Czech J. Genet. Plant Breed., 45, 2009 (1): 31–34

CONFERENCE REPORT

IV International Conference on Legume Genomics and Genetics

Puerto Vallarta, Mexico, December 7–12, 2008

The legumes (*Fabaceae*) represent the third largest of all plant families, with around 18 000 species distributed across some 650 genera. The family includes colonizers of a wide range of diverse habitats, from the cold, short-season polar region to the warm humid tropical forests. The distinguishing feature of the legumes, and the one which has long attracted the attention of plant biologists, is its ability to symbiotically fix atmospheric nitrogen. Several legume species were domesticated early in the process of the development of agriculture, and the family is historically linked to the birth of genetics, thanks to Mendel's choice of the garden pea (*Pisum sativum*) as his main experimental model for investigating the rules governing heredity and hybridity. The foremost grain legume species contributors to human nutrition are soybean (*Glycine max*) (although the species is often classified for statistical purposes as an oil crop), common bean (*Phaseolus spp.*), chickpea (*Cicer arietinum*), field (or broad) bean (*Vicia faba*) and pea. Less visible, but nevertheless of major importance, are the forage legumes, led by lucerne (*Medicago sativa*) and clover (*Trifolium spp.*). Finally, a number of economically important tree species are leguminous; many of these are of tropical origin and are used as a source of timber, but others are prospective sources of biofuel (in particular, the Asian species *Pongamia pinnata*).

In 2002, the international legume genetics and biology community initiated a series of biannual meetings, the first of which was held in Minneapolis-St. Paul (USA) the second in 2004 in Dijon (France) and the third in 2006 in Brisbane (Australia). Such a retrospective was provided by CARROLL VANCE from University of Minnesota, one of the "godfathers" of legume conferences. A particular reference was made to the map-based cloning of receptor kinases controlling nodulation (as well as some other mycorhizal processes). Gene identification in the current era has been greatly simplified by the availability of the recently completed genome sequences of the two model legumes, *Medicago truncatula* and *Lotus japonicus*. These, supported further by the draft soybean genome sequence generated by the US agencies DOE and USDA, have enabled a raft of comparative genomics studies of the major legume species. The necessity of validating gene function has led the legume community to develop both transgenesis and insertional mutagenesis as tools, along with the sequencing of ESTs and the characterization of the proteome. The audience was asked a number of leading questions: "Where will legume genomics go from here? Will the characterization and analysis of paralogs reveal divergence in gene function? Will agronomically important QTL be mendelized by the emerging study of genome-wide genotype/phenotype associations? Will microRNAs represent the next generation technology behind basic legume genetics? And finally, will fundamental discoveries feed through into crop improvement?" Following this provocative presentation, the more than 350 scientists (from about 55 countries across all five continents) spent the following five days discussing the current status of legume science in a programme structured into eleven sessions.

Session 1: Whole genome: structure and comparison

This session reflected upon the three current legume genome sequencing projects: *M. truncatula* (NEVIN YOUNG, University of Minnesota, USA), *L. japonicus* (SHUSEI SATO, Kazusa Institute, Japan) and soybean (JEREMY SCHMUTZ, Joint Genome Institute, USA). These genomes are largely similar to one another with respect to their gene content and genomic landscape, and the differences which have emerged may be an artefact of the choice of different sequencing methodologies. The euchromatic fraction of *M. truncatula* has been sequenced using a BAC-by-BAC strategy, providing over 300 Mbp of sequence. Of the 39 000 gene models extracted, 10 000 encode a hypothetical protein and a proportion of these may be legume-specific. About 30% of the genome comprises repetitive sequences, mainly retrotransposons. Curiously, there is less evidence for genome duplication than in the other plant genomes sequenced so far. Synteny between *M. truncatula*, soybean and *L. japonicus* is extensive, and colinearity on a broad scale is the norm with pea. Some synteny has been retained with *Arabidopsis thaliana* and *Populus trichocarpa*. The *L. japonicus* genome has been sequenced using a strategy of shotgun clone-by-clone sequencing in selected genomic regions. This has delivered > 320 Mbp of sequence so far, representing ~ 75% of the full genome, and nearly 11 000 complete and 20 000 partial protein-coding genes have been annotated. Traces of genome endoduplication have been established, and some synteny has been retained with soybean. The soybean genome is being sequenced using a whole genome shotgun approach. A draft sequence, based on 7× genome coverage, has been published at http://www.phytozome.net. Reconstituting the soybean genome is challenging because of its history of multiple rounds of polyploidization, the frequency of segmental duplications and the relatively large size of the genome. As a result, many genes are represented by at least four homoeologous copies. As explained by Scott Jackson (Purdue University, USA), the rather smaller, but – importantly – diploid common bean genome (~ 500 Mbp), can be used to resolve some problematic regions. The plastomes of four legume species (*M. truncatula*, chickpea, soybean and white clover – *Trifolium repens*) have now also been fully sequenced, and these sequences are providing a valuable phylogenetic tool, as shown by Stefanie Ickert-Bond (University of Fairbanks, USA). A personal highlight was the contribution of Jiří Macas (IPMB České Budějovice, Czech Republic) who targeted the repetitive, rather than the genic fraction of the pea genome. For this purpose, 454 sequencing technology was used to characterize about 30 Mbp (out of ~ 4 000 Mbp) of the pea genome sequence, and this was exploited to define the repetitive sequence families representing about 50% of the genome.

Session 2: Defining gene function

The predominant approaches described for defining gene function were insertion mutagenesis, as presented for *M. truncatula* by Pascal Ratet (CNRS, France); and TILLing, as demonstrated in soybean by Meksem Khalid (Southern Illinois University, USA). Armed with the model legume genome sequences, positional cloning in the legumes has become easier and more efficient, as shown by Rebecca Dickstein (University of North Texas, USA), who described her programme to isolate the NIP gene, required for rhizobial invasion during *M. truncatula* nodulation. An excellent talk (as usual) was given by Julie Hofer (John Innes Centre, UK) covering tendril formation and branching in the legume leaf. Based on a long term study focusing particularly on the contact-sensitive filamentous tendrils, she has successfully cloned the *tendriless* (Tl) gene, and characterized it as a homoeodomain-zipper. The conversion of tendrils into leaflets in the mutant type demonstrates that the pea tendril is a modified leaflet inhibited from completing its laminar development by Tl. The gene is transcriptionally regulated by the floral meristem identity gene *Unifoliata/LEAFY*. She has also shown that the semi-leafless type, which dominates mechanised pea production thanks to its improved standing ability and disease resistance, arises through the action of afila, a recessive Zn-finger protein gene.

Session 3. Pathogenesis and disease resistance

The plant response to biotic stress is a focal topic of both applied and basic research, so was unsurprsingly represented by numerous contributions. Karam Singh (CSIRO, Australia) described a gene responsible for bluegreen aphid resistance in *M. truncatula*. The same research team is also mapping QTL for resistance against several soil-borne pathogens, especially *Rhizoctonia solani* and *Fusarium oxysporum*, which are responsible for serious root diseases in a broad range of legumes. Ben Rosen's (University of California, USA) lab is working on the highly variable NBS-LRR disease resistance genes, and have identified 77 TIR resistance gene homologs (RGHs) and 78 non-TIR RGHs in *M. truncatula*. They have exploited these sequence data to develop a degenerate primer PCR assay to amplify homologs across the *Fabaceae*, aiming to provide some insight into the diversification of RGHs in the context of speciation, as well as to illustrate the evolution of disease resistance genes.

Session 4: Genome enabled agriculture

The important role in world agriculture played by legumes was highlighted by Francisco Aragão's (EMBRAPA, Brazil) talk, which dealt with the development and marketing of virus (BGMV) resistant common bean. The resistance was engineered by an RNAi strategy, and the transgenic plants are being subjected to a prolonged biosafety evaluation prior to their release for commercial use. Tom Clemente (University of Nebraska, USA) gave a notable presentation on the modification of soybean oil for feed and fuel. The upping of oleic acid content to 85% increases car tank life, and reduces the emission of NO_x in the exhaust. On the other hand, the the omega-3-fatty acid content of the oil can be raised to 60%, making it highly desirable as part of the human diet. Finally, Paul Scott (University of Queensland, Australia) introduced the tropical/subtropical tree *Pongamia pinnata* as a promising source of biofuel. As Rudolph Diesel (1858–1913) remarked in words which are remain relevant today "the diesel engine can be fed with vegetable oil and thus can support agriculture". Given a production of about 5 t/ha of oil, Australia would need to establish 20 036 km^2 plantations on marginal land to entirely supply its petroleum demand from *Pongamia*!

Session 5: Abiotic stress

Crop losses due to drought and soil salinity are becoming increasingly severe and commonplace – it is estimated that ~ 40% of crop production is lost each year in the USA. Genetic tolerance is generally under polygenic control, and a number of lectures covered the genetics and discovery of candidate genes for abiotic stress tolerance in bean, soybean and *M. truncatula*.

Session 6: Development

Absolute, "Hollywood style" astonishing presentation given by Robert Goldberg (University of California, USA) focused on the number of genes required to make a soybean/legume seed. It has been forecast that the world will have to produce more food in the next 50 years than it has produced during the entire history of humankind, using for this endeavour an ever-decreasing area of land. Thus, a major challenge for 21st century agriculture is to radically increase crop productivity. A combination of gene chip technology, 454 DNA sequencing and laser-assisted dissection was exploited to estimate that of the 13 000 distinct mRNAs present in each soybean and *A. thaliana* seed compartment, ~ 1 000 are transcription factors. Some 100 mRNAs are seed-specific, but are present in adjacent seed layers. How genes active in distinct parts of the seed are organized into regulatory networks to program seed development remains to be determined. In a second attention-grabbing presentation, Elisabeth Dun (University of Queensland, Australia) expanded on the strigolactones, a novel class of phytohormones, which have only recently been introduced in a publication in Nature.

Session 7: Translational genomics

As the quantity of genomic information grows, the challenge is to effectively exploit it to advance both applied and theoretical research aims. Douglas Cook, the organizer of next conference to be held in California in 2010, explained his vision in which genomic data will be used as means of deducing the evolutionary history of the *Papilionoideae* genomes, as well as to develop tools for applied legume research. His approach is to use low copy genes with a simple evolutionary history to define ancestral genome segments, and multi-copy, fast-evolving NBS-LRR genes to understand the evolution of sequences within a species. Both these approaches require species-specific BAC libraries to provide a reservoir of genetic markers for linking genetic and physical maps. Robust database systems have become a vital component of the legume research effort, as illustrated by the Legoo bioinformatics gateway, developed by Jerome Gouzy (INRA, France).

Session 8: Whole genome expression analysis

DNA chip technology, in conjunction with high throughput sequencing capacity, has turned whole genome expression analysis into a flourishing research field. Expression atlases have been assembled from various tissues of various species, subjected to various experimental conditions in order to explore transcription. Helge Kuster (University of Hannover, Germany) presented the use of Truncatulix software to identify novel components of symbiotic signalling in the arbuscular mycorrhizal roots of *M. truncatula*. Transcription analysis has shown that small RNAs play a number of important roles in certain developmental processes. A comparison of soybean near isogenic lines differing in response to iron deficiency (which can cause substantial yield losses) was made by Randy Shoemaker (USDA-ARS, USA) using the Affymetrix chip platform. He went on to foreshadow how the 835 candidate sequences identified could be sifted and validated by QTL mapping and comparative genetics.

Session 9: Evolution and diversity

It is now clear that most angiosperms have undergone one or more rounds of polyploidization in the course of their evolution. Jeff Doyle (Cornell University, USA) discussed the evolution of soybean and its relatives, and was able to make a link to Darwinian fitness by pointing out that the photoprotective ability of polyploid soybean species is higher than that of the diploids. Transcriptomic analysis was able to demonstrate differences between the expression patterns of homoeologous genes. A comparative analysis of the genomic region of the *Rpg1* disease resistance gene in soybean, its diploid relative *G. tomentella* and the common bean, being undertaken by a team from the University of Minnesota, USA, led by Nevin Young, has shown that following polyploidization, one cluster of homoeologous genes was lost, while another was expanded by the insertion of retrotransposons.

Session 10: Symbiosis

Symbiotic nitrogen fixation has been widely studied, both with respect to the host plant and its microbial symbiont. Thanks to various induced mutants, several nodulation genes have been identified and the nodulation network is gradually being teased apart. The complex, but finely tuned interplay of kinase and associated phosphatase cascades was explained by Peter Gresshoff (University of Queensland, Australia). Legumes have developed a systemic regulatory system ("autoregulation of nodulation") which requires communication between the shoot and the root via a root-derived and a shoot-derived long-distance signal. Masayoshi Kawaguchi (Kazusa DNA Institute, Japan) presented data obtained from *L. japonicus* showing that genes in the pathway connecting *CLAVATA1* and a *CLAVATA3* peptide ligand may be involved in this mechanism.

Session 11: Recognition and signalling

This session developed from the previous one, aiming to provide a more detailed picture of the peptide signalling occurring between the host plant and the microbial symbiont. Cascades similar to the shoot meristem *CLAVATA3* pathway have been revealed, as shown by Jens Stoogaard (University of Aarhus, Denmark) and Peter Gresshoff (University of Queensland, Australia). That calcium signalling also plays an important role was shown by Giles Oldroyd (John Innes Centre, UK). Calcium acts as a diffusible signal perceived both by the plant and by the microbe, activating the symbiosis pathway through a calcium/calmodulin-dependent kinase.

In all, the formal part of the meeting consisted of 74 oral lectures and 136 posters, and was held over five days at the Bougainvilleas-Sheraton Hotel and Convention Centre, Puerto Vallarta, Mexico. Despite the nearness of the beach, the lectures were well attended throughout, and discussions outside of the formal proceedings were lively and lengthy. The conference was generally seen as a success, and the organizers were thanked by a generous round of applause in the closing session. Delegates left Mexico in an optimistic frame of mind for the future of legume research.

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