

## Legume biology: the basis for crop improvement

Rajeev K. Varshney<sup>A,B,C</sup> and Himabindu Kudapa<sup>A</sup>

<sup>A</sup>International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502324, India.

<sup>B</sup>School of Plant Biology, University of Western Australia, Crawley, Perth, WA 6009, Australia.

<sup>C</sup>Corresponding author. Email: [r.k.varshney@cgiar.org](mailto:r.k.varshney@cgiar.org)

**Abstract.** Legumes represent the most valued food sources in agriculture after cereals. Despite the advances made in breeding food legumes, there is a need to develop and further improve legume productivity to meet increasing food demand worldwide. Several biotic and abiotic stresses affect legume crop productivity throughout the world. The study of legume genetics, genomics and biology are all important in order to understand the limitations of yield of legume crops and to support our legume breeding programs. With the advent of huge genomic resources and modern technologies, legume research can be directed towards precise understanding of the target genes responsible for controlling important traits for yield potential, and for resistance to abiotic and biotic stresses. Programmed and systematic research will lead to developing high yielding, stress tolerant and early maturing varieties. This issue of *Functional Plant Biology* is dedicated to ‘Legume Biology’ research covering part of the work presented at VI International Conference on Legume Genetics and Genomics held at Hyderabad, India, in 2012. The 13 contributions cover recent advances in legume research in the context of plant architecture and trait mapping, functional genomics, biotic stress and abiotic stress.

**Additional keywords:** candidate genes, chickpea, functional genomics, *Lotus*, *Medicago*, peanut, soybean, trait mapping, transcriptome.

Legumes are extensively grown in dry or semiarid regions of the world, usually under rainfed rather than irrigated agriculture. Grain legumes are rich source of dietary protein (Duranti and Gius 1997), especially for the largely vegetarian population of sub-tropics. Despite a large growing area under these crops, productivity remains low and has been declining for the last few decades. Yields of these crops are reduced due to severe effect of several biotic and abiotic stresses. For example, in soybean, peanut and chickpea, drought is an important abiotic stress constraint and major biotic stresses include anthracnose, angular leaf spot, bean rust, bacterial blight in common bean, *Ascochyta* blight and *Fusarium* wilt in chickpea (Miklas *et al.* 2006; Kulcheski *et al.* 2011; Varshney *et al.* 2013a). Climate change is another converging force, which will potentially decrease crop productivity (Varshney *et al.* 2010; McClean *et al.* 2011). Combating the stresses to produce cultivars resistant to biotic or abiotic stresses together with ability of adapting to changing climate and increase crop productivity is needed to meet the increasing demands for food.

Grain legumes have a narrow genetic base since they are self-pollinated (though cross-pollination does take place, it is at very low frequency). Thus, there is a need to study legume genetics and genomics in depth to understand the biology of legume crops, widen the genetic base, support legume breeding programs and introgress traits of interest. With the advent of next generation sequencing (NGS) technologies, increased throughput together with reduced sequencing costs dramatically changed the sequencing scenarios of plant genomes (Varshney *et al.* 2009;

Thudi *et al.* 2012). To date over 55 plant genome sequences are sequenced (Michael and Jackson 2013) and many more are under way (<http://www.onekp.com/>).

Legumes have certain unique features such as ability for symbiotic nitrogen fixation, protein rich physiology, secondary metabolism, etc., and hence are different from other plant species. Therefore it is important to sequence legume genomes in addition to model crops (e.g. *Arabidopsis*, rice) to understand the special features of legume species (Cook 1999). With this understanding, two legume plant species namely, *Medicago truncatula* (Barker *et al.* 1990) and *Lotus japonicus* (Handberg and Stougaard 1992) were selected as model systems for legumes. This resulted in genome sequencing of *Lotus* (Sato *et al.* 2008) and *Medicago* (Young *et al.* 2011). In addition to the two model legumes *Medicago* and *Lotus* discussed above, soybean was the first legume crop for which reference genome was assembled (Schmutz *et al.* 2010). Recently, draft genome sequences have become available for pigeonpea (Varshney *et al.* 2012) and chickpea (Varshney *et al.* 2013b). In addition, common bean genome sequence has also been assembled (Scott Jackson, pers. comm.; <http://www.phytozome.net/commonbean.php>) and significant efforts are underway in sequencing other important legume genomes such as peanut (<http://www.peanutbioscience.com/>), pea and lentil. The availability of the genome sequence in model legumes as well as above mentioned legume crop species serve as a useful resource for legume crop improvement. In addition to genome assemblies, *de novo* transcriptome assemblies were developed in

several legumes including *Medicago*, soybean, chickpea, pigeonpea, etc (Cheung *et al.* 2006; Deschamps and Campbell 2010; Hiremath *et al.* 2011; Garg *et al.* 2011; Kudapa *et al.* 2012).

As part of analyses of legume transcriptome and genome sequencing projects, several type of markers such as simple sequence repeats (SSRs) and single nucleotide polymorphism (SNPs) have been identified at genome level. The genomic resources developed facilitate discovery of genes and association of genes with phenotypes and eventually help enhancing legume molecular breeding program.

*The VI International Conference on Legume Genetics and Genomics* (VI ICLGG) held in Hyderabad, India in 2012 focussed on different disciplines in legumes ranging from basic science to applied aspects such as symbiosis and development, evolution and diversity, nutrition and quality, next generation genomics, abiotic stress, pathogenesis and disease resistance, genomic resources and trait mapping, genomics assisted breeding, and translational genomics. The conference covered model legume species along with crop species like soybean, cowpea, chickpea, lentil, common bean, pigeonpea, faba bean and mung bean. Some selected high-quality papers related to plant biology themes presented in this conference have been included in this special issue on 'Legume biology: the basis for crop improvement' in *Functional Plant Biology*. In parallel, some papers presented in the VI ICLGG related to genetics and molecular breeding themes have been included in the special issue on 'Legume Genomics' in *The Plant Genome* journal.

This issue comprises six reviews and seven research articles contributed by eminent international legume researchers. Articles in this issue are categorised to four different themes *viz.*, plant architecture and trait mapping (two articles), functional genomics (four articles), biotic stress (three articles) and abiotic stress (four articles). Each theme consists of both review and research articles. The first article by Putterill *et al.* (2013) is a review that describes recent efforts in uncovering flowering-time regulators in *Medicago* using candidate gene approaches. They explain how plants integrate flowering signals from a range of different internal and external cues in order to flower and set seed under optimal conditions. Networks of genes controlling flowering time are summarised with reference to the flowering models *Arabidopsis*, wheat, barley and rice. Investigations revealing important commonalities such as FT genes that promote flowering in all of these plants, as well as regulators that are unique to some of them are also discussed, in addition to the effect of miRNA in root growth and nitrogen fixing nodule number of *Medicago*. It is stated that less is known overall about flowering control in other important groups of plants such as the legumes and this review discusses flowering-time regulators in legumes highlighting the importance of a *Medicago* FT gene, *FTa1*, in flowering-time control. The following article by Bustos-Sanmamed *et al.* (2013) demonstrated the involvement of miRNA in auxin-dependent regulation of nodule organogenesis in *Medicago*. Earlier studies state that the phytohormone auxin plays fundamental roles in plant development, including the formation of symbiotic nitrogen-fixing nodules in legumes. An important conclusion from this study is that microRNA160 represses the expression of five

transcription factors and its overexpression in the root affects both root growth and nodule number.

The significant role of functional genomics in controlling biotic and abiotic stresses of legumes is considered in two papers. A comprehensive review on legume functional genomics, most importantly the role of novel approaches in studying stress responses in crop legumes, is presented by Kudapa *et al.* (2013). Identification of genes conferring resistance to biotic stresses and tolerance to abiotic stresses that can be used to both understand molecular mechanisms of plant response to the environment and to accelerate crop improvement have been broadly discussed in this review. A range of approaches such as the sequencing of genomes and transcriptomes, gene expression microarray as well as RNA-seq based gene expression profiling, and map-based cloning for the identification and isolation of biotic and abiotic stress responsive genes in several crop legumes have been reported. An overview on recent advances in the functional genomics of 10 crop legumes that includes the discovery as well as validation of candidate genes have been presented in this review. Another review article authored by Pflieger *et al.* (2013) presents Virus-Induced Gene Silencing (VIGS), an important technology for functional genomics studies in legumes. This review focuses on the urgent need for reverse-genetics tools highlighting the role of VIGS as a powerful alternative technology to validate the function of unknown genes, most importantly genes that contribute to yield and product quality. Several VIGS systems have been developed for legume species, including those based on *Bean pod mottle virus*, *Pea early browning virus*, and *Apple latent spherical virus*. The use of these systems in reverse-genetics studies of a wide variety of plant biological processes is debated in this article and an overview on successful applications and limitations of VIGS systems in legume functional genomics studies is presented.

Li *et al.* (2013) developed transcriptome profiles, a functional genomics approach to better understand the molecular mechanisms underlying the peanut gynophore gravitropism. Until recently, the genetic basis underlying gravitropic bending of gynophores is not well understood. This study facilitated to identify genes related to gynophore gravitropism, gene expression profiles (up- and down-regulated) of gynophores cultured *in vitro*. The differentially expressed genes identified in the study were assigned to 24 functional categories and twenty pathways including carbon fixation, aminoacyl-tRNA biosynthesis, pentose phosphate pathway, starch and sucrose metabolism were recognised in the study to help understand peanut gynophore gravitropism.

The article presented by Domoney *et al.* (2013) shows the utilisation of mutagenised population for identification of candidate genes associated with different traits like seed metabolism and storage with pea (*Pisum sativum* L.) as an example. To enable the identification and isolation of genes underlying particular traits and processes, a fast neutron mutagenised population was generated in pea and the present study suggest that large deletions affecting one or more loci can be non-deleterious to the pea genome, yielding mutants that could not be obtained by other means. Furthermore, unique opportunities to identify the products of complex and related gene families have been discussed in this article. It is stated that

rapid reverse genetic screens of the fast neutron mutant populations supports conclusions on the frequency of deletions based on phenotype alone when compared with forward genetics.

The importance of functional genomics approaches has been discussed in the above articles and the following seven articles touch upon biotic and abiotic stresses. Recent advances in plant-aphid interactions and the mechanisms underlying resistance to aphids in legumes are reviewed by Kamphuis *et al.* (2013). They report that sap-sucking insects such as aphids cause substantial yield losses in agriculture by draining plant nutrients as well as vectoring viruses and the main method to control them is through the application of insecticides. They also mentioned that aphids rapidly evolve mechanisms to detoxify these and thus is important to develop durable plant resistance to these damaging insect pests. Hence the review focuses on aphid interactions with legumes, but relevant work on aphid interactions with other plants, particularly *Arabidopsis* and tomato, is included. This review covers advances on the plant side of the interaction and also on the aphids side of interaction, including the identification of major resistance genes and quantitative trait loci conferring aphid resistance in legumes, defence signalling following aphid infestation and the role of specialised metabolites, aphid effector proteins and aphid detoxification enzymes. Significant impacts based on the understanding of plant-aphid interaction, for the delivery of durable resistance to aphids in legume crops are emphasised.

Another major abiotic stress of legume crops is Fusarium wilt. The current situation in understanding of molecular defence responses of chickpea during *Fusarium interplay* are dealt within the next article (Gupta *et al.* 2013). This review presents the expandability and applicability of the defence responsive experimental findings from the model plant *Arabidopsis thaliana* to other crop species highlighting the important food legume, chickpea. Dynamic complexities of chickpea-*Foc1* interplay and sustainable solutions in wilt management programs have been briefly discussed in this review.

The research article by Morgante *et al.* (2013) focuses on the identification of candidate genes involved in peanut resistance to root-knot nematodes. Quantitative real time PCR (qRT-PCR) approach has been used in this study to look into the transcription expression profiles of selected candidate *Arachis* species genes. Significant differential expression of sixteen genes (observed in infected compared with non-infected roots) was reported in this article, providing information on candidate resistance genes involved in wild peanut resistance to *Meloidogyne* for use in peanut breeding program.

A review on water stress tolerance research emphasises water as the most important molecular component of research rather than ‘silver bullet’ single genes. The probability of success of specific traits or trait combinations across water stress scenarios is discussed in this review (Vadez *et al.* 2013). Comprehensive approaches to revitalise the development of breeding materials that are adapted to water stress considering realistic water limitations and their link to yield when designing biotechnological experiments are discussed in the review. The genetic aspects of traits related in part to increased, better or more conservative uses of soil water are emphasised.

Evaluation of peanut genotypes for identification of drought responsive mechanisms identified potential molecular mechanisms utilising a candidate-gene approach in five peanut

genotypes with contrasting drought responses (Dang *et al.* 2013). Two research articles addresses heat stress and drought stress in chickpea. Kaushal *et al.* (2013) screened a large core-collection of chickpea against heat stress and identified two heat-tolerant (ICC15614, ICCV. 92944) and two heat-sensitive (ICC10685, ICC5912) genotypes. These observations were confirmed by repeating the phenotypic observations in two season sowings. Important observations related to stomatal conductance, leaf water content, chlorophyll, membrane integrity and photochemical efficiency, pollen function impaired fertilisation and poor pod set were studied with respect to heat tolerant and heat-sensitive genotypes in this article. This study eventually resulted in identification heat tolerant and heat sensitive chickpea genotypes. Krishnamurthy *et al.* (2013) studied carbon isotope discrimination ( $\Delta^{13}\text{C}$ ) an important component of yield under drought conditions in chickpea reference collection. Observations on reduced shoot biomass by 36 to 39% and grain yield by 23% was reported in this article stating the importance of the component in drought stress. Furthermore association of carbon isotope discrimination with pod numbers and seed size under drought stress was confirmed in this study providing a significant finding to control drought stress in chickpea.

This issue provides a consideration of a range of topics, including available resources for legumes, their utilisations to discover novel genes, approaches to identify genes candidates, and understanding of the resistance mechanisms for different stresses. As such legume biology is worthy for further investigation to identify genes for traits of interest using ideal approaches for use in legume crop improvement.

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