leaf; C) roots and root nodules formed on a supernodulating mutant; D) cross-section of a mature nodule filled with nitrogen-fixing bacteroids; E) mature pods; F) mature seeds; G) whole (left) and dissected (right) flower; H) field of soybean plants.

Common Bean: a model food legume for international agriculture

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Common beans (*Phaseolus vulgaris* L.) is the most important grain legume for direct human consumption (23 M tons/year) and provide major amounts of protein and mineral nutrients to people in tropical and sub-tropical countries of Africa and Latin America (3). Their vitamins and complex starches have health benefits as well. The crop represents 57% of the food legumes consumed worldwide, is widely adaptable over a range of production sites, is grown on every continent except Antarctica and is consumed both in developed and developing countries. Annual consumption per capita can reach as high as 66 kg/year in parts of highland Africa where the nutritional role of beans is second only to maize. Meanwhile Brazil is the largest producer overall followed by Mexico and large exporting countries include Argentina, Canada, China and the United States. Apart from farms in the developed world, most bean production is for subsistence or regional sale and is produced on small farms ranging from 1-10 ha in size often in marginal environments such as drought prone areas, acid/low fertility soils or on steep hillsides.

Due to the crop's widespread acceptance and ample genetic resources consisting in two gene pools and up to seven races, varieties and landraces of common bean are grown in many environments at different altitudes and with different climate regimes (8). As a crop species, common bean was domesticated separately in South and Central America giving rise to the Andean and Mesoamerican gene pools and then spread to many secondary centers of diversity. Notably, some genotypes are adapted to drought and others to abiotic stresses such as aluminum toxic soils or low phosphorus levels while many sources of resistance to specific diseases and insects have been found over the past 75 or more years of research on the crop. Co-adaptation of disease races and common bean gene pools allows researchers to exploit inter-gene pool crosses to find resistance. Meanwhile new sources of resistance can be searched for in the 75,000 plus accessions of common bean and close relatives held in germplasm banks around the world such as at the International Center for Tropical Agriculture (CIAT).

Common bean has a relatively small genome (650 Mbp), an autogamous mating system and is a true diploid (2n=22) without the complex chromosomal rearrangements, wide-scale duplications and genome expansion found in some other legumes (5). All of this has simplified genetic analysis of the species and benefits rapid progress in molecular assisted breeding and selection (MAB or MAS). Common beans are also closely related to other cultivated *Phaseolus* species (*P. acutifolius* [tepary bean], *P. coccineus* [scarlet runner bean] and *P. lunatus* [lima bean]) and to legumes in the *Phaseoleae* tribe (*Glycine max* [soybean]; *Cajanus cajan* [pigeonpea]; *Vigna unguiculata* [cowpea], *V. radiata* [mungbean],) and as such are useful for comparative genomics (4). The remainder of this review describes common bean genetics and genomics from the perspective of international agricultural research and the role that genome technologies, sequencing and model status can have on crop improvement for and in developing countries especially as they relate to nutritional quality and adaptation to abiotic and biotic stresses.

The first genome technology to have an impact on common bean has been genetic markers and maps. Common bean has a well-developed genetic map and a large number of molecular markers specific to the species. These include a core of RFLP probes that represented among the first sequences cloned in the species and more recently added microsatellites and single nucleotide polymorphism loci. Both genomic and genic microsatellites have been developed from database searches, enriched libraries for $(GA)_n$ and $(ATA)_n$ repeats, cDNA and EST sequences as well as direct or *in silico* screening of un-enriched libraries or BAC end sequences. SNP markers have been derived from EST sequencing efforts and have been implemented with various platforms such as heteroduplex digestion and bead arrays. In terms of genetic tools to support marker development and gene discovery, over 80,000 EST sequences have been developed for the species and over 10 BAC libraries have been constructed for wild and cultivated accessions of the crop which can be useful for targeted map-based cloning of traits from specific interesting or promising genotypes of common bean. In addition, a physical map has been constructed for common bean based on one of these BAC libraries and a whole genome sequencing project has begun at the JGI/DOE facility in the United States with additional sequencing planned through a consortium of Latin American countries. Overall, common beans have an active research community around the world which means that the pace of genomic discovery has been accelerating.

The genetic tools described above have been put to use in common bean for a large number of gene tagging studies and quantitative trait loci (QTL) analysis (1, 7). To the bean breeding community this research is immediately applicable for crop improvement and has been of high priority. In the context of developing country stresses, gene identification has led to tagging of important viral (bean common mosaic, bean golden yellow mosaic and related geminiviruses), fungal (angular leaf spot, anthracnose, ascochyta blight, root rots, white mold) and bacterial (common bacterial blight, halo blight) diseases as well as several insect pests (bruchids, pod weevils). QTL analysis has been conducted for these biotic resistances as well as for abiotic stress tolerances (aluminum toxicity, intermittent and terminal drought, low phosphorus) and nutritional quality (mineral and phytate content) traits. CIAT has implemented a marker assisted selection program for specific resistances to fungal and viral diseases, with ramping up to evaluate up to 10,000 individual segregants per season, and is planning selection for QTL to improve drought tolerance and nutritional quality. Finally, QTL and gene tagging studies have been important components of degree training in various countries and within regional networks for the crop.

Candidate gene identification for the traits mentioned above is leading to better markers for use in MAS. The scheme for the discovery and validation of gene-based markers with their subsequent use in traditional and marker-assisted or "molecular" breeding is outlined in Figure 1 with examples of drought tolerance and nutritional quality traits that are important in terms of the international agricultural context of common beans. While complex in terms of

physiology, drought tolerance mechanisms could be explored by understanding the underlying transcription factors and stress response pathways involved in adaptation to this stress (6). Functional genomics may also assist in exploring nutrient uptake which affects the nutritional quality of common bean or the secondary metabolites of common bean seeds many of which are health related compounds (2). Detailed genetic studies have already made headway in understanding the symbioses of this crop with *Rhizobium* and mycorrhizae. Furthermore, next generation sequencing and recently developed TILLING and EMS mutagenesis protocols hold the promise of speeding up gene discovery through accumulation of sequence information and mutant analysis. The only limitation to functional genomics in the crop is lack of knock out or transformation system for common bean.

Finally, marker repertoire and genetic understanding of common bean can be enhanced by comparisons with other related Phaseolid legumes in the process known as comparative genomics. Both PCR and hybridization-based markers have been transferable to a certain extent between common bean, cowpea and soybean depending on the degree of sequence conservation of the genes being studied. Recent examples of synteny analysis show alignment between regions of the common bean and soybean genomes even at disease resistance loci that are fast evolving and interestingly, most loci in common bean have a pair of orthologs in the soybean genome, since this is an ancestral tetraploid with a duplicated genome. In conclusion, common bean is both a crop and model legume with important scientific and societal reasons for further study and results from genetic research will have an impact on a crop that is very important to small farmers for income generation, food security and agricultural sustainability. ■

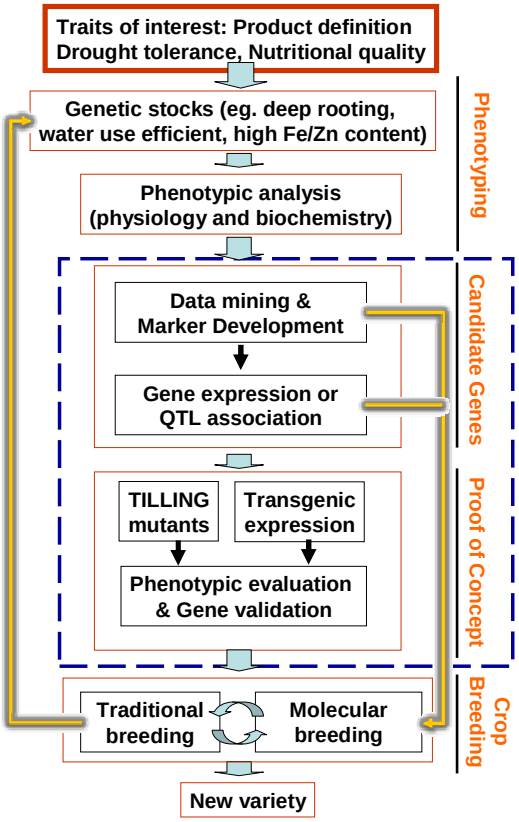

Figure 1. Scheme for translational genomics using candidate genes for drought tolerance and nutritional quality based on initial product definition followed by a phenotyping phase, a gene discovery and validation phase and a crop breeding phase. Arrows indicate feedback and feed-forward flows of genotypes (right) and gene-based markers (left).

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Pea as a model system

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Any experiment is necessarily involving a model: it is a simplification for the purpose of description. The biological 'model systems' seem to be more than this; they provide a justification for study based on facility, the consequence of resources developed by a community of coordinated researchers. This can have a profound impact on the science that is undertaken simply because science requires funding and funding agencies seek value for money as well as scientific excellence. Pea is not well positioned in this political context because its research community is small and the crop is not one of the top ten commodities.

The recent 'Grain Legumes' Integrated Project sought to forge an alliance between arable crop legume research and models. Whether this will, in the end, be of benefit remains to be seen, but along the way some significant resources for pea were developed, notably a TILLING population (4), a BAC library (used in 6) and characterisation of the relationship between accessions in European *Pisum* germplasm collections (in prep). No doubt too, as the cost of the generation of sequence-based marker sets declines, this will eventually be funded in pea allowing us to build on the syntenic relationship that has been established between pea and the sequenced legume genomes (1, 3, 7, 11). These resources should help in the facilitation of research activities, but still fall short of establishing pea as a 'model system'. Nevertheless pea does have some areas where it is an exemplary research system, and these encouraged Cannon *et al.* (2) to include pea among those legumes described as 'models'.

Here I draw attention to areas where I think pea is indeed an exemplary experimental system and where significant biological insight has been made in the last few years.

The area of most recent excitement has concerned the characterisation of strigolactone hormones in the regulation of bud outgrowth (5, 8). This seems especially interesting as it has emerged at least in part from the interaction between those studying pea and newly emerged 'model' species rice and *Medicago truncatula.* This hormonal system regulates not only bud outgrowth, but signals between plants and their mycorrhizal symbionts, hinting that the regulation of growth may be a component of a set of co-regulated processes that were important for plants in the initial colonisation of land.

Pea genetics was for many years the model system for understanding the regulation of flowering time. Studies in *Arabidopsis thaliana* then permitted the molecular characterisation of many genes involved in this process, but recently there has been a resurgence of interest and insight gained from pea where mutants are now characterised at the molecular level (see 10 for an excellent review). For flower development per se, zygomorphy has been gained and lost on multiple occasions, so comparison of the genes involved in the regulation of floral symmetry in diverse organisms can also shed light on independent evolutionary pathways as has been studied by Wang *et al.* (9).

Another evolutionary process represented by multiple gains and losses is the emergence of compound or dissected leaf forms from simple (entire) leaves and vice versa. A consequence of the taxonomic distribution of these forms is that structures given the same name may not necessarily be strictly homologous. Hofer and colleagues have developed pea as a genetic system in which these processes can be studied at a molecular level and shown its similarities to, and differences from, related developmental programmes either in other species or other organ types. Most recently this work has taken a direct approach to the isolation of the pea *Tendril-less* gene, known only by its phenotype and absent form, or uncharacterised in, model systems with extensive genome sequence (6).

The control of flowering is a consequence of the interaction between those processes that recognise environmental cues and those that regulate floral (or inflorescence) development. As such we can expect that, for diverse organisms, those genes that contributed to either process in a common ancestor will be shared components of this regulation. However the way in which these interact may be different in different organisms reflecting independent evolutionary paths. This is clearly seen in the comparison of the genetic control of flowering in pea and *Arabidopsis thaliana* (10). ■

Picture of a pea flower used as model system to study the genetics of the regulation of flowering time

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Maps to find treasures: from genome landmarks to the identification of genes that contribute to phenotypes of interest

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A genetic map is a representation of genetic markers on a chromosome in linear order, with distances between them expressed as percent of recombination or centimorgans. A physical map is defined as an ordered set of large DNA fragments, usually BAC clones, representing the physical linear order of genes along the genome with distances expressed as base pairs. Hence genetic and physical maps put landmarks on a genome and are valuable tools for gene hunting.

Maps as molecular tools that allowed the onset of the *Medicago truncatula* genome sequencing project.

The backbone needed for the sequencing project of *M. truncatula* was provided by a genetic map of 93 F_2 individuals from a cross between A17 and A20 (1). Genetic and cytogenetic analysis produced eight linkage groups, correlated with eight chromosomes by means of FISH with mapped BAC clones (3).The up-to-date version of this map is available on http://www/medicago.org. Large DNA fragment libraries of the Jemalong-A17 genome (BAC libraries mth1 (6) and mth2) allow the generation of the physical map. Adding 378 BAC-derived microsatellites markers anchored genetic and physical maps (5). The physical map now comprises 1370 contigs (44 292 BACs) and represents 466 Mb, i.e. 93% of the genome length. A total of 571 of these contigs are embedded into the actual sequence pseudomolecule (genome assembly V3, http://www.medicago.org).

Maps as genetic resources for gene hunting.

Linkage maps record segregating alleles and identify positions of loci/genes that contribute to phenotype expression. Two distinct strategies are possible. The first involves producing high density linkage maps of large F_2 populations in which the phenotype segregates, such as those used by Yang *et al.* (10). However, this strategy is time- and resource-consuming, and inadequate for the study of phenotypes that require replicate experiments because of strong environmental effects. An alternative solution is based on a set of medium-density linkage maps of Recombinant Inbred Line (RILs) populations centred on Jemalong-A17 (figure 1A). The parental lines were chosen for their phenotypic diversity and their contrasted pedo-climatic sites of origin (9 and Huguet *et al.*, unpublished). The maps are anchored on the *M. truncatula* sequence: each MTE genetic locus consists of a set of microsatellite sequences located on a given BAC, putatively polymorphic in different crosses. This approach makes it straightforward to compare different maps to detect congruency of QTLs position and effects. The colinearity of the maps allows immediate access to candidate genes underlying a QTL in a given cross by identifying the corresponding region on the Jemalong-A17 sequence (figure 1B). This set of genetic maps was developed in collaboration and is now widely used as it allows access to several alleles for each locus (e.g. 2, 7, 8). The physical maps further define the DNA sequence between genetic markers and are essential to rapid identification of genes at the locus of interest (e.g. 4).

Association genetics based on re-sequencing a large number of *M. truncatula* lines (HapMap project) is a promising new method for identifying interesting genes. Genetic and physical maps will be essential to validate this approach. Combining both will lead to high efficiency in the hunt for gene treasures in the model legume.■

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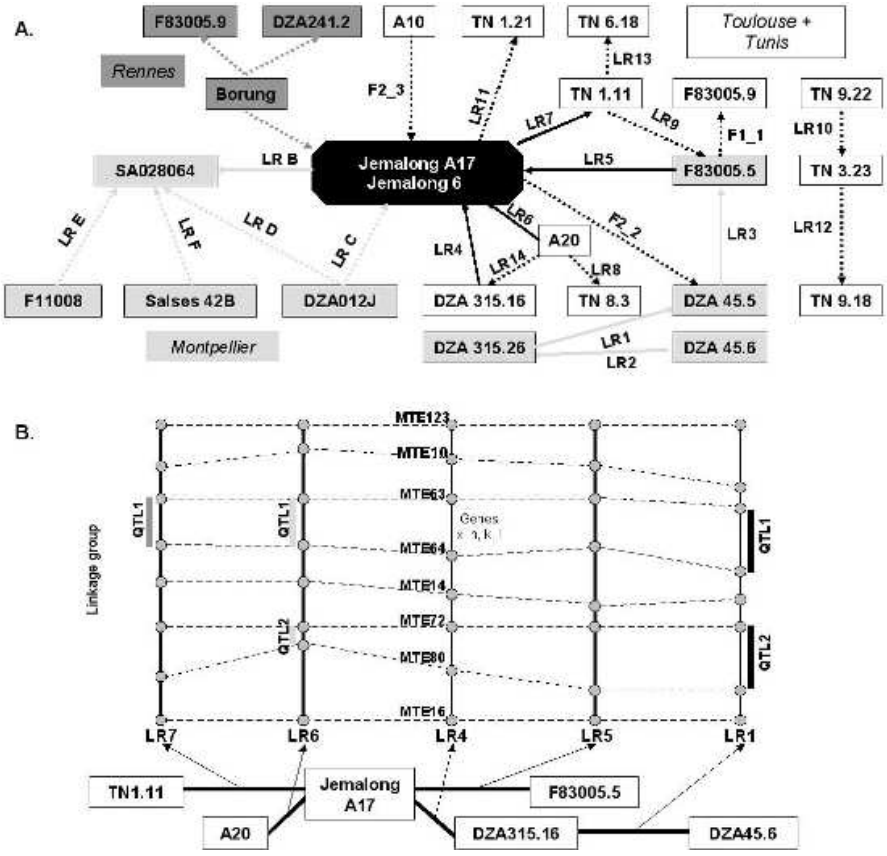

Figure 1. Genetic mapping in *Medicago truncatula* : from markers to candidate genes.
A. Set of available RILs populations. Montpellier: populations developed by Prosperi *et al.*, Rennes: populations developed by Baranger *et al.*, Toulouse - Tunis: populations developed by Huguet *et al.* and Aouani *et al.* Dashed arrows: in progress, dark arrows: F_5 generation or more.
B. Congruency of QTLs positions and subsequent inference of candidate genes from populations using sequenced Jemalong-A17 line as parental line.

Tools developed to reveal genome conservation between legumes

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Comparative genomics is important for plant breeding because most cultivated species have large and/or polyploid genomes, making them difficult to study at genetic level. For some, such as groundnut, this is confounded by low levels of polymorphism due to a genetic bottleneck at the time of domestication. Model plants, for experimental convenience, can provide information for crops quickly and cheaply. Comparative genomics can predict gene order in large segments of a genome if collinearity is demonstrated. *Arabidopsis thaliana* is a model for many aspects of plant biology, but *Medicago truncatula* and *Lotus japonicus* serve as models for legumes that share other attributes (such as root symbiosis) and they also serve as structural genomic models.

Comparative genetic mapping should use orthologous DNA sequences. Such these are coding regions which have not undergone genomic rearrangement and are under conservative selection maintaining function and a high degree of sequence identity. Orthologous sequences can be detected (i) by cross-species genetic mapping or (ii) by computational methods. (i) In several legume studies, intron-targeted (IT) PCR-based genetic markers were used to map orthologous genes (1, 3 and Seres *et al.* in prep.). IT markers have primer pairs complementary to exon sequences which flank one or more introns. Using conserved exonic sequences guarantees that the same genes, or the members of a same gene family are amplified, and the amplification of less conserved intron sequences assures a high degree of polymorphism. Primer design is a crucial consideration and generally it is carried out as follows:

The template sequences for IT primers are cDNA, EST and tentative consensus (TC) sequences of single and low copy number genes from several species. Their copy number is generally determined by forming homology clusters; sequences with few homolog members are selected for further analysis. Using computer programs the consensus sequences are aligned to genomic sequences to identify introns. If there is no legume genomic sequence available, then *Arabidopsis thaliana* can be used because, in most cases, the exon-intron structure of homologous genes is conserved. PCR primers designed to the exons flanking introns are used to generate genetic markers in different species. Their map positions are analyzed to reveal collinear genomic segments. IT primers have been generated by this approach in a comparative mapping program of the Grain Legumes Integrated Project (GLIP; funded by the EU) to analyse syntenic relationship between grain legumes (2 and Seres *et al.* in prep.).

As more genomic sequence becomes available for diverse species, computer-based methods (ii) are also used to compare content and order of genes between species. Sequence alignment identifies homologous positions and the content and proximity of homologs can define orthologous genomic regions. ■

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Functional genomics in legumes: reversing the grain drain

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For many years, generating phenotypic variation in crops has relied on induced mutations, but producing novel variation to feed into breeding programmes is becoming more and more difficult. In the post-genomics era, as we are forced to move away from empirical approaches, understanding how genes work (functional genomics) has become paramount to future crop improvement. Leading the way for legumes has been development of reverse genetics at the whole genome level in both of the adopted legume models.

In conventional mutagenesis programmes, breeders look for particular traits or phenotypes in a population of mutagenised plants (so called 'forward genetics'), but in 'reverse genetics' one can target particular genes and obtain mutants for them. Since all plant species are susceptible to mutagenesis this allows knowledge about genes from models to be transferred directly to crops. In this process of understanding gene function, one particular method has been extremely helpful since it involves non-GM technology, and that method is TILLING (Targeting Induced Local Lesions IN Genomes). TILLING relies on chemically induced mutations that can be identified by comparing the mutant sequence to that of the wild type. Since the researcher has identified their gene of interest, this is relatively straightforward (see insert). By screening a population of several thousand mutagenised plants one can normally identify a spectrum of mutations in one's gene of interest. The high throughput nature of the methods means that such technologies can be offered as services for the research community (e.g. http://revgenuk.jic.ac.uk). There are populations of plants available for the models *M. truncatula* (1) and *L. japonicus* (2) (Figure 1) and now for a number for legume crops as well (3). TILLING has been used to look at the function of many genes to date, especially those involved in symbioses (4), and provides a suite of mutant alleles particularly useful in structure-function studies. The disadvantage of TILLING is that it produces a series of alleles, of which roughly 5% will give a complete loss of gene function, so one may not be able to unmask the true function of the gene.

Other forms of mutagenesis have been used in the models to develop reverse genetics platforms (3), but perhaps the one proving to be most effective is insertional mutagenesis whereby a small piece of DNA (from a bacterium or transposable element) is inserted into a gene to disrupt it and generate null alleles. Although TILLING is available in *M. truncatula*, insertional mutagenesis has also proved very valuable in this model. Researchers have been able to use a retrotransposon from tobacco to disrupt gene function efficiently (5) so that a large population of mutagenised plants could be generated for community screening (3). Other forms of reverse genetics such as post-transcriptional gene silencing (RNA interference; RNAi) or virus-induced gene silencing (VIGS) have been used successfully in both models (6, 7), but are not suitable to high throughput methodologies and whole genome analyses (8). A major disadvantage for manipulating crops in the current climate using insertional mutagenesis is that it involves genetic modification. All plants are susceptible to chemical mutagenesis, however, so now we have a choice to transfer knowledge gained about gene function from models to legume crops by either GM or non-GM technologies. ■

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TILLING requires a population of mutagenised plants from which DNA is extracted and seed is stored. Specific primers are designed against the target sequence and each carries a fluorochrome required for DNA sequencers. PCR products from a population of mutant DNAs and DNA from the wild type are mixed, heated and re-annealed to form duplexes. Those from one WT and one mutant strand form a mismatch because of the mutant sequence and this mismatch can be recognised by a particular cleavage enzyme known as CEL1. Treating the PCR mix with CEL1 produces labelled products of reciprocal sizes that add up to the original size of the target sequence. The cleavage products can be separated on a DNA sequencer and the mutation identified, which can then be tracked back to an individual mutant plant.

Figure 1. A M_2 population of *Lotus japonicus* plants. These individual plants will provide the seed families from which mutant plants for target genes will be isolated by TILLING.

Insertion mutant collections as genetic tools in model legumes

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Insertional mutagenesis allows mutagenesis of an organism using a known DNA element (such as a transposon) that is inserted randomly in the genome of this organism. It is a powerful genetic tool that has been used in several organisms to disrupt and tag genes. Having insertion mutant collections allows the easy identification of the tagged genes and consequently the elucidation of their functions in the organism being studied. In plants, insertional mutagenesis using either T-DNA, transposons or retrotransposons, in *Arabidopsis*, rice, maize and *Antirrhinum*, has been very helpful for identifying developmental mutants and the associated molecular functions. These collections can also be used as reverse genetic tools in order to obtain mutants in any gene under study for which the sequence is known (3).

In legume plants, mutagenesis programs were first developed in order to decipher the complex interaction with rhizobia. These mutant collections are now used to understand other aspects of legume biology. TILLING and De-TILLING platforms for legumes were established for *Medicago truncatula*, *Lotus japonicus*, *Pisum sativum* and soybean using Ethylmethane Sulfonate (EMS) mutagenesis, fast neutron bombardment and gamma rays (see Wang T. in this issue).

Insertional mutagenesis has been used in both *M. truncatula* and *L. japonicus* to isolate mutants affected in symbiosis or development. T-DNA and transposon tagging using Ac/Ds elements allowed the isolation of a limited number of mutants (2, 3). However, the *copia*-like retrotransposon from tobacco *Tnt1* has been used successfully in *M. truncatula* and generated a plethora of insertion mutants (1, 6, 7, 10, 11 and figure 1). *Tnt1* transposes only during tissue culture with insertions preferentially in transcribed regions of the genome (4, 9). *Tnt1* can be used for both forward and reverse genetic screens in *M. truncatula* and an international consortium has permitted the generation of 15,000 independent *Tnt1* lines which are estimated to contain approximately 375,000 inserts (10, http://bioinfo4.noble.org/mutant/). In addition, endogenous active retroelements have been recently identified in *M. truncatula* (*MERE1*) (8) and *L. japonicus* (*LORE*) (5). In *Medicago*, *MERE1* is a *copia* type retroelement that transposes only during tissue culture and which can be used as a complementary genetic tool to the already existing mutant collections.

We expect that the growing amount of information and discoveries made using insertional mutagenesis in model legumes should be transferable to other legumes species where genetic and genomic approaches are not easily achievable if not impossible. ■

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Figure 1. A *Medicago truncatula Tnt1* tagged *Stamina pistilloidia* mutant.
The flower in this mutant develops normal first whorl organs (sepals) but it is indeterminate in the second whorl; instead of developing petals a proliferation of new flower meristems appears. These new meristems develop into flowers which follow the same pattern as the primary flower. As a result, the flower is mainly composed of a proliferation of sepals. (P. Ratet's laboratory unpublished)

Tools developed for model legumes - Transcriptomics

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Transcriptomics is a modern technology of experimental genomics that allows to monitor the expression of thousands of genes in parallel in a single experiment, ideally resulting in genome-wide snapshots of gene expression. Traditionally, transcriptomics platforms rely on microarrays that carry gene-specific probes. These microarrays are queried using fluorescence-labeled transcript sequences from tissues of interest, and the recorded fluorescence per probe is proportional to the expression of the corresponding gene.

The development of widely available transcriptomics tools for model legumes dates back to the "First International Conference on Legume Genomics and Genetics: Translation to Crop Improvement" in Minneapolis-St. Paul/USA, held in the year 2002. During this conference, a group of European and US researchers decided to order a set of 16,000 70mer oligonucleotides from a commercial facility, at that time Qiagen-Operon, to set up a community microarray for the model legume *Medicago truncatula* (5). These oligos were targeted at the complete expressed genome available from the DFCI *Medicago truncatula* Gene Index. Over the years, several thousand oligonucleotide microarrays were printed in the US in the frame of an NSF project and at the Center for Biotechnology, Bielefeld University. Microarray production and access in Europe from the beginning was organized in the frame of several European collaborative projects, building on networks established for the earlier development of cDNA arrays (6). Data integration and data availability developed into a key issue in the forthcoming years, and web-based platforms such as EMMA (2) and Truncatulix (3) were set up to allow data mining.

In recent years, legume transcriptomics tools were upgraded towards a more genome-wide scope, with GeneChips being current working horses both for the model legume *M. truncatula* and also for soybean. In case of the model legume *Lotus japonicus*, efforts to construct transcriptomics tools also relate to the development of GeneChips, although these are not available from the Affymetrix community programme. Efforts at the Samuel Roberts Noble foundation led to the development of a *Medicago* Gene Expression Atlas, a database of expression profiles that turned out to be a popular and easy-to-use tool for mining *M. truncatula* transcriptomics data (1). Parallel to microarrays and GeneChips, a real-time RT-PCR platform has been designed for *M. truncatula*. This platform ultimately aims at targeting all transcription factor genes and provides an unprecedented sensitivity as well as specificity in monitoring the expression of key regulators during legume development (4).

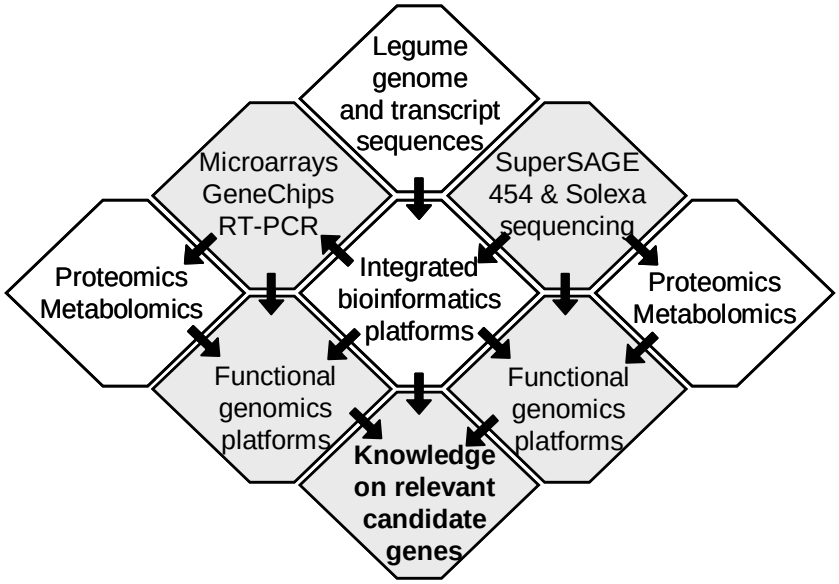

Figure 1. Legume gene identification modules. Relevant experimental techniques and their interdependencies are depicted with pathways related to transcriptomics shown in grey. Integrated via bioinformatic platforms, knowledge on candidate genes relevant for particular conditions of legume biology can be derived.

Whereas array and RT-PCR approaches represent closed platforms relying on known sequence information, techniques such as serial analysis of gene expression (SAGE) are now available that are able to decipher legume transcriptomes without prior sequence knowledge (7). This approach, together with the high-throughput sequencing of conventional cDNAs, benefits from the current revolution in ultrafast DNA sequencing. Here, technologies such as 454- and Solexa-sequencing yield up to several million reads in a few days, providing the opportunity to characterize the genome-wide transcriptome of any legume without the need for an available genome sequence.

Together, and combined with proteome and metabolome data via integrated bioinformatics platforms, transcriptomics has the potential to deliver sets of candidate genes for functional studies (Figure 1), making use of legume mutant collections. ■

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Proteomics in model legume species: a case study in seeds

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In the 1990s, significant advances in proteomics, including the refinement of two-dimensional gel electrophoresis (2-DE) and the development of sensitive mass spectrometry methods for protein identification, were made. In the last decade, major genomic resources have become available for *Glycine max* (soybean), *Lotus japonicus* (japanese trefoil) and *Medicago truncatula* (barrel medic), thus allowing the proteomics technology to be successfully applied to legume plants.

A detailed analysis of the proteins present in various tissues at different developmental stages (leaf senescence, root symbiosis, seed development and germination) has been performed for several legume species (e.g. *G. max*, *Pisum sativum* and *M. truncatula*). 2-DE maps are now available for these tissues (1) and can be used in two different ways. The one aims at identifying proteins whose level of accumulation changes during specific stages of development. These proteins form the molecular signature of the corresponding tissue at a particular stage. Their function can be further validated by means of mutants for the corresponding genes. This approach contributes to our understanding of how metabolic networks are regulated at the protein level during development. The second concentrates on the analysis of the protein complement of the tissue at a given stage. In particular, this approach aims at identifying proteins that were subjected to post-translational modifications. To illustrate the information gained by proteomics in the legume biology field we will take as an example the dissection of seed protein composition, in which we have been particularly involved.

Proteomics to investigate processes controlling seed protein accumulation

Most legume seeds are large, rich in protein (from 20% to as much as 40%), and thereby used as protein sources for human food and animal feed. The protein composition (i.e the proteome) of the seed varies according to environmental conditions and genetic constitution. Such changes often modify the user value of seeds. By allowing a dissection of seed protein composition and of the establishment of the different protein fractions during seed development on the mother plant, proteomics is expected to accelerate discoveries of the genetic and molecular determinisms of seed protein accumulation. Such discoveries will help to improve seed quality and/or to stabilize it in a fluctuating environment.

2-DE maps have been assembled for seeds of several model legumes (*M. truncatula, L. japonicus)* and crops (*P. sativum*, *G. max*, *Lupinus albus* L.*)* (2-5). For example, the *P. sativum* and *M. truncatula* seed proteome maps comprise 156 and 224 identified proteins, respectively. These maps are available at http://www.inra.fr/legumbase/peaseedmap/ and : http://www.inra.fr/legumbase/medicago-seed-proteome/

A cross-comparison of the 2-DE seed maps for *P. sativum* and its closely related model species *M. truncatula* revealed similarities in storage protein accumulation/deposition. The most abundant protein spots were detected between 30 and 80 kDa. They correspond to the principal storage proteins, namely the 7S (vicilin, convicilin) and 11S (legumin) globulins. The Figure 1 shows a 2-DE reference map for mature *M. truncatula* seeds and the reconstruction of post-translational modifications leading to the deposition of mature vicilins. Unlike legumes, the seed proteome of Arabidopsis (an oilseed *Brassicacea*), adopted as the universal model system for plant biologists, is mainly composed of 12S (cruciferin) globulins of molecular weight ranging between 16 and 35 kDa (6). The differences in storage protein composition between legume species and Arabidopsis reinforce the usefulness of developing a legume-specific model to investigate processes controlling seed protein accumulation in these plants.

Proteomics to investigate the genetic determinism of seed composition

Genetic variations can be detected by the position of proteins in 2-DE gels, as influenced by sequence polymorphism, and/or by the variation in amount of a given protein in different genotypes (genetically determined quantitative variations). A proteomic study in this area dealing with genetic variability for seed protein content and composition in the model legume plant *M. truncatula* was performed. Fifty lines of *M. truncatula*, derived from ecotypes or cultivars of diverse geographical origin, were grown under uniform conditions, and variation in seed protein composition and quantity was investigated (7). Lines contrasting for qualitative traits and seed protein content were identified that could be used to derive mapping populations and characterize PQLs (protein quantity loci) that explain part of the variability in spot intensity (8, 9). This powerful approach combining plant proteomics and plant genetics can help us elucidate the genetic control of seed protein composition, which can be exploited for legume crop improvement. ■

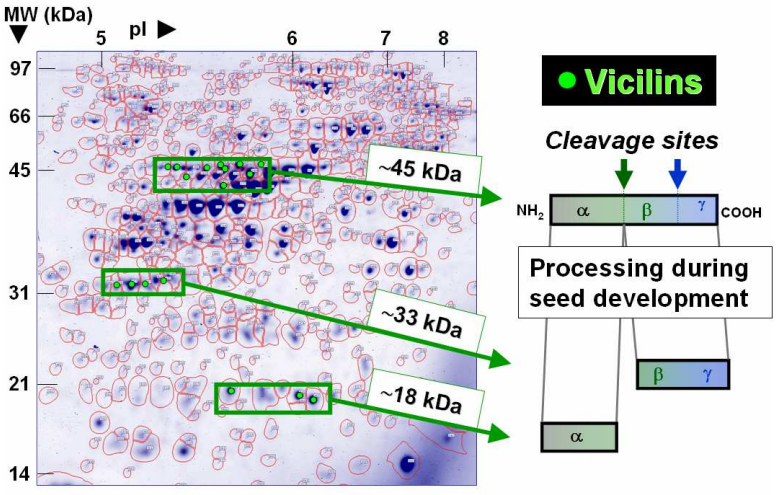

Figure 1. 2-DE gel of total soluble proteins from dry mature seeds of *M. truncatula* showing the different polypeptides constituting the mature vicilin.

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Legume metabolomics

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It is estimated that plants contain approximately 10,000 to 15,000 metabolites per species and over 200,000 different metabolites across all plants species. Over half still remain chemically uncharacterized. The key difference between metabolites, proteins, and DNA is the relatively smaller molecular size of metabolites. Primary metabolites serve as the basic building blocks of life and are important energy sources. For example, living organisms utilize amino acids to construct proteins and enzymes (proteins that catalyze specific chemical reactions). Fats and carbohydrates, also known as sugars, serve as key energy sources important for growth and survival.

Plants also contain a diversity of secondary metabolites which are not critical for sustaining life, but provide a competitive advantage for the plants in their environment. These compounds function as specialized chemical defense agents and/or as important chemical signals. Examples of antimicrobial (i.e. chemicals that are deterrents to bacterial and/or fungal infection) and antiherbivory compounds (deterrents to animals or insects eating the plants) include alkaloids such as nicotine and caffeine. Chemical attractants such as volatiles emitted by flowers to attract pollinators or compounds secreted into the soil which are recognized by Rhizobia during root nodulation are examples of beneficial signaling molecules.

Metabolomics is the large-scale qualitative (i.e. the chemical identification/naming of each metabolite) and quantitative (i.e. measuring the quantity of metabolites present) profiling of primary and secondary metabolites that provides a high resolution biochemical phenotype that helps scientists better understand the physiological and biochemical status of plants. Much like a physician samples blood to profile glucose as an indicator of health or disease, metabolomics is used to profile hundreds to thousands of metabolites to better understand health and disease in plants.

Metabolomics is proving to be a novel tool for understanding metabolism and even more so when integrated with other 'omics' technologies that profile large numbers of proteins (proteomics) and mRNA (transcriptomics). Currently, metabolomics is being used to identify novel metabolites and specific genes involved in their biosynthesis (1) in the model legume *Medicago truncatula*. This technique is identifying differential chemical defense response mechanisms in response to herbivores and fungi (2, 4). Metabolomics has been used to describe drought tolerance mechanisms in transgenic alfalfa (7) and clover (3). It is revealing insights into salt tolerance in the model legume *Lotus japonicus* (5, 6). Metabolomics is being used to study the specialized biochemistry of specific plant organs such as root hairs and border cells during nodulation, the biochemical composition of pollen, and the biosynthetic capacity and chemical content of leaf trichomes.

In summary, metabolomics is one of the youngest 'omics' tools. However, it is definitely proving to be a valuable tool in current plant biotech. It is certain that metabolomics will continue to serve as a key discovery and descriptive technology well into the future. ■

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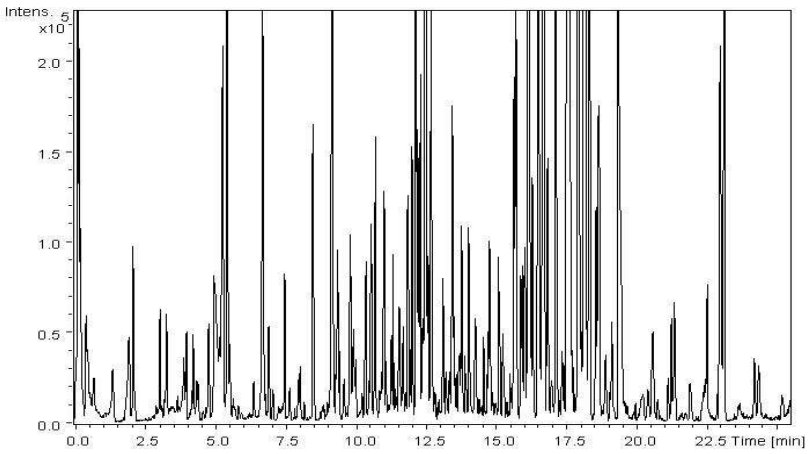

Figure 1. Metabolomics uses series of sophisticated instrumentation, such as ultra high pressure liquid chromatography coupled to mass spectrometry (UHPLC-MS), to profile complex mixtures of metabolites. A profile is provided for a representative legume extract. Each peak represents a least one unique metabolite and the height of each peak is representative of its relative abundance in the complex mixture. The cumulative metabolite profiling data represents a high resolution biochemical phenotype extremely useful in understanding fundamental biochemical responses to genetic engineering, biotic stress, abiotic stress, symbiosis, and plant disease/defense.

Tools developed for model legumes - Ecophysiology

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Ecophysiology aims at analysing and modelling the relationships existing between plants and their environment. The environmental factors can be abiotic (solar radiation, temperature, water, mineral nutrients) or biotic (pests, diseases, beneficial or detrimental micro-organisms) and they can also include the impact of agricultural practices. Plant growth is characterised by a set of different major "integrated functions" such as water or nutrient (mainly carbon and nitrogen but also phosphorus and sulfur) uptake, water, nutrient or metabolite allocation within the plant, seed reserve formation, etc. The relative importance and/or focus associated to each of these functions depend widely on the species and upon the issue addressed. From a spatial scale point of view, the organ level is preferentially targeted, but it can be narrowed down to the cell level or widened to the plant or crop level. From a time scale point of view, the analysis accounts for dynamic patterns, with a daily to weekly time interval, across specific growth periods or during the entire growth cycle. The ecophysiological analysis involves identifying, for a specific plant function, the meta-mechanisms determining the plant's response to environment through response curves to either environmental factors or plant signals (6). As such, ecophysiological models reveal emerging properties at the whole plant level resulting from the interaction between processes either within the plant or with the environment.

In legumes, the yield and quality of the harvested seeds not only depend on carbon uptake by photosynthesis but also on nitrogen uptake through the legume-Rhizobia symbiosis, which is strongly dependent on soil nitrate availability. In our team, the ecophysiological analysis is therefore focused on the characterisation of the interacting carbon and nitrogen fluxes within the plant, from the uptake of nutrient in the environment, to their accumulation in the finally harvested seed. Pea has been and remains our first model plant (4) as it has been the major legume crop in Western Europe from the 1980's onward. Since 2000 our knowledge is being transferred to *Medicago truncatula* because of its evident interest as a model plant for the genomic community. As such, ecophysiological tools were first developed to characterise *M. trunctula* shoot vegetative and reproductive development as a function of air temperature and vernalisation (1, 2). The analysis of carbon and nitrogen fluxes within the plant provided an evaluation of the *Medicago-Sinorhizobium* symbiosis performance according to light and nitrate conditions (3). These tools are of importance in standardizing and monitoring expected growth conditions in the 'omics' studies. In particular, it has been shown that the environmental conditions (in terms of light and nitrate) under which the *Medicago-Sinorhizobium* symbiosis is usually grown can result in N deficiency (3). Moreover, ecophysiological models provide a framework analysis to break up complex variables, such as yield, into intermediate variables representing physiological processes (such as nitrogen uptake as a function of nodule biomass, carbon uptake as a function of leaf area and light) (3, 5, 7). As such, our models are useful tools for quantitative genetics and plant breeding. A genotype can therefore be defined as a specific combination of model parameters of mathematical functions representing either specific meta-mechanisms or response curves to the environment. Detection of QTLs can be conducted on these model parameters, thus implicitly accounting for the genotype–environment interaction. Finally, ecophysiological modelling by accounting for interactions between processes and with the environment allows identifying key processes in the crop N nitrogen performance, building *in silico* ideotypes and thus identifying essential combinations of traits for breeding. ■

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The rhizobium-legume nitrogen-fixing symbiosis

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Beneficial from all points of view

The rhizobium-legume symbiosis is established between certain nitrogen-fixing soil bacteria collectively called rhizobia, and plants of the legume family (*Fabaceae*). Recognition between partners leads to nodulation – the development of root nodules, infected with rhizobia. In these structures the rhizobia fix atmospheric nitrogen in return for a carbon source derived from photosynthesis. As a result, plant growth is independent of a mineral nitrogen source in the soil, which explains why legume plants are often among the first to colonise poor soils and why legume grain, forage and fodder crops can be grown with no added nitrogen fertiliser. It has been estimated that, world-wide, biological nitrogen-fixation in agricultural systems alone fixes 40-60 million tonnes of nitrogen annually, compared to the 100 million tonnes of nitrogen fertiliser that is chemically synthesised (3). Considering that the production and transport of nitrogen fertilisers account for a substantial part of the crops' total energy inputs and for 1-2% of the world's annual energy consumption, the culture of legume plants is clearly economically and environmentally advantageous. Also, although much of the nitrogen fixed by legume crops is harvested for feed or fodder, the remainder becomes available in the soil for subsequent plants grown in rotation. A wider use of legumes together with increased legume crop productivity and quality under low fertiliser conditions are obvious steps in the necessary transition to sustainable agricultural systems. In this regard, improving symbiotic nitrogen fixation is an important target for research. Recent work on model legumes is unravelling the mechanisms leading to symbiosis and should lead to new strategies to make symbiotic nitrogen fixation more efficient.

Rhizobium-legume recognition involves chemical signalling

Genome sequencing has revealed that rhizobia are a collection of largely unrelated bacterial species that have acquired the potential to nodulate legumes and to fix nitrogen, through the acquisition of specific genes, called the *nod* and *nif* genes (4). Many rhizobia can only nodulate a few legume species whereas others have a much broader host range. Genetic studies on rhizobia and model legumes have established that the ability of rhizobia to be recognised and to nodulate a legume is governed by chemical signalling between the partners. The key signals for most plant-rhizobium couples are Nod factors, lipo-chito-oligosaccharidic molecules (LCOs) produced by rhizobia in response to legume root exudates. Differences in Nod factor structure are largely responsible for host specificity, although surface or secreted polysaccharides are also bacterial components necessary for infection, possibly as suppressors of the plant defence system. In the past few years, genetic studies largely on model legumes have identified key components of Nod factor perception and signal transduction (5). These include a certain type of receptors (LysM-RLKs) involved in recognising Nod factors, which are related to receptors involved in defence responses to chito-oligosaccharides produced by pathogenic fungi. How legumes discriminate between pathogens and symbionts using similar receptors is currently under study. Remarkably, several genes acting just downstream of Nod factor receptors also control establishment of the symbiosis with endomycorrhizal fungi, indicating that the rhizobium-legume symbiosis has evolved from this ancient and widespread symbiosis.

Hundreds of genes are involved in nodule development and function

When rhizobia infect their host plants they are kept separated from the plant cell by a membrane, a fact that may be important for maintaining the symbiotic relationship. In the nodule the bacteria differentiate into forms, called bacteroids, that express only a sub-set of genes, mostly those involved in nitrogen fixation and adaptation to the plant micro-environment (1). These include the *nif* genes leading to activity of the nitrogen-fixing nitrogenase enzyme that has a high energy requirement and sensitivity to oxygen. Legumes have evolved mechanisms to accommodate these properties through the structural and biochemical characteristics of the nodules.

Nodules are highly differentiated organs, organised into zones with different functions. There is considerable variation in their size and structure among legumes, but the two major model species, *Medicago truncatula* and *Lotus japonicus*, are representatives of legumes forming the two main nodule types. Transcriptomic and proteomic studies have identified hundreds of genes that are activated (or repressed) during nodulation and which are associated to different developmental stages and to different zones of the nodule. Plant genes which are essentially expressed in nodules are called nodulin genes. They often represent special-

Positive impact of rhizobium-inoculation on soybean growth in the absence of nitrogen fertiliser (George Sommer/INRA)

ized members of multi-gene families and are involved in a variety of processes including cell division and differentiation, infection, signalling, defence responses and metabolic exchange.
In a mature nodule, essential membrane transporters are involved in the efficient exchange of carbon to the bacteroids and fixed-nitrogen to the plant. Then, nitrogen-rich amides and ureides are synthesized for transport to the shoot. Genetic and physiological studies have shown that the plant and bacterial metabolisms are intimately coordinated at the nodule level and are also regulated by the photosynthetic activity of the shoot and the developmental stage of the plant.

Nodulation, a highly regulated process

Nodulation is very sensitive to environmental conditions, such as soil acidity, salt, heavy metal, drought and biotic factors such as pest and pathogen attack. Nodule formation is inhibited by the presence of mineral nitrogen sources in the soil and is further controlled by endogenous plant processes. In this way the plant controls its investment in the energy-demanding processes of nodulation and nitrogen fixation, according to its needs. Recent genetic studies have shown that a mechanism of auto-regulation of nodule number also mediates N-inhibition of nodulation and involves systemic signalling between the root and the shoot. A key component is a shoot LRR-RLK receptor that is involved in detecting a signal sent from the root and then sends back a signal to the root to inhibit nodulation. Although not all the details of this regulation are known, plant hormones could be involved as mobile signalling components. More generally plant hormones are important mediators of nodulation and infection, with either positive (cytokinin and auxin) or negative (ethylene, abscisic acid and methyl jasmonate) roles.
In the nodule, further controls operate on the symbiosis. The plant has been shown to control the differentiation of rhizobia into bacteroids and has evolved mechanisms to sanction bacteria that do not efficiently fix nitrogen. Despite recent progress, many of the key components underlying these mechanisms remain to be identified.

Future challenges and applications

Nitrogen fixation in the field is notoriously variable, depending on the bacterial strain, the plant genotype and the environmental conditions. Rhizobial inoculants often improve nodulation, but their effectiveness can be limited by the presence in the soil of competing, less-efficient rhizobia and by residual nitrogen. Generally legumes have not been bred with symbiotic efficiency in mind. There is thus considerable potential for genetic improvement in both partners and in identifying efficient partner combinations (2).
The discovery of Nod factors has already led to a useful application, since rhizobial inoculants enriched in the appropriate Nod factors have been used since 2005 on over a million hectares of agricultural land to improve yield of major crops such as soybean, peanut and alfalfa. Better knowledge of Nod factor signalling and of the mechanisms regulating nodule formation and activity could be further exploited to favour nodulation by specific rhizobial strains, improved for symbiotic efficiency in different environmental conditions. The relationship between nodulation and mycorrhization could also be exploited to produce mixed inoculants to improve phosphorus and other nutrient uptake in addition to nitrogen nutrition. Transcriptomics and proteomics approaches on model legumes and their symbionts have revealed the complexities of the gene networks leading to an effective symbiosis. A major challenge is to identify those genes that could lead to an improvement in symbiotic efficiency: the development of functional genomics platforms and efficient ecophysiology and phenotyping methods in model legumes are providing methods in which this can be done. In addition, the exploration of natural variation, greatly facilitated by next generation sequencing technologies, represents a promising strategy, notably for identifying genes involved in the adaptation of symbiosis to various environments. ■

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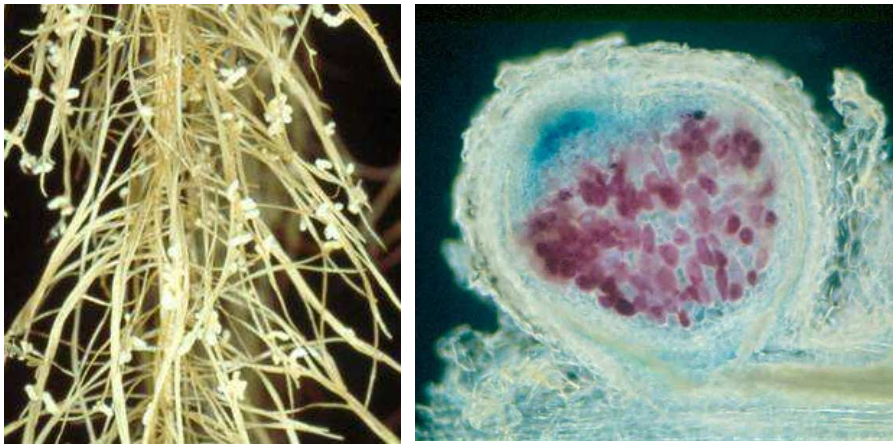

Nodulation of the model legume *Medicago truncatula* with a nodule section (right) showing rhizobia coloured in red and the expression of a Nod factor receptor gene in blue.

The contribution of model legumes to arbuscular mycorrhiza research

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The large majority of cultivated and wild plant species harbour arbuscular mycorrhiza (mükes=fungus, rhiza=root) in their root systems. Legumes are no exception. These fungi, belonging to the phylum Glomeromycota, exist in symbiosis with vascular plants in most terrestrial ecosystems throughout the world. Apart from species of *Lupinus*, all genera in the Fabaceae benefit from arbuscular mycorrhiza, which enhance nitrogen fixation and primary productivity by providing plants principally with a means of better soil exploitation for nutrients (particularly Pi). In this context, arbuscular mycorrhiza research initially focussed on crop legumes because of their economic impact. Soybean and clover were early models for physiological studies of mycorrhiza-legume interactions, followed by pea with the first discovery in the 1980s of mycorrhiza-deficient genotypes in a non-nodulating mutant background. However, these crop legumes have large genomes and it was necessary to wait for the implementation in the 1990's of the model legumes *Medicago truncatula* and *Lotus japonicus*, which have small genomes and are amenable to forward and reverse genetic analyses, for large scale research into plant genetic programmes regulating the mycorrhizal symbiosis. Although the picture is still fragmentary, the identification of simultaneous mycorrhiza and nodulation mutants also in *M. truncatula* and *L. japonicus* has confirmed that genetic programmes driving the two root symbioses partially overlap (2, 4, 7). The arbuscular mycorrhiza symbiosis appeared in terrestrial plants over 400 million years ago, well before the hypothesized origin of nodulation in legumes (60-70 million years ago). This, together with the existence of some shared genetical, transcriptional and ultra-cytological features between the two root symbioses in legumes, has repeatedly led to the suggestion that part of the plant cell programme accommodating nitrogen-fixing bacteria in nodules was recruited from pre-existing functions in arbuscular mycorrhiza.

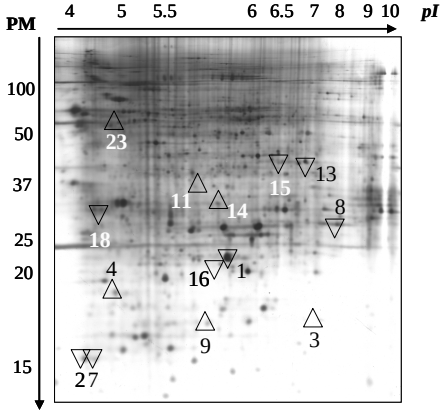

Figure 1. Two dimensional gel electrophoresis of differentially accumulated proteins in membrane enriched fractions (microsomes) of mycorrhizal compared to non-mycorrhizal roots of *Medicago truncatula* : D and Ñ indicate up- and down-accumulated proteins (Abdallah, Recorbet & Dumas-Gaudot, unpublished data).

Although arbuscular mycorrhiza are non-specific and legumes can associate with a broad array of Glomeromycota species, the processes of root colonization and symbiosis development are conserved between different plant species. Schematically, a hyphae of the fungal symbiont coming from a spore or a mycelium network in the soil differentiates an appressorium at the root epidermis, from which a penetration hyphae enters the root. Once inside the root, intercellular hyphae grow into the inner cortex where the fungus forms highly branched structures, called arbuscules, within root cortical cells. The formation of arbuscules induces transcriptional and metabolic activation of host cell contents which undergo rearrangement with the proliferation of organelles and membrane systems. This culminates in the formation of an extensive symbiotic interface between plant and fungal cells, assumed to be the site of nutrient exchange between the two symbionts.

Screening of mutant collections, principally in the model legumes *M. truncatula*, *L. japonicus* and pea, has been instrumental in the identification of distinct plant loci which are required for different steps of mycorrhiza establishment. At present, three homologous genes required for early steps of root penetration by a mycorrhizal fungus have been cloned and a predicted function identified in *L. japonicus* (*LjSYMRK, LjPOLLUX, LjCCaMK, LjSYM15*), *M. truncatula* (*MtDMI1, MtSYM2/DMI2, MtSYM13/DMI3*) and pea (*PsSYM19, PsSYM8, PsSYM9*), and an additional three have been identified in *L. japonicus* (*LjCASTOR, LjNUP85, LjNUP133*). They encode proteins that are directly or indirectly involved in signal transduction networks necessary for mycorrhiza establishment since their inactivation gives a phenotype in which fungal development is limited to the outer epidermal or exodermal cell layers of roots. Their function argues for a role of calcium-sensing in the perception of fungal signals ('Myc' factors) during early interactions between roots and arbuscular mycorrhizal fungi. However, not all plant mutants have stable phenotypes and variations between mutated alleles for mycorrhiza development have been reported for some of the *L. japonicus* and *M. truncatula* genes (4, 7). More infrequently described are mutants which allow colonization of the inner root cortex but where arbuscule formation is defective (*Ljcyclops*, *Pssym36*), rate of arbuscule turnover/senescence is altered (*Ljmcbco*, *Pssym33, Pssym40*) or mycorrhiza colonization is increased (*sunn*) (2, 4, 7). In most cases, the mutated gene, or the function of the encoded protein, has not yet been identified.

Over the past few years, studies mainly in *M. truncatula* and *L. japonicus* have identified a broad number of plant gene categories that are transcriptionally modulated during the development of arbuscular mycorrhiza, giving clues as to how cell functions may be regulated during interactions. The most comprehensive data so far are related to plant-derived reprogramming for compatibility between mycorrhizal partners. They include nodulin-related gene activation, attenuated or localized defence responses with root penetration or intracellular arbuscule formation, and membrane intrinsic transporters essential for phosphate transfer at, and maintenance of, the symbiotic interface (3, 4, 7). Mycorrhiza-defective mutants of model legumes are valuable tools for pinpointing plant and fungal cell programmes involved in symbiotic mycorrhiza interactions. Research has mainly focussed on plant mutants affecting the initial events leading to root penetration where inactivation of the corresponding plant genes interferes with signal-perception responses to the symbiotic fungi and extinguishes plant

stimulatory effects on fungal gene activity (4, 5, 7). By introducing mycorrhiza-defective *M. truncatula* and *L. japonicus* mutants into large-scale gene expression studies, based on tools issuing from transcriptome (3, 4) and genome sequencing (www.medicago.org/genome/; www.kazusa.or.jp/lotus/; www.affymetrix.com/support/technical/datasheets/medicago_datasheet.pdf), it will be possible to further unravel the role of plant genes in signal exchange pathways within the symbiosis, propose maps of metabolic circuits which drive the symbiotic interactions and define those which specifically respond to symbiotic stimuli. The availability of genomic databases and plant mutants are also providing opportunities for large-scale profiling of the mycorrhiza-related proteome, ranging from wide display proteomics to more focussed characterization of subcellular membrane compartments (6, Figure 1). Research input based on these different strategies to *M. truncatula* has, for example, identified several appressoria-responsive proteins belonging to categories involved in recognition and defence responses, as well as membrane proteins related to the functioning of the established mycorrhizal symbiosis (4, 6).

Legumes have also been instrumental in research on the ability of arbuscular mycorrhiza to confer additional benefits other than phosphate acquisition to plants, including resistance or tolerance to biotic and abiotic stresses. For instance, using pea as a model system, it has been shown that arbuscule development is necessary for the mycorrhizal symbiosis to reduce root infection and damage by the destructive pathogen *Aphanomyces euteiches*, for which no highly resistant cultivars or efficient control methods are available (Rispail et al., this issue). A link between the presence of arbuscules, mycorrhiza-related chitinase and chitosanase isoforms and bioprotection has suggested a possible role of these proteins in this phenomenon. In this context of bioprotection, pea and *M. truncatula* have also been exploited as model plants to investigate how arbuscular mycorrhiza impact on plant tolerance to soil pollutant stress (Figure 2). Investigations of the molecular basis of the protective effect against cadmium pollution have so far concluded that toxicity may be counteracted through the mycorrhiza-dependent synthesis of proteins involved in alleviating damage from oxidative stress thus conferring low sensitivity to the metal (1).

Model legumes will continue to provide opportunities for identifying cell circuits driving plant-fungal compatibility essential to mycorrhizal formation and functioning. They are also instrumental for evaluating similarities and evolutionary divergence between cell programmes in nodule and mycorrhiza symbioses. In addition, deeper analysis of signalling networks regulating plant defence responses to arbuscular mycorrhizal fungi will contribute to delimiting boundaries in the symbiosis-pathogenesis continuum. Furthermore, the extensive synteny of pea and alfalfa with the *M. truncatula* genome (Kaló *et al.*, this issue) makes extrapolation of on-going basic mycorrhiza research to agriculturally important crop legumes feasible. ■

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Figure 2. *Medicago truncatula* growing in a heavy metal-contaminated sand/soil substrate: NM, without mycorrhiza; M, with mycorrhiza (courtesy E. Jacquot-Plumey).

Abiotic stress: regulation of nitrogen fixation in the model legume *Medicago truncatula*

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Plants are exposed to a number of environmental stresses both under natural and agricultural conditions. In plant physiology, stress is usually defined as an external factor that exerts a disadvantageous influence on the plant. Some of the most common environmental factors causing stress in plants include water deficit, salinity, chilling and freezing, heat, and flooding. Among them, water deficit is considered one of the environmental factors more severely influencing plant growth and crop productivity. According to the "World Atlas of Desertification", 47% of the terrestrial land surface can be considered dry land, with one sixth of them are classified as hyper-arid, including deserts such as the Sahara (6). Furthermore, one out of five developing countries is estimated to suffer from water shortage for agriculture by 2030 (2).

Plant responses to water deficit are complex, involving adaptative changes and/or deleterious effects (1). The process that is most affected by water deficit is plant cell growth. The more severe the water deficit is the larger the number of cellular processes affected, including inhibition of cell division, cell wall formation and protein synthesis. As a consequence of loss of turgor pressure, growth is inhibited and a limitation in leaf expansion occurs. The second most important consequence of decreased water availability for plants is a reduction of photosynthesis due to abscisic acid-mediated stomatal closure.

Nitrogen-fixing legumes are especially sensitive to water deficit and other environmental stresses. Figure 1 shows the metabolite exchange in nitrogen-fixing temperate legumes and carbon and nitrogen metabolism in nodules. Under drought, symbiotic nitrogen fixation (SNF) is rapidly inhibited, previous to the decline in photosynthesis. A negative impact on SNF has been also shown for other abiotic stresses such as salt, low temperatures and flooding. The effects of a particular abiotic stress on SNF can occur at different steps of the symbiotic interaction: infection, nodule development and nodule functioning. Under drought, both formation of new root hairs and elongation of previously differentiated root hairs are limited and, as a consequence, the development of new plant-bacteria interactions and infection threads is greatly reduced. Although in general nitrogen-fixing bacteria are more tolerant as free-living cells than under symbiotic conditions, the success of the symbiotic system largely depends on the plant stress tolerance.

The regulation of SNF under drought stress involves a number of factors, namely, internal O_2 availability, nitrogen feedback regulation and carbon limitation, whose interactions are not fully understood. In addition, the regulation of SNF has been recently shown to depend on the plant species under study, as differential response mechanisms to drought have been found in grain and pasture legumes (Table 1).

Regulation of SNF under drought stress in grain legumes

Signalling for SNF regulation has been related to a nitrogen feedback mechanism. Several molecules have been suggested to play a role in this regulation, although so far evidences for potential candidates are indirect and speculative. To date, most of these studies have been carried out in soybean and there is scarce information about the role of these compounds in temperate legumes. Moreover, the decline of SNF under drought has been shown not to involve a systemic signalling mechanism, but a regulation at the local level, as demonstrated for both tropical and temperate legumes (4). This local regulation suggests that the search for nitrogen compounds exerting a role on the regulation of SNF should be mainly focused at root nodule level.

Another proposed regulatory mechanism is *via* control of carbon flux within nodules. Nodule-enhanced sucrose synthase, a key enzyme metabolising sucrose toward glycolysis, has been shown to be the first nodule enzyme activity that declines under drought in tropical and temperate grain legumes. This sucrose metabolisation decline leads to an accumulation of this substrate and a concomitant depletion of organic acids, mostly malate, in nodules. Therefore, a limitation of carbon supply for bacteroid respiration would be responsible for the observed decline in nitrogenase activity (4).

Regulation of SNF under drought stress in the model legume: *Medicago truncatula*

In pasture legumes such as *Medicago sativa* and *M. truncatula* the drought-induced down-regulation of nodule sucrose synthase has been shown to occur after SNF inhibition and concomitant to an accumulation of

	Grain legumes	Pasture legumes
Water potential at severe drought	-1.5 MPa	-2.5 MPa
Degree of inhibition of SNF	High	High
Degree of inhibition of photosynthesis	High	Moderate
Oxidative damage	High	Moderate
Effects on nodule metabolism:		
Sucrose	Accumulation	Accumulation
Sucrose synthase	Strong decline	Moderate decline
Malate	Strong decline	Accumulation
Most likely cause of the inhibition	Carbon limitation for bacteroid respiration	Impairment of bacteroid metabolism and nitrogenase

Table 1. Summary of the general physiological and metabolic responses to drought stress of several legumes. This overview is based on progressive drought experiments carried out under controlled conditions using soybean (*Glycine max*) and pea (*Pisum sativum*), as representatives of grain legumes, and barrel medic (*Medicago truncatula*) and alfalfa (*M. sativa*), as pasture legumes (see reference 5 for more detailed discussion). SNF, symbiotic nitrogen fixation.

malate. In a recent study, the regulation of SNF in drought-stressed *M. truncatula* plants has been analysed using a combination of physiological, metabolomic and proteomic approaches. It was then concluded that the inhibition of SNF in *M. truncatula* under drought stress appeared to be related to an impairment of bacteroid metabolism and nitrogen-fixing capacity, rather than to a limitation of carbon respiratory substrate to fuel bacteroid nitrogenase (3). In this sense, the regulation of SNF in *M. truncatula* during drought shows more similarities with other species such as *M. sativa*, suggesting a possible common regulatory response among pasture legumes. In addition to this different SNF regulation model, a number of physiological and biochemical studies strongly suggest that medics are more tolerant to drought than grain legumes. *Medicago* species are able to reach lower water potentials than grain legumes, maintaining better photosynthetic rates and plant biomass production.

A better understanding of these regulatory mechanisms will enable the optimisation of legume SNF under different abiotic stresses, positioning legumes, even more, as essential crops for a sustainable agriculture. ■

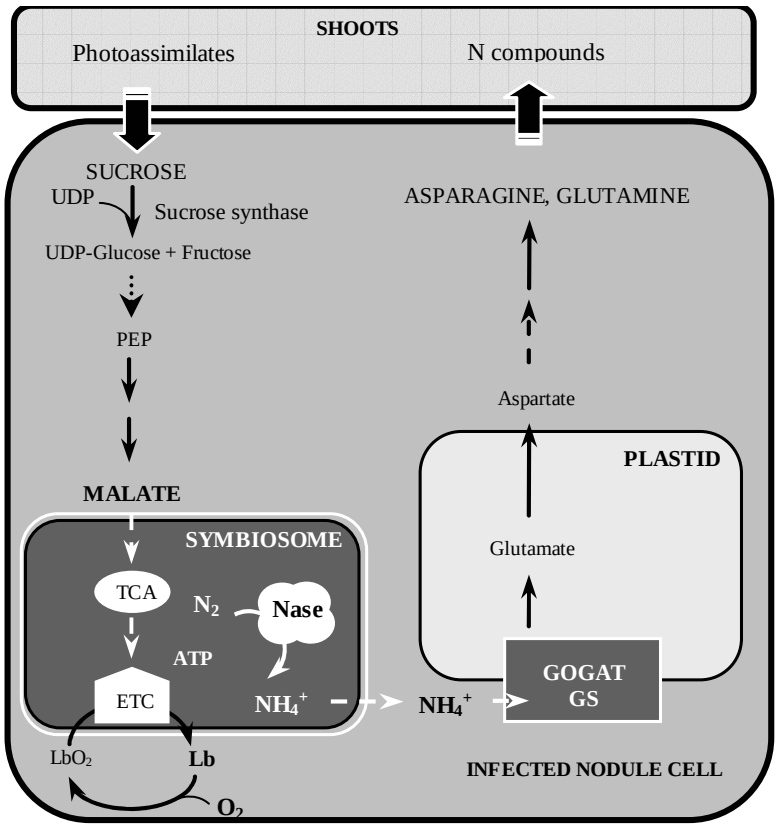

Figure 1. Simplified representation of the metabolic exchange and carbon and nitrogen metabolism in nodules in nitrogen-fixing temperate legumes. ETC, Electron Transport Chain; Lb, Leghemoglobin; Nase, Nitrogenase; OAA, Oxaloacetate; PEP, Phosphoenolpyruvate; TCA, Tricarboxylic Acid Cycle.

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Use of the model legumes for improving legume resistance to pathogens and pests

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Grain and pasture legumes such as pea and alfalfa are challenged by many pathogens and pests which strongly affect their yield worldwide. Genetic resistance is considered the most desirable control method since it is more cost effective and environment-friendly than the alternative chemical-based methods. Mining legume crop germplasm collections has identified many resistance sources in legume crops for which the corresponding genetic loci, or quantitative trait loci (QTL), have been located on genetic maps (e.g., 16). However, the large genetic distance existing in most cases between the identified genetic markers and the resistance QTLs, and the general lack of knowledge on resistance mechanisms in legumes limit greatly the use of genetic markers to confer resistance to grain legumes. The model legumes *M. truncatula* and *L. japonicus* are affected by most pathogens and pests limiting grain and pasture legume yield (Figure 1). These legume species are self-fertile with small diploid genomes which are nearly completely sequenced and for which many genomic tools have been generated (13). Thus, they offer a great opportunity to improve our understanding on legume resistance mechanisms and to identify effective resistance genes acting against these pathogens and pests.

Fungal and oomycete pathogens are the largest and most diverse group of pathogens. They are responsible for the most dramatic legume crop yield losses worldwide. *M. truncatula* is a potential host for a large variety of necrotrophic and biotrophic fungal and oomycete diseases including anthracnosis, fusarium wilt, root rot, ascochyta blights, mildews and rusts (13) (Figure 1). *M. truncatula* genotypes showing differential responses to these pathogens from susceptible to resistant have been identified (e.g., 9, 12). These serve as a basis for the characterisation of underlying resistance mechanisms at the cellular and molecular levels as well as for the identification of defence genes and QTLs responsible for resistance. In some instances single resistance genes have been identified, such as for resistance to *Phoma medicaginis* and *Colletotrichum trifolii* (13). By contrast, resistance to *Aphanomyces eusteiches*, *Mycosphaerella pinodes*, *Uromyces striatus*, *Peronospora trifoliorum* or *Erysiphe pisi* appears mainly partial and controlled by multiple QTLs associated with different defence mechanisms. Resistance to *E. pisi* was shown to be controlled by several defence mechanisms including papilla formation, hypersensitive response and post-colony establishment mechanisms (12) (Figure 2). A root stele reinforcement mechanism controlled by a QTL potentially encoding a proteasome-related protein was also recently shown to be responsible for the partial resistance of the *M. truncatula* A17 genotype to *A. eusteiches* (5). Interestingly, this QTL mapped to chromosome 3 where a second major QTL for *A. eusteiches* resistance, named *AER1*, was also identified (11). At the molecular level, transcriptomic and proteomic studies indicated that the efficient defence reaction of *M. truncatula* requires the coordinated expression of several defence-related proteins. These studies also highlighted the crucial role played by the pathogenesis-related (PR) proteins, and in particular PR10, as a resistance determinant at least against *A. eusteiches* and *C. trifoliorum* (13). Several studies also showed that phytohormones including ethylene and ABA, and signalling molecules such as H_2O_2 are important defence determinants against fungal pathogens (2, 4, 10).

Legumes are also affected by bacterial pathogens. *M. truncatula* and *L. japonicus* can be infected by several bacterial pathogens including *Ralstonia solanacearum*, *Pseudomonas syringae* and *Xanthomonas campestris*. A recent study showed that one *M. truncatula* line, F83005.5, was resistant to most *R. solanacearum* isolates (18). The associated resistance mechanism, controlled by several QTLs, restricted bacterial infection to the root tip by preventing vascular bundle colonization of the pathogen (17, 18). In a recent study, Bozsó and coworkers (1) compare the gene expression pattern induced by the most important defence mechanisms, that is the basal defence mechanism, and the hypersensitive response during the non-host interaction *M. trunca-*

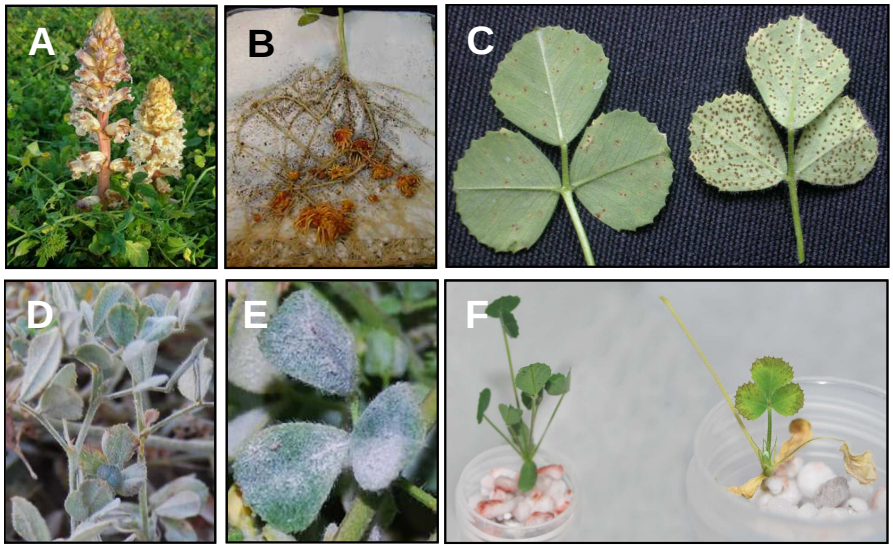

Figure 1. Photographs of the model legume *Medicago truncatula* infected by different diseases. A, Flowering spikes of *O. crenata* infecting *M. truncatula*.B. Early stage of *O. foetida* on *M. truncatula* roots. C. *M. truncatula* – *Uromyces striatus* interaction in resistant or susceptible genotype. Resistance is detected by HR-related necrotic spots on the leaf surface (left leaf) while susceptible genotype is covered by rust pustule and colony (right leaf). D, E. *M. truncatula* - *Erysiphe pisi* susceptible interaction. The picture show susceptible genotype covered by powdery mildew colony at whole plant (D) or leaf level (E). F. *M. truncatula*- *Fusarium oxysporum* f. sp. *medicaginis*, The picture compares healthy non infected plants of *M. truncatula* (left plant) with diseased plant showing necrotic and dead leaves (right plant) (Photo A and B courtesy of M. Fernández-Aparicio).

tula – Pseudomonas syringae. As expected, they found that infection with the bacterial pathogen led to profound and largely overlapping changes in gene expression and involved genes from several functional groups including cell signalling, transcription factors and defence-related genes such as PR10 (1).

Nematodes are also an important cause of yield losses in legumes. *M. truncatula* and *L. japonicus* can be colonised by several types of nematodes including the stem nematode *Ditylenchus dipsaci* and the root-knot and cyst nematodes of the *Meloidogyne* and *Heterodera* genera (3, 13). Screening *M. truncatula* and *L. japonicus* collections revealed differential infection responses, ranging from susceptible to resistant, to several of these nematodes. Such genetic diversity is being used to map and identify genes and/or QTLs involved in resistance to nematode infection, and to better characterise the associated resistance mechanisms (3).

Although less studied, legumes are also susceptible to viral diseases. Despite the damage they cause, very little is known about virus resistance mechanisms. In legumes, virus resistance has been mainly targeted in the economically important and emerging model legumes pea and soybean for which several resistance QTLs have been found and in some cases pyramidized (e.g., 8). For *M. truncatula* and *L. japonicus*, the only reports published to date indicated that *M. truncatula* could be infected by the *Pea early browning virus* (PEBV) and *Subterranean clover mottle virus* (SCMoV) while *L. japonicus* could be infected by *Arabis mosaic virus* (ArMV) and *Tobacco ringspot virus* (TRSV) but not by legume-infecting virus (6, 15). The screening of a *M. truncatula* core collection against SCMoV identified one resistant line expressing the typical hypersensitive response which was found to be controlled by a single gene located on chromosome 6 in a region containing typical resistance gene sequences (14).

In semi-arid regions worldwide, parasitic plants of the *Orobanche* spp. including *O. crenata*, *O. aegyptiaca* and *O. foetida* drastically decrease legume yield. *M. truncatula* has been proposed as a model to study the *Orobanche* spp. – legume interaction (7) (Figure 1). To improve our understanding of the *M. truncatula* - *O. crenata* interaction, transcriptomic and proteomic approaches were performed allowing the identification of many candidate genes for *O. crenata* defence (13). These approaches indicated that *M. truncatula* resistance to *O. crenata* involved genes belonging to different functional categories mainly associated with defence including genes of the PR families, cell wall modification, hormone-associated genes and transcription factors.

M. truncatula and *L. japonicus* were initially selected as model legumes to gain insight into molecular aspects of the symbiotic interaction with rhizobium and AM fungi. More recently, the use of these model legumes has been enlarged to study other aspects of legume biology, such as grain quality or resistance/tolerance to stresses taking advantage of the many tools developed for them. Collections of these species have been screened for resistance against the most important legume pathogens and pests allowing identification of several sources of resistance. The initial characterisation of these new resistance sources located useful resistance QTLs, that can serve to confer resistance in legume crops, and allowed more fundamental research to decipher legume resistance mechanisms. Interestingly, response to most pathogens and pests led to the activation of common pathways involving amongst others PR genes and phytohormone signalling. These studies identified a series of candidate genes with potential for improving disease resistance in legumes. These candidate genes should be validated through functional analysis before their use for genetic improvement of legume crops either directly through genetic transformation or indirectly by MAS. ■

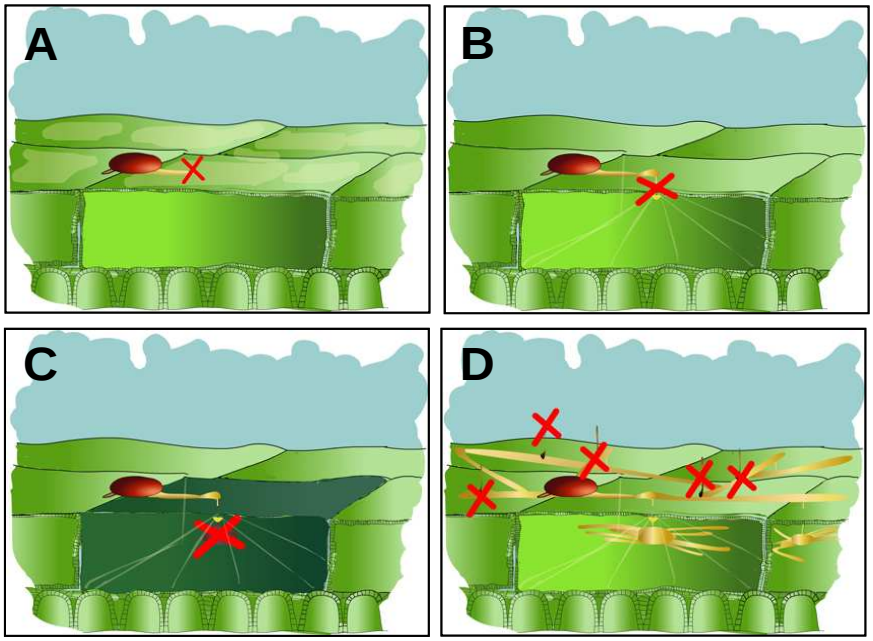

Figure 2. Resistance mechanisms acting against aerial fungal pathogens. The cartoons exemplify the resistance mechanism encountered during the *M. truncatula – Erysiphe pisi* as an illustration of the resistance mechanism acting in air-borne fungal pathogens. The mechanisms include the pre-penetration resistance mechanism (A), the penetration mechanism (B), the hypersensitive response (C) and the post-colony establishment mechanism (D).

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Genome conservation between model legumes

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In the last three decades comparative mapping studies revealed that species - depending on their evolutionary relationship - preserved similarities in the content and order of genes in their genomes consistent with similarity by descent, but increasingly disrupted as time passes. Originally comparative genome analyses used genetic maps developed with molecular markers but increasingly genome sequences (www.plantgdb.org/prj/Genome_browser.php) make comparisons direct and more extensive. At first heterologous markers (markers derived from one species but used in another) were used to compare genetic maps, but later comparison of the function of mapped genes in different species and analysis of their relationship came to the fore. The availability of extensive EST and genomic sequence data enables computer based analysis of a vast number of genes and genomic regions. These analyses align almost the whole genomes of different species and so give precise insights into chromosome evolution. The difference between macrosynteny (at the level of genetic maps) and microsynteny (within small-scale genomic regions) is becoming indistinct using *in silico* methods.

The three legume (Fabaceae) subfamilies - *Ceasalpinioideae*, *Mimosoideae* and *Papilionoideae* – contain about 20 000 species, almost all the economically important grain and forage legumes fall into the seven clades of the Papilionoideae subfamily. Genetic studies are principally focused on these species, so cross-species markers and genetic maps have been developed only for papilionoid species.

Legume genomics focuses mainly on two economically important legume groups (Doyle and Luckow 2003); (i) two Hologalegina legumes, *Medicago truncatula* and *Lotus japonicus*, for which tools for functional genomics have been developed and the sequenceing of the gene-rich euchromatic parts of these genomes is underway and (ii) due to its economical importance comprehensive genomic resources have been developed for soybean (*Glycine max*, a *Millettioid* legume) (www.soybase.org).

The Hologalegina clade is split into two lineages, the first is represented by *Lotus* for genomic studies, and the second contains well known legumes such as *Medicago*, *Trifolium*, *Melilotus*, *Lens*, *Pisum* and *Vicia*. The species in Hologalegina and Millettioid clades separated around 54 million years ago while the divergence of *Medicago* and *Lotus* is dated about 51 million years ago (4). The split between the *Vicieae* (containing *Pisum*) and *Trifolieae* (containing *Medicago*) tribes is estimated at 20-25 million years ago. This phylogenetic relationship is reflected in the corresponding genomic collinearity; the most conserved synteny was detected between the most closely related species.

Comparative genomics between model legumes

The most comprehensive macrosynteny analysis between legume genomes was reported by Choi *et al.* (3) and Zhu *et al.* (16). Cross-species gene specific markers were used to identify homologous genome segments among eight legume species (*M. truncatula*, alfalfa, *L. japonicus*, pea, chickpea, soybean, mungbean and common bean). Using the *M. truncatula* genetic map as a reference genome these eight genomes were aligned and a simplified legume consensus map created. Based on the analysis of the gene content of 63 sequenced BAC (large insert containing) clones, Choi *et al.* (3) reported collinearity between the two model legume species *M. truncatula* and *L. japonicus* for the first time. The map position of the corresponding BAC clones showed that the genomes of both model legumes are highly syntenic and the chromosomal rearrangements resulting in different chromosome number (*M. truncatula* 2n=16; *L. japonicus* 2n=12) was interpreted as translocation of chromosome arms. The high quality sequence of the reference legume genomes allowed the computer-based comparison of large genomic regions by analysing vast numbers of map-anchored BAC or TAC clones (2) which reinforced and completed the comparative study performed by Choi *et al.* (3). The schematic presentation of large-scale chromosomal rearrangements between the two model species produced by the integration of these studies is shown in Fig. 1. The difference in centromere number between the two species is the result of chromosomal rearrangements between *Medicago* chromosomes 3, 7, 5 and 6 and *Lotus* chromosomes 1 and 2 (Fig. 1). Ana-

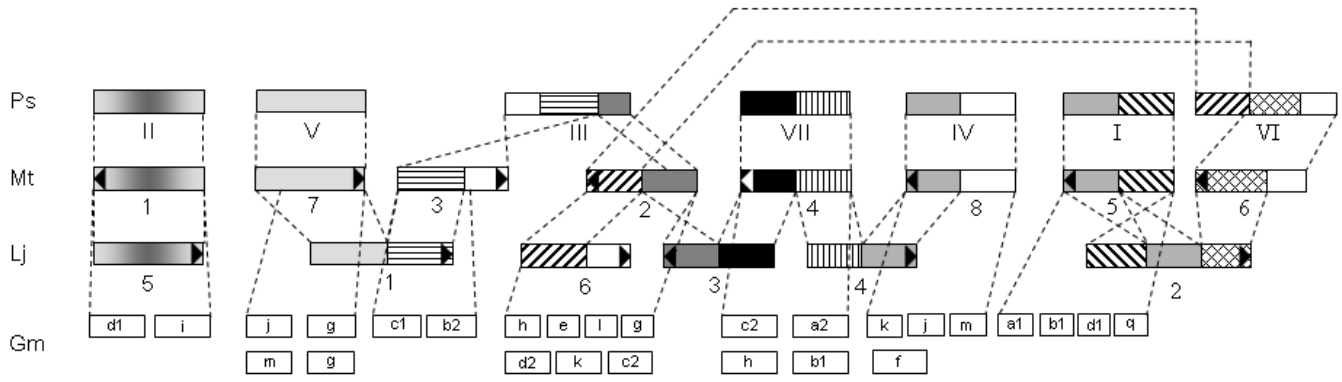

Figure 1. Schematic representation of large-scale synteny blocks between *M. truncatula* (Mt), *L. japonicus* (Lj), *Pisum sativum* (Ps) and *Glycine max* (Gm) chromosomes and chromosome segments. Bars with the same color or pattern show homologous chromosomal regions and arrows in the boxes indicate the orientation of the chromosomes (short arm – long arm) in the case of *Medicago* and *Lotus*. The bars do not reflect the relative sizes of chromosome or chromosome segments and the break points of chromosomes are indicated approximately.

lyzing the gene content of ten pairs of large insert clones showed almost identical gene content and the homologous genes showed the same order and orientation (3). Comparison of the microstructure of 276kb of the *MtDMI2(NORK)/LjSYMRK* region similarly revealed a nearly complete conservation in gene content and order (6, 10, 16).
Soybean (2n=40) has a highly duplicated genome and one genome duplication followed its divergence from other *Phaseoleae* species (12). These genetic rearrangements resulted in a partially duplicated genome which makes the detection of macrosynteny between soybean and other legumes complicated. However, the study performed by Choi *et al.* (3) revealed eleven collinear segments between the soybean and *M. truncatula* genomes (Fig. 1). The macrosynteny between *L. japonicus* and soybean was analysed by mapping the same cDNA clones on the genetic map of both species (13) which also detected several synteny blocks. Despite the lower level of collinearity at chromosome level, several studies confirmed the high degree of microsynteny between *M. truncatula* and soybean (11, 15). The draft genome sequence of soybean was released in 2008 and the gene-rich space of the other model legumes *M. truncatula* and *Lotus japonicus* will be finished in the near future.
The substantial conservation in gene order between *Medicago* species and pea identified genetic rearrangements between the two genomes which account for the difference in their chromosome number (8 for *Medicago* and 7 for pea; Fig. 1) (3, 9). The structural rearrangements between *Medicago* and *Pisum* chromosomes indicated that the 10-fold difference in their genome size is not the result of large scale genome multiplication in pea.

Synteny as a tool in gene isolation

Given what we know about the collinearity of legume genomes we might expect this to have been used widely in the isolation of legume genes. However, this has not yet been fully implemented, and to date collinearity has simply been a clue as to which genes are likely orthologues that can be further analysed. For example this was the case for the pea symbiotic genes *Sym2*, *Sym10* and *Sym19* (reviewed in 5) and *Sym35* (1) and similarly genes involved in the regulation of flowering and flowering time (reviewed in 7). A project aimed at the isolation of the pea *Afila* gene using comparative mapping and positional cloning has been underway for some time, and although extensive microsynteny has been found this work has yet to be published. At the same time direct approaches to gene isolation using deletion mutants has been successful (8, 14), and as genomic and post genomic tools become more widely available mapping by synteny will no doubt become a tool integrated with others facilitating the characterisation of gene function in legumes. ■

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Collection and conservation of Iberian *Medicago* spp. germplasm

by D. RUBIALES[1*], M. FERNÁNDEZ-APARICIO[1], A. MORAL[1] and A. PUJADAS[2]

Annual medics (*Medicago* spp.) are important pasture crops, native of Mediterranean Basin. Annual medics are also naturalized in southern Australia, where they accidentally introduced in the nineteenth century. Their spread there was encouraged initially by the application of superphosphate and then by the breeding and release of improved cultivars. Annual medics have also been introduced later on to other parts of the world with Mediterranean-type climates, e.g. parts of South Africa and Chile. Nowadays annual medics are grown on over 4.5 million hectares with a number of cultivar registered and available to farmers (2). However in their area of origin, their cultivation and utilisation has been neglected with risk of genetic erosion.

In addition to this obvious interest of annual medics as pastures, one of them, *M. truncatula* has become a model for studying various aspects of legume genomics and biology (1, 3). Both the economic interest as pastures and as model legume, reinforce the need to collect and preserve endangered germplasm from the Iberian Peninsula. We performed several field trips during 2005, 2006 and 2007 across Spain and Portugal collecting samples. A dry plant specimen from each population was deposited at the Herbaria of the Botanical Garden of Cordoba. Mature pods were collected from as many plants as possible from each population, with an average of 20-30 pods. Seeds have been multiplied in field plots and sent to Centro de Recursos Fitogenéticos, CRF-INIA, Madrid. ■

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Species	No. of collected populations	No. of populations with seeds available
M. arabica (L.) Hudson	18	6
M. ciliaris (L.) All.	3	3
M. doliata Carming.	50	30
M. intertexta (L.) Miller	6	5
M. italica (Mill) Flori	16	9
M. marina L.	3	1
M. littoralis Rohde ex Loisel.	3	0
M. minima (L.) Bartal.	45	5
M. orbicularis (L.) Bartal.	89	44
M. polymorpha L.	97	63
M. rigidula (L.) All.	31	26
M. sativa L.	35	0
M. scutellata (L.) Miller.	5	5
M. truncatula Gaertner	48	18

Proteomic and metabolomic analysis of drought-stressed nodules: new players in the regulation of nitrogen fixation in *Medicago truncatula*

by E. LARRAINZAR

Legume plants are unique in their ability to establish symbiotic relationships with soil bacteria (rhizobia), allowing them to live in nitrogen-poor soils thanks to the process of nitrogen fixation. However, nitrogen fixation is quickly inhibited under a range of biotic and abiotic stresses, particularly drought. The regulation of nitrogen fixation under drought is a complex process in which a number of factors interact. One of the possible regulation mechanisms suggested is a reduction of carbon supply available for the differentiated form of the symbiotic bacteria (bacteroid), due to a decrease in one of the plant enzymes responsible for the cleavage of sucrose in nodules; sucrose synthase. Although the carbon limitation mechanism has been experimentally shown to operate in legumes such as pea and soybean, this model has been recently challenged in the case of alfalfa. Is the limitation on nodule carbon supply specific to grain legumes? The main objective of this thesis work was to analyse the response to drought stress of the pasture legume *Medicago truncatula* at the nodule level in order to gain further insights into this regulation process. The proteomic analysis of drought-stressed nodules allowed us to identify a number of drought-responsive proteins. Among them, the content of nodule sucrose synthase was found to decline, but not as dramatically as previously observed for other legumes. An integrated proteomic and metabolomic analysis of nodules subjected to drought and a subsequent recovery provided evidence of a possible impairment of bacteroid metabolism and nitrogen-fixing capacity as the most plausible cause of the inhibition of nitrogen fixation in *M. truncatula*. On the other hand, the involvement of other nodule enzymes such as plant methionine synthase, essential for the synthesis of sulphur-containing amino acids, led us to investigate the effects of drought stress on sulphur metabolism in nodules. In summary, this work has provided evidence for a differential regulation of nitrogen fixation in the pasture legume *M. truncatula* under water-limiting conditions.

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Date: June 2009

Understanding the mechanisms involved in the response to cadmium of *Medicago truncatula* in symbiotic interaction with *Glomus intraradices*: physiological and molecular analyses

by A. ALOUI

Cadmium (Cd^{2+}) is a toxic heavy metal whose accumulation is increasing in ecosystems. Environmental contamination by this metal has major consequences for agriculture and public health. Phytoremediation is a potential route for the decontamination of polluted soils. The colonization of plant roots by arbuscular mycorrhizal fungi (AM) can improve this process and increase the tolerance of the colonized plants to metal stress. In this work, we have combined physiological and molecular approaches to decipher the mechanisms involved directly or indirectly in the triple interaction "*Medicago truncatula-Glomus intraradices*-cadmium". To have a global vision, the approaches mentioned above have been used both for roots and aerial organs. The morphological and physiological studies have revealed the sensitivity of the legume *M. truncatula* Jemalong 5 towards Cd^{2+} (2 ppm), resulting in reduction of root and aerial biomasses. Furthermore, this is accompanied by a reduction in the number of leaves, the surface area of aerial organs and the levels of pigments (chlorophylls a and b and carotenoids). In addition to an improvement in all the physiological parameters studied, we have shown that arbuscular mycorrhizal symbiosis (AMS) preferentially allocates biomass production to aerial organs rather than to roots, and increases the capacity of *M. truncatula* to accumulate Cd^{2+}. AMS, therefore, conferson the plant a certain tolerance towards cadmium stress, attenuating phytotoxic effects of Cd^{2+} by the dilution of its content in plant tissues, and directing the plant to a phytoextraction strategy. The non-targeted proteomic approach revealed proteins whose accumulation showed significant changes following the various treatments, including 30 proteins in roots and 23 proteins in aerial organs that were identified by LC-MS / MS and MALDI-TOF. Following analysis of the functional distribution of these proteins, we have demonstrated the involvement of AMS in the up-accumulation of proteins potentially responsible for the alleviation of Cd^{2+} toxicity by decreasing oxidative stress. The targeted study of the expression of genes involved in defence, has ruled out a global regulation of these genes in response to AMS. The identification and quantification of isoflavonoids in roots of *M. truncatula* following the various treatments also highlighted the potential involvement of these molecules in response to cadmium stress.

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Use of transposable elements to discover symbiotic genes and *vice versa*

by A. RAKOCEVIC

The symbiotic interaction between legumes and *Rhizobia* leads to the formation of a new organ: the nodule. The molecular mechanisms involved in this interaction are not yet all characterized. To better understand this process, two *M. truncatula* insertion mutant collections (T-DNA and *Tnt1*) have been generated. We have characterized two mutants from these collections.

The nod$^-$ phenotype of the mutant *tnk148* results from the *tnt1* insertion into the *MtNIN* gene. We have demonstrated, in collaboration with G. Oldroyd's laboratory (UK) that this mutant is blocked in the late steps of the Nod factor signalling pathway and that *MtNIN* plays a crucial role in infection thread formation and in nodule organogenesis (1). We have also phenotypically and molecularly characterised the somaclonal early nod$^-$ mutant *ms219*. The mapping of the mutation led to the identification of an active *M. truncatula copia*-like retroelement called *MERE1-1* for *Medicago RetroElement1-1*, as an insertion in the symbiotic *NSP2* gene. This 5.3 kb retroelement is the first active retroelement described in *M. truncatula. MERE1-1* activation occurs only during *in vitro* culture. The methylation status analysis of this element showed that the hypomethylation during *in vitro* culture correlates with the activation of expressionv of this retroelement (2).

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Nitrogen nutrition of winter pea-wheat intercrops (*Pisum sativum* L. – *Triticum aestivum* L.): analysis, modelling, and propositions for N-management strategies

by C. NAUDIN

Cereal-legume intercrops are gaining increasing interest in Europe because of low-input preoccupations, preservation of environment and biodiversity. Previous studies have shown that intercropping performances are highly dependent on soil N availability. This practice could provide interesting applications to develop crops for multi-service outcomes along with lower inputs (particularly N). However, very few references are currently available to guide N management of intercrops for contrasted production targets.

The objectives of this work were (i) to use current knowledge on winter pea-wheat intercropping in order to study whether N fertilization could be a helpful tool to manage such intercropping towards production targets under a conventional agriculture; (ii) to improve knowledge on the response of intercropping to different N-availability scenarios (N-resource sharing; inhibition and reversibility of Symbiotic Nitrogen Fixation (SNF)); (iii) to give propositions towards decision rules about N-management of pea-wheat intercrops for different production goals.

Our field experiments demonstrated that N-fertilization is an efficient tool for managing pea-wheat intercropping, notably for the contribution of the cereal to the total intercrop yield, which is nowadays a badly controlled criterion. N-supply favoured cereal growth to the detriment of the legume. The cereal is more competitive than the legume for mineral N resources if N-application occurs before the beginning of pea seed filling. However, the intensity of the response to the date of N-fertilizer application is variable depending on the gap between the intercrops in growth dynamics and stage of development. This is determinant for N-sharing and SNF response.

Experiments under greenhouse conditions investigated the inhibitory effect of nitrate and the ability of pea SNF to recover through a structure – function analysis according to different stages of nitrate exposure. Nitrate reduced the rate of nodule establishment when nodulated roots were exposed to nitrate during vegetative phases while it damaged existing nodules when applied during flowering and seed filling. Nitrate exposure always decreased the specific activity of nodules. Moreover, the ability to recover SNF after nitrate removal is partly dependent on the level of C availability to nodules. Thus, pea SNF can recover when a short-term inhibition by nitrate occurs before seed filling stages, which is in agreement with our own field observations.

A crop model, AZODYN-IC, was built to simulate pea-wheat intercropping. It is based on two single crop models, (AZODYN and AFISOL for wheat and pea, respectively). Its main interests are (i) to be able to satisfactorily simulate response of such intercrops to various soil mineral N availability dynamics in order to be used as a decision support tool; (ii) to use simple formalisms to simulate resource sharing (light, water, and nitrogen), tightly linking light sharing and N-acquisition, (iii) to run with the sole-crop parameters. This model was used to extend field experimentations by simulating a wider range of N-management strategies (sowing densities x N-supply rates x N-supply dates) using numerous climatic datasets. This work was able to give propositions towards decision rules for N-management of intercrop according to soil N mineral content at the end of winter, contribution of wheat in intercrop biomass at the end of winter, estimation of N-mineralization from the end of winter to harvest, and the rate and date of N-application.

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Identification of genes and QTLs related to domestication and yield-related traits in *Vicia faba* L. and synteny with other legume crops

by S. CRUZ-IZQUIERDO

There is tremendous interest in using comparative mapping to identify genes responsible for quantitative variation of complex traits of agricultural and evolutionary importance. The aims of this study were: (i) to produce the most saturated gene-based genetic linkage map of faba bean (*Vicia faba* L.) reported so far, using orthologous cross-species markers from *Medicago truncatula, Pisum sativum, Lupinus albus,* and *Glycine max*, and to assign the linkage groups (LGs) to specific chromosomes, (ii) to analyse the syntenic relationship between faba bean and *M. truncatula*, *L. culinaris*, *P. sativum*, and *L. albus*, (iii) to identify putative QTLs associated with domestication and yield-related traits, and (iv) to study the stability of the detected QTLs for their future application in marker assisted selection of *Vicia*.
The genetic linkage map was constructed using 124 F_6 lines derived from a cross between lines Vf6 (*equina* type) and Vf27 (*paucijuga* type). A number of intron-targeted gene-based anchor markers developed within the comparative mapping program of GLIP (http://www.eugrainlegumes.org/) and additional markers provided by Dr. Oliver´s group (2) were used in the analysis. Grip Map was used to compare the genetic maps of *V. faba* and *M. truncatula*, *L. culinaris*, *P. sativum*, and *L. albus.* Ordered loci from faba bean and other legumes LGs were listed vertically and horizontally, and dots positioned at the intersection of the locations of the corresponding markers in each of the genetic maps. Composite interval mapping (CIM) and multiple interval mapping (MIM) were used to check the presence of a putative QTL with QTL-Cartographer v. 2,5 software.
Two hundred and fifty-eight markers were attributed to 16 LGs and five of the largest arrays could be assigned to specific chromosomes thanks to the use of 199 common markers previously mapped in *Vicia*. The total length of the map was 1.875,1 cM. The average distance between markers was 7,26 cM.
The inclusion of 167 ESTs markers of *Medicago*, *Pisum*, *Lupinus* and *Glycine* allowed a comparison of our map with some of those legumes. The common orthologous markers were distributed uniformly principally along the main LGs that were essentially colinear. Thus, markers originally designed from genes on the same *M. truncatula* BACs were found to be grouped together in corresponding syntenic areas in lentil and faba bean, although faba bean and lentil shared a common rearrangement relative to *M. truncatula.* The outcome suggests shared ancestral chromosomal changes in faba bean and lentil compared to *M. truncatula* and confirms their phylogenetically closer relationship as reported before (2). A similar pattern was observed with *P. sativum* and *Lupinus*, but the reduced number of common markers prevented us from verifying unambiguously the collinearity.
Sixty-five QTLs were identified and characterised for 20 domestication and yield-related traits. Twelve QTLs, controlling five traits, (beginning of flowering, flowering length, pod length, number of ovules per pod, and number of grains per pod), were stable in the two years of evaluation. Through comparative mapping with previous reports in *V. faba* (1), in *L. japonicus* (3) *P. sativum* (4), and *L. culinaris* (5) it was possible to identify possible homologies with similar QTLs detected in other species although these outcomes should be ratified in future studies.

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Regulation of soybean nodulation induced by CLE peptides

by D. REID

The ability of legumes to enter into a symbiotic relationship with nitrogen fixing soil bacteria called rhizobia is one of their defining characteristics and is essential to their ability to grow in limiting nitrogen conditions. The host plant regulates nodule numbers to avoid excessive nodulation, which is detrimental to plant growth. This regulation can be triggered by rhizobia infection events or by the perception of nitrogen in the soil. Following infection, the initial nodule cell divisions triggered by the bacteria induce a systemic Autoregulation Of Nodulation (AON) mechanism in the plant. The current model for AON involves a root-derived elicitor (Q) of AON that is perceived in the shoot by an LRR receptor kinase called NARK. This triggers the production of a shoot-derived inhibitor (SDI) that is transported back to the roots where it prevents further nodule development. Due to the high degree of similarity between NARK and the LRR receptor kinase, CLAVATA1 in *Arabidopsis*, it has been proposed that NARK may perceive a similar ligand/s to that interacting with CLAVATA1, a small peptide called CLAVATA3. My PhD research focuses on Q and the role that CLAVATA3/ESR related (CLE) peptides play in the regulation of nodulation in the model legume, soybean. We have identified candidate CLE genes that respond to inoculation and/or nitrate treatments and propose that these induce AON in soybean. We are using reverse genetics approaches such as gene silencing and overexpression studies to learn more about the role these candidates play in both systemic rhizobia-induced AON and local nitrate-induced nodule regulation. The availability of the complete soybean genome has enhanced our ability to conduct non-targeted gene expression studies by means of next-generation sequencing technologies using the Illumina GAII platform. We will use this technology to further develop a bioassay to detect long-distance molecular signals involved in AON as well as to identify novel genes acting downstream of NARK in the leaf.

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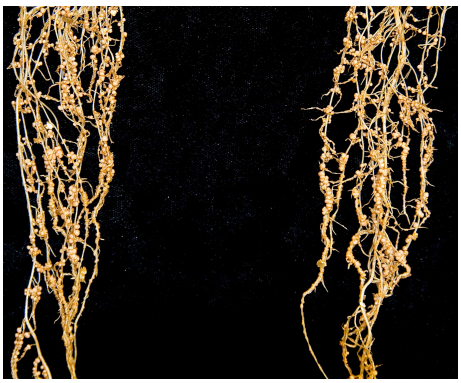
Characterising the inhibition of legume nodulation by low pH conditions

by M.H. LIN*

Through symbiosis with soil bacteria called rhizobia, legumes are able to form nitrogen-fixing root organs known as nodules. Nodule formation is tightly regulated by the plant and can be inhibited by a number of external factors, such as soil acidity. This is of great importance as much of the world's legume crops, such as soybean, are grown in acidic soils. Despite this, the precise mechanism by which acidic conditions inhibit soybean nodule development remains poorly characterized. Indeed, from a plant perspective, the effects of acid soils on nodulation events are relatively unknown, having largely been overlooked to date. Previous investigations have typically reported the problem to be associated with either disrupted plant and rhizobia growth, plant-rhizobia signaling and/or rhizobia infection. We have identified that the inhibition of nodulation by acidic conditions may be controlled by the plant. Moreover, this control may have a systemic component based on findings from studies using split-root, mutant-based, and petiole feeding approaches. Furthermore, we show that a critical step during nodule initiation and development after which nodulation is no longer inhibited by soil acidity. Next generation deep sequencing will be used to determine expressional changes and identify novel genes functioning in acid-stressed, rhizobia-infected, soybean roots. The role of the soybean Nod Factor Receptor, *GmNFR1a*, under acid stress will also be investigated using the reverse-genetics technique, RNAi.



Non-acid treated (left) and acid treated (right) roots showing no inhibition of nodulation of the soybean supernodulation mutant, *nod4*, by acid soil.

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Tissue and cell type analysis of molecular events during the early stages of soybean nodulation

by S. HAYASHI

Legumes develop nitrogen-fixing root nodules through a symbiotic association with soil bacteria commonly known as rhizobia. Nodulation provides legumes with nitrogen for growth and development, allowing them to grow in nitrogen-poor environments and making them valuable biological nitrogen fertilisers. In recent decades, nodulation research has led to the discovery of a number of genetic and biochemical components that are required for nodule development. These studies have indicated that a coordinated development between epidermal and cortical/pericycle cells of the root during the early stages of nodulation is necessary for the formation of fully functional nodules. However, there is still very little known about how these different root cell layers interact and synchronise their responses in order to form functional nodules. In addition, many of the nodulation genes reported are yet to be identified in crop legumes, such as soybean (Glycine max). Based on sequence homology and expression analysis, we have identified a number of candidate genes in soybean that act during early stages of nodule development. To identify novel genes acting during the early stages of soybean nodulation, we have undertaken root transcriptome analysis via high-throughput next-generation sequencing technology using the Illumina GAII platform. The genes identified using this approach are not only likely to be important for nodulation, but may also provide clues regarding the components involved in cell-to-cell interactions occurring during the early stages of nodule development. Detailed expression analysis of candidate genes will be performed to help identify their cellular expression (i.e., epidermis, pericycle or cortex) and the precise timing that they are activated during nodulation.

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Development of a bioassay for the characterisation and identification of the soybean nodulation inhibitory factor SDI

by Y.H. LIN*

Nodule development in legumes is an energy and resource expensive process. Consequently, the plant has a control system in place to regulate nodule numbers. This control system is known as Autoregulation of Nodulation (AON). During the early stages of nodule development, an elicitor signal, Q is produced. Q is subsequently transported to the leaf where it is detected by Nodulation Autoregulation Receptor Kinase (NARK) in the leaves. This leads to the production of a new signal called the Shoot-derived Inhibitor (SDI), which is transported to the root where it arrests further nodule development. We have developed a petiole-feeding bioassay for the characterisation and identification of SDI in soybean plants (Fig. 1). This assay enables the feeding of aqueous solutions to test plants, including dyes and radiolabelled tracers to determine the movement of the fed constituents, hormones and leaf extracts to identify the suppressive effect on nodule numbers. Feeding leaf extracts from *Bradyrhizobium japonicum*-inoculated wild type (*Glycine max*. cv. Bragg) or *NARK* mutant plants to *NARK* hypernodulating mutant (*nts1116*) test plants demonstrated that suppression activity is only present in wild type leaf extracts. We have also demonstrated that SDI is small, NARK-induced, stable, *Rhizobium* and Nod factor-dependent and likely not an RNA or protein molecule. Analytical techniques, including mass spectrometry and NMR, are currently being used to identify the SDI molecule in soybean leaf extracts.

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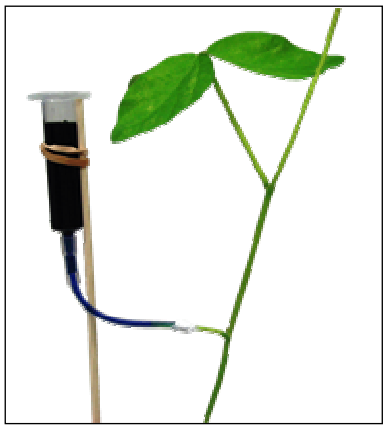

Figure 1. The petiole feeding bioassay allows the feeding of aqueous solutions to be continuously fed into recipient plants without the need for constant user supervision. It is being used to identify the Shoot-derived Inhibitor signal involved in regulating legume nodule numbers

Root Plastids involvement in arbuscular mycorrhizal symbiosis. Cellular and molecular characterization

by Z. DAHER

Despite the recognized importance of non-photosynthetic plastids in a wide array of plant processes, the role of root plastids in arbuscular mycorrhizal symbiosis remains to be explored. Using electronic microscopy, we have clearly identified in cortical cells of *M. truncatula* roots four main types of plastids with a predominance of amyloplasts. In contrast, AM-colonized cortical cells had a proliferation of plastids without internal membranes and plastids with internal tubular membranes at the expense of amyloplasts. This morphological change suggests therefore a remarkable specialization of plastid metabolism in cells colonized by AM fungi. The metabolism of these plastids is involved in the synthesis of a yellow pigment, mycorradicin, the accumulation of which we have measured by HPLC in mycorrhizal roots. Mycorradicin accumulation is concomitant with fungal arbuscule development. This correlation, coupled with morphological changes of root plastids occurring in mycorrhizal roots, suggests that the development of AM symbiosis requires a profound upheaval of plastid metabolism. The study of the root plastid proteome then proved fundamental in allowing us, using GeLC-MS/MS, to establish the first repertory of a root plastidome: 266 proteins of known or putative plastidial origin were identified, including 30 proteins for which no homolog had previously been identified as plastid-localized. These new candidates might play a role in the sentinel function that plastids may use in plants to react to biotic and abiotic stress. The qualitative comparison of mycorrhizal and non mycorrhizal root plastidomes identified 29 plastid proteins as induced or up-regulated in response to the AM symbiosis. These proteins are involved for their great majority in fatty acid and amino acid metabolism. The stimulation of lipid metabolism in mycorrhizal roots confirms the results of our ultrastructural observations of a shift from carbohydrate metabolism (amyloplasts) to a more pronounced lipid metabolism (plastids with tubular membranes). Taken together, the data obtained suggest plastids to be key organelles for maintaining functional AM interaction.

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COVER PHOTOS :
The model legumes: leaves, flowers, pods, seeds and nodules of *Medicago truncatula, Lotus japonicus, Glycine max* and *Pisum sativum* on an experimental field of *Lotus japonicus.* (Photos of *M. truncatula* courtesy of R. Thompson, P. Gamas, INRA, France and D. Rubiales, CSIC, Spain; photos of *L. japonicus* courtesy of E. Tuck, IBERS, University of Aberystwyth, UK, N. Rispail, CSIC, Spain and M. Rebuffo, INIA. Uruguay; Photo of soybean courtesy of B.J. Ferguson and P.M. Gresshoff, ARC-Centre of Excellence for Integrative Legume Research, Australia; Photo of pea courtesy of N. Ellis, JIC, UK)

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