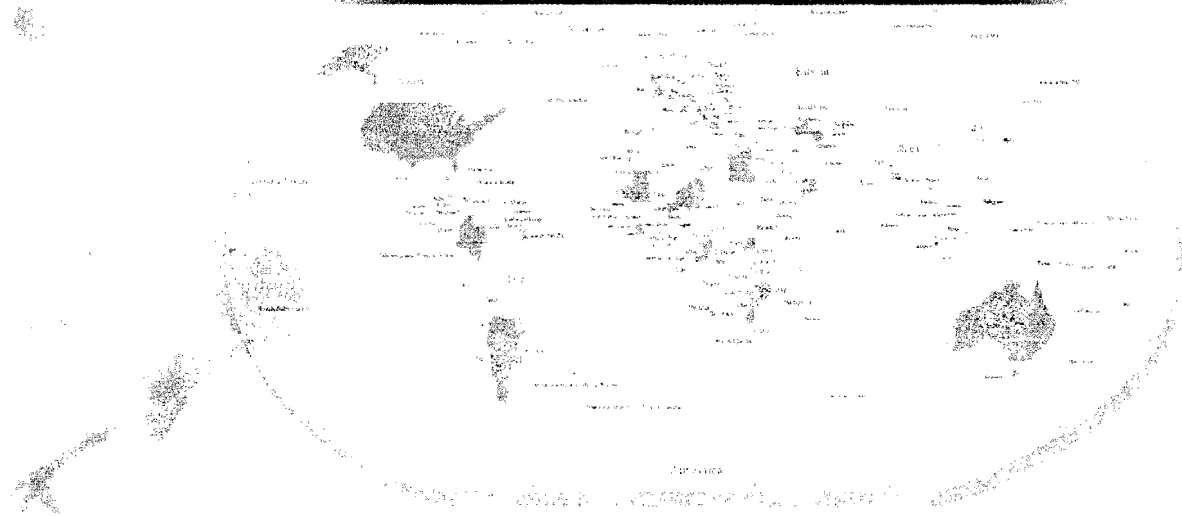


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Enhancing the value of legume genetic resources using core/mini core and applied genomic tools

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ABSTRACT

Grain legumes are rich resource of dietary proteins, minerals, and vitamins; however, productivity remains low, narrow genetic base of the cultivars being one of the several reasons to low productivity.

Worldwide approximately half a million legume germplasm accessions are preserved in genebanks. However, there has been limited use of these resources in crop breeding. Core collection (10% of the entire collection), a subset of accessions representing at least 70% of the genetic variation in the entire collection of the species, has been suggested as a gateway to enhance utilization of germplasm. Core and mini core (10% of core) collections have been reported in several legumes that when evaluated identified new sources of variation for agronomic traits including resistance to biotic and abiotic stresses.

Legumes genomics resources in the past lagged behind cereals. However, situation dramatically changed with emergence of *Medicago truncatula*, *Lotus japonicus*, and *Glycine max* as models for comparative genomics within legume family. Several genomic resources including markers, maps, transcriptomics, proteomics, metabolomics, and bioinformatics resources have been developed. These resources in model plants will not only allow investigation of basic processes important to legumes, but also open the possibility to transfer those processes to- or locate them in other crop species including several legumes. Several genomic projects are developing tools for less-studied legumes which are economically important in Africa and Asia.

These genetic and genomic resources represent major milestones in the history of legumes research, which will help understand the evolutionary events that shaped legume genomes, and provide avenues for genetic enhancement for sustainable agriculture.

I. Introduction:

Legumes represent the second largest family of higher plants after cereals. Leguminosae consists of about 20,000 species across 700 genera, traditionally divided into three subfamilies: Caesalpinoideae, Mimosoideae and Papilionoideae (Doyle and Luckow 2003). The two major groups of cultivated species in the Papilionoideae are the tropical or 'phaseoloid' legumes (*Phaseolus*, *Vigna*, *Glycine*, and *Cajanus*) and the temperate or 'galeoid' legumes (*Melilotus*, *Trifolium*, *Medicago*, *Pisum*, *Vicia*, *Lotus*, *Cicer*, *Lens*, and *Lathyrus*). Groundnut is somewhat distinct from the phaseoloid

and galegoid groups of grain legumes (Figure 1). Grain legumes are a rich source of protein, lysine, and essential vitamins and minerals. It also contains beneficial secondary compounds with significant health-promoting properties.

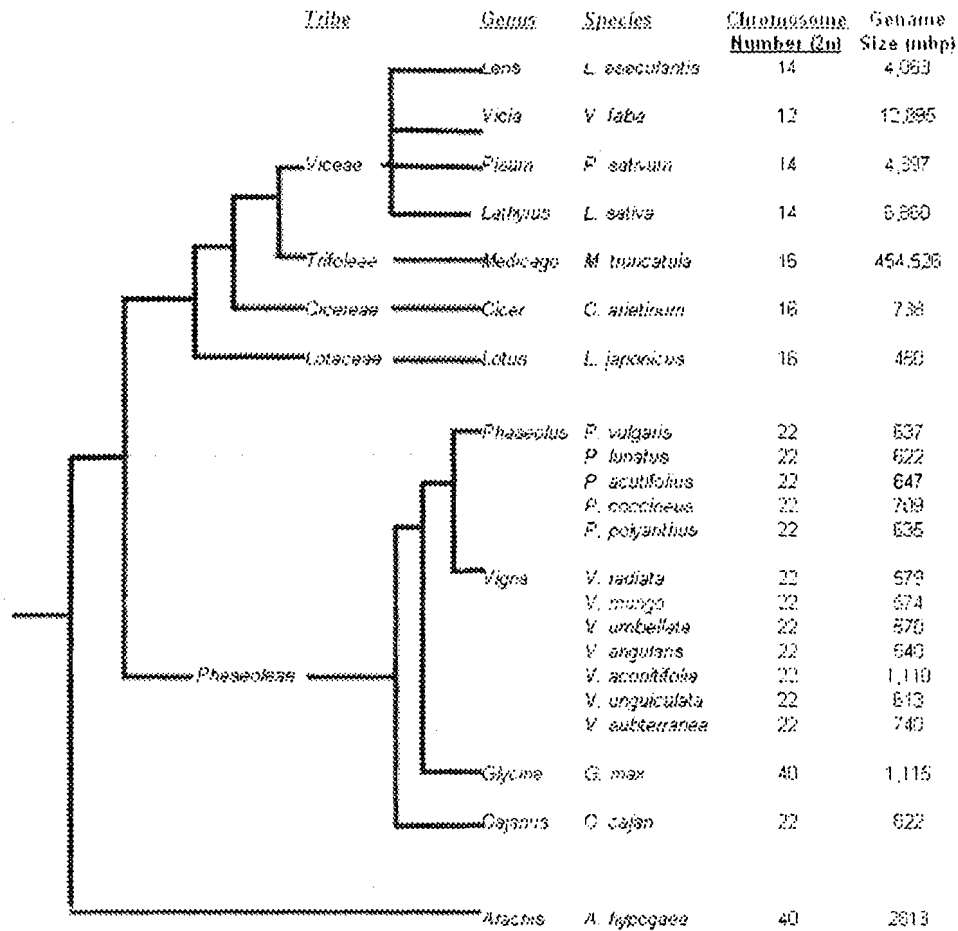


Figure 1. Phylogenetic relationship between the model legumes and major temperate and tropical legumes (Crouch and Dwivedi 2005)

The use of plant genetic resources in crop improvement is one of the most sustainable ways to conserve valuable genetic resources for the future, and simultaneously to increase agricultural production and food security. Key to successful crop improvement is a continued supply of genetic diversity in the breeding programs, including new or improved variability for target traits. Collectively, the CGIAR centers possess about 600,000 samples, of which, grain legumes represent about 30%, second only after cereals (65%). The Patancheru-based ICRISAT genebank in India contains 49,344 germplasm accessions of its three legume crops (chickpea, groundnut and pigeonpea), of which, 48,004 belong to cultivated and 1,340 wild species accessions.

The genetic base of legumes is narrow. In addition to bottlenecks associated with the origin and domestication events of these crops there has been limited use of germplasm in breeding programs in spite of the relatively large collections maintained in the genebanks, and because of the repeated use of the few germplasm/advanced lines for the development of new cultivars (reviewed in Upadhyaya et al. 2009). The reasons for the underutilization of germplasm include i) lack of accurate and precise large

scale multi-location evaluation of germplasm, (ii) the lack of rational systematic entry points into the vast international collections, and the lack of robust cost-effective tools to facilitate the efficient utilization of exotic germplasm in plant breeding programs (Dwivedi et al. 2005). Clearly, there is a need to identify germplasm with beneficial traits for the diversification of the genetic base in these crops.

II. Core and mini core collection: Crop germplasm collections held in genebanks are the best genetic resources for detailed characterization of important traits such as tolerance to biotic and abiotic stresses, yield, nutrition, and grain quality. However, the size of most crop-related global germplasm collections is simply too vast for systematic evaluation in replicated multilocal trials. The development of core collections (Brown 1989) has been shown to be a particularly powerful strategy for providing crop breeding programs with a systematic yet manageable entry point into global germplasm resources. Using passport, characterization and agronomic evaluation data, the core is constituted from the 10% of the entire germplasm collection, representing over 70% of the collections variability in that collection. Core collections are a cost-effective means of identifying accessions with desirable agronomic traits as well new sources of disease and pest resistance or abiotic stress tolerance. However, in crop species with several thousands of germplasm accessions, even a core collection would be unwieldy for evaluation by the breeders in the multi-location replicated trials. To overcome this, Upadhyaya and Ortiz (2001) suggested mini-core collection approach which is a core of core (10% of core or 1% of entire collection) representing the species diversity. Mini-core is established after evaluating the core subset for various morphological, agronomic, and seed quality traits, and selecting about 10% accessions from the core subset. At both stages in selection of core and mini-core collections, standard clustering procedures are used to separate groups of similar accessions combined with various statistical tests to identify the best representatives. Conventional core and mini core collections have been developed in chickpea (Upadhyaya et al. 2001; Upadhyaya and Ortiz 2001), groundnut (Holbrook et al. 1993; Upadhyaya et al. 2002, 2003; Holbrook and Dong 2005; Dwivedi et al. 2008a), and pigeonpea (Reddy et al. 2005; Upadhyaya et al. 2006). Core collections have also been reported in pea, lentil, common bean, and soybean (Dwivedi et al. 2005). More recently, genotype-based reference sets have also been developed in some legumes including chickpea, groundnut and pigeonpea (<http://www.generationcp.org>). Availability of these genetic resources sets offers immense opportunities to identify new sources of variation for use in crop breeding.

III. New sources of variation: Evaluation of core and mini-core collections has been suggested as the most efficient and reliable means of carrying out an initial search of germplasm collections for desirable traits. Such efforts have led to the identification of diverse germplasm with desirable agronomic traits including resistance to biotic and abiotic stresses in chickpea, groundnut, and pigeonpea (Table 1; ICRISAT Project 2 Archival Report 2008). New sources of variation have also been reported for agronomic traits including resistance to diseases and pests in pea, lentil, and common bean (Dwivedi et al. 2005).

IV. Enhancing trait value using wild relatives: When resistance to a particular disease or pest is not available in the cultivated germplasm, wild relatives of cultigens become often very handy. Resistance to many pests and diseases have been successfully transferred from wild relatives to agriculturally important crops including legumes (Dwivedi et al. 2005, 2008b). Wild relatives have also contributed alleles for agronomic traits in tomato and rice (Tanksley and McCouch 1997). Studies at ICRISAT and elsewhere are in progress to demonstrate this proof of concept in legumes. More recently, synthetic polyploids have been successful at generating diversity in wheat and *Brassica* species. The work is in progress to re-synthesize the cultivated groundnut using ancestral and related species for the introduction of new genes while minimizing the problem of sterility and suppression of recombination, both major constraints in the utilization of wild relatives in breeding (ICRISAT Project 2 Archival Report 2008).

V. Genomic resources in model and crop legumes

A. Model legumes: *Medicago truncatula* and *Lotus japonicus* have emerged as models for plant genomic research in legumes, relatively with compact genomes of approximately 470 Mbp. Scientists now study these species to investigate a range of questions from disease resistance to environmental tolerance and from bacterial and fungal symbiosis to complex secondary metabolism. However, both belong to temperate legume. For tropical legumes, soybean has emerged as a model genome. *M. truncatula* (<http://www.medicago.org/genome/>), *L. japonicus* (<http://www.kazusa.or.jp/lotus/>) and soybean (<http://www.phytozome.net/soybean>) genomes are currently the subject of independent large-scale sequencing projects. In addition, large-scale transcriptomics, proteomics, metabolomics, phenomics, and bioinformatics resources and reverse genetic tools have been developed. The characterization of these three legume genomes will undoubtedly enhance ongoing comparative genomic analyses (Szczyglowski and Stougaard 2008; Cannon et al. 2006; Anè et al. 2008; Rose 2008). The value of model systems will be enhanced by the ability to connect model systems to crops at structural and functional genome levels, for example, synteny between model and crop species should

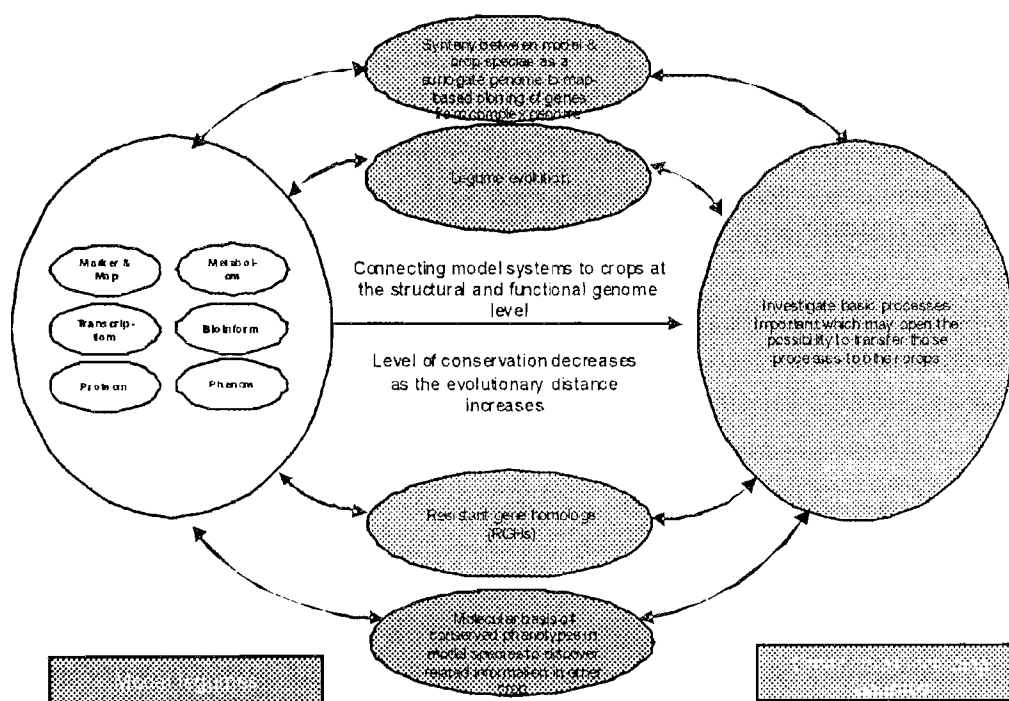


Figure 2. Translational genomics from model to crop legumes

allow the use of model species as a surrogate genome for map-based cloning of agronomically important genes in crops with complex genomes (Rose 2008) or identifying legume anchor markers to link syntenic regions across legumes (Hougaard et al. 2008). Thus, the genetic, genomic, and molecular tools available to these model plants allow not only investigation of basic processes important to legumes, but also transfer that processes to other crop species (Anè et al. 2008; Figure 2).

B. Crop legumes: Legumes which form a coherent taxonomic group with frequent and widespread macro- and micro-syteny, however, have huge variation in nuclear genome size, ranging from 370 million base pair (Mbp) in *Lablab niger* to 13,000 Mbp in *Vicia faba* (Arumugunathan and Earle 1991; <http://www.rbgekew.org.uk/cval/homepage.html>). Black gram, mung bean, common bean, lima bean, tepary bean, and cowpea have the smallest genomes (574 Mbp to 647 Mbp); pigeonpea (784, 882 Mbp) and chickpea (738 Mbp) have slightly larger genomes; soybean has a relatively large genome (1115 Mbp); while pea and lentil (4063 Mbp to 4397 Mbp) and broad bean (12,603 Mbp) have massive genome sizes. The whole genome duplication and segmental duplications appear to have played a significant role in creating new diversity in higher plants including the legumes (AGI 2000; Vision et al. 2000). Over the years, there have been several publications reporting the development of a range of genomic resources including markers; ESTs and BAC libraries; genetic, cytogenetic and physical maps; identification of QTL associated with beneficial traits, and use of these resources in applied breeding. In recent years, tremendous progress has been made towards developing genetic markers (especially SSRs and SNPs) and/or construction of high-density genetic linkage maps in chickpea, groundnut, and pigeonpea (Table 2; Varshney et al. 2009), which will enable researchers to more rapidly and precisely characterize genetic diversity, identify trait-based genetically diverse germplasm, target genes underlying key agronomic traits, and develop molecular assays that are both relevant and of appropriate scale for breeding applications. More importantly, high throughput and cost effective genotyping platforms, combined with automation in phenotyping methodologies, will increase the uptake of genomic tools into breeding programs, and thus usher in an era of genomics-enabled molecular breeding in legumes (Varshney et al. 2009).

Table 1. New sources of variation identified after evaluating core/mini core subsets in chickpea, groundnut, and pigeonpea

Trait	Chickpea	Groundnut	Pigeonpea
Diseases	FW - 67 AB - 3 BGM - 55 DRR - 5	GRD - 3 ELS - 1 BW -14 Aflatoxin - 5	SM - 28 FW - 4 PB - 78 Multiple resistance -2
Drought	18	18	
parSalinity	12	12	16
High temperature	5		
Low temperature		158	
Early maturity	28	21	20
Seed size	16	7	
Grain yield	39	60	54
Protein content	5	5	

FW= Fusarium wilt, AB=Ascochyta blight, BGM=Botrytis gray mold, DRR=Dry root rot, GRD=Groundnut rosette disease, ELS=Early leaf-spot, BW=Bacterial wilt, SM=Sterility mosaic, PB=Phytophthora blight

Table 2. Genomic resources in chickpea, common bean, cowpea, groundnut and pigeonpea (Source: Modified from Varshney et al. 2009)

Genomic resource	Cowpea	Chickpea	Groundnut	Pigeonpea	Common bean
Ploidy	2n = 22	2n = 16	4n = 40	2n = 22	2n = 22
Genome size (Mbp)	620	740	2890	858	637
SSR	768	~2000	~2700	~3200	~500
BAC	6-17X	3.8 - 10X	4X - 7.4X	11X	10-20X
BAC-end sequences	50,120 (36.7Mbp)	46,270 (33.2Mbp)	41,856 (28.6 Mbp)	85,785 (56.5 Mbp)	89,017 (62Mbp)
Genetic maps (bc)	++	++	AA (2X) genome: ++	No	++
Genetic maps (nc)	+	+	BB (2X) genome: ++ AABB (4X): +	No	+
Physical map	Yes	No	In progress (AA genome)	No	Yes

bc - broad crosses; nc - narrow crosses

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