# Legume Crop Genomics

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Chapter 4

# Genomics and Genetic Diversity in Common Bean

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## The Agronomic and Experimental Importance of *Phaseolus vulgaris*

Common bean (*Phaseolus vulgaris* L.; *2n = 2x* = 22) is the most important edible food legume. It represents 50% of the grain legumes consumed worldwide. In some countries, such as Brazil and Mexico, it is the primary source of protein in the human diet. As such, common bean is a very important nonprocessed food crop in third world countries and contributes significantly to the world's protein diet. The impor tance of this crop as an international protein source is reflected by the fact that the dry bean export market alone has a value of $1.8 billion to the U.S. economy (1). In addition, the cash value of the crop at the U.S. farm gate is $1 billion. Lately, domestic bean consumption has increased because of the rising importance of ethnic foods, high levels of certain minerals and vitamins, and the perceived health bene fits related to the blood-cholesterol-lowering effects of beans (2,3).

Many genomic features contribute to the attractiveness of common bean as an experimental crop species. The genome size, estimated to be about 450 to 650 million base pairs (Mb)/haploid, is comparable to rice (4), which generally is considered to have the smallest genome among major crop species. Nearly all loci are single copy (5-7), and the traditionally large families, such as resistance gene analogs (8) and protein kinases (9), are of moderate size.

From a population genetics perspective, the major subdivisions of wild common bean progenitors are known, and the domesticated gene pools have been defined. Based on phaseolin seed storage protein variation (10,11), marker diversity (12,13), and morphology (14), two major gene pools of wild common bean were identified. The Middle American gene pool extends from Mexico through Central America and into Colombia and Venezuela, whereas the Andean gene pool is found in southern Peru, Chile, Bolivia, and Argentina. The two domesticated gene pools appear to converge in Colombia (10). Based on a novel phaseolin type, a third, possibly ancestral gene pool based in southern Ecuador and northern Peru was described (15.16).

Two major domestication events, one in Mesoamerica (possibly west-central Mexico) and the other in the southern Andes, appear to have resulted in the Middle American and Andean gene pools that mirror the geographic distribution of the wild progenitors. Following domestication, gene pool divergence led to the development



of three races within each of the two major domesticated gene pools (17). The Middle American gene pool, consisting of races Durango, Jalisco, and Mesoamerica is represented by the medium and small seeded pinto, pink, black, white, and some snap beans. The Andean gene pool, consisting of races Nueva Granada, Peru, and Chile, is represented by the large-seeded kidney, cranberry, and many snap beans.

*Phaseolus* research benefits from the existence of two extensive germplasm collections: one at the USDA Plant Introduction Station in Pullman, WA (about 13,001 accessions), and the other at the International Center of Tropical Agriculture (CIAT) in Colombia (about 25,000 accessions). Each collection is freely distributed. Both collections include a broad sample of wild and domesticated *P. vulgaris,* and core collections representing the genetic diversity of the species are available (18,19). Such collections represent a rich source of genetic variability to study species-wide divers it and to apply new genetic marker tools to uncover new sources of disease resistance.

## *Phaseolus* Phylogeny

Recent plant phylogenies are primarily derived using sequence data from genes representing each of the three plant genomes. As with all nitrogen-fixing species, th data have placed *Phaseolus* as a member of the Fabaceae (also known a Leguminosae) family. The family is one of three (along with Polygalaceae an Surianaceae) that comprise the order Fabales. In turn, the Fabales are one of the four orders of the Eurosid I clade. The Fabaceae is subdivided into three subfamilies, an *Phaseolus* is a member of the Papilionoideae subfamily. This is by far the largest subfamily, consisting of 476 genera (20), and all of the main economic legume cropare found among the nearly 14,000 species of this subfamily. It is estimated that this subfamily appeared about 50 million years ago (my a) (21), and it resolves as monophyletic with weak support (22,23).

Four main groups are found within the Papilionoideae subfamily. Two of these, the Hologalegina and Phaseoloid/Millettoid groups, appear to be sister taxa that appeared 35 to 40 mya. Tribes that contain important economic species are assigned to eac group. The primary tribe within the Hologalegina group, the IRLC tribe, contains the *Pisum, Cicer, Trifolium,* and *Medicago* genera. Estimates place the appearance of th tribe at about 25 mya. The Phaseoloid/Millettoid group contains the Phaseoleae and Millettieae tribes. The Phaseoleae tribe is economically important because it contains *thePhaseolus, Vigna,* and *Glycine* genera. Further analysis places *Phaseolus* and *Vigna* in the Phaseolinae subtribe, whereas *Glycine* is a member of the Glycininae subtribe.

The most thorough phylogeny of the genus *Phaseolus w*as developed by Delgado-Salinas *et al.* (24) using 5.8S rDNA, the corresponding internal transcribed spacer regions one and two, and morphological data. The two species *Macroptilium atropurpureum* (DC) Urb. and *M. erythroloma* appear to be the most related species to the genus *Phaseolus.* Further, all of the 55 species in the analysis formed a monophyletic clade. Within this clade, *P. microcarpus* forms the earliest branch. A total of nine species groups were defined. Among the cultivated species, *P. lunatus* L. was in

a group with other South American and oceanic island *Phaseolus* species. The other four cultivated species, *P. vulgaris* L, *P. coccineus* L, *P. polyanthus* (L) B.S.P., and *P. acutifolius* A. Gray were members of the same group. This research is significant because it provides an experimental basis upon which future hypotheses regarding *Phaseolus* phylogeny can be drawn when other genes are considered.

## DNA Sequencing

To compare sequence data available for *Phaseolus* with that available for other members of the Fabaceae family, we performed a series of searches of the Genbank database (summarized in Table 4.1). Currently, nearly 700,000 Fabaceae nucleotide sequences are found in GenBank. Because of the significant recent funding, 87% of the sequences are expressed sequence tag (EST) products. Of total Fabaceae sequences, 76% are *Glycine max* (L) Merr. and *Medicago truncatula* Gaertner EST sequences. A limited number of EST sequences are available for *Phaseolus.* Twenty thousand one hundred twenty ESTs represent globular stage gene expression in *P. coccineus* (25). The next largest class of sequences in the database represents sequences obtained from genomic surveys. The vast majority of these are end sequences of bacterial artificial chromosome (BAC) and transformation competent bacterial artificial chromosome (TAC) clones. For this class of sequences, 60% represent TAC end sequence data from *Lotus corniculatus* L (26), whereas 26,000 BAC sequences are available for G. *max* (27). For *Phaseolus*,

**TABLE 4.1**

Summary of Fabaceae Nucleotide Sequence Found in GenBank (13 Dec., 2003) (Nucleotide Queries Submitted to Entrez at NCBI, www.ncbi.nlm.nih.gov)

| | Total[a] | EST[b] | Genomic survey[c] | Gene CDS[d] | Gene complete CDS[e] |
|---|---|---|---|---|---|
| Fabaceae | 696,895 | 601,418 | 77,152 | 5,592 | 1,858 |
| *Arachis hypogaea* | 1,563 | 1,346 | 0 | 67 | 24 |
| *Cicer arietinum* | 513 | 25 | 0 | 217 | 0 |
| *Glycine max* | 374,174 | 344,542 | 26,208 | 1,411 | 642 |
| *Lotus corniculatus* | 83,450 | 36,311 | 46,569 | 162 | 45 |
| *Lupinus* spp. | 2,897 | 2,492 | 0 | 159 | 81 |
| *Medicago sativa* | 1,548 | 879 | 0 | 329 | 106 |
| *Medicago truncatula* | 192,804 | 187,763 | 3,885 | 241 | 74 |
| *Phaseolus* spp. | 22,028 | 20,807 | 162 | 443 | 119 |
| *Phaseolus vulgaris* | 1,592 | 575 | 162 | 375 | 96 |
| *Pisum sativum* | 4,623 | 3,037 | 154 | 747 | 305 |
| *Vigna* spp. | 888 | 207 | 89 | 224 | 115 |
| *Vicia* spp. | 588 | 1 | 65 | 164 | 35 |

[a]query: species [orgn].
[b]query: species [orgn] est cdna.
[c]query: species [orgn] genomic survey.
[d]query: species [orgn] cds NOT est.
[e]query: species [orgn] complete NOT est.



all of the genomic survey sequence data represent the work of Murray *et al.* (28). They sequenced and annotated both ends of the Bng RFLP clones originally used to develop the Florida linkage map (5).

EST data provide a very useful glimpse of the expressed portion of genes in the genome. The data provide a catalog of potential genes to be found once a genome is sequenced. Because EST sequences are named by standard annotation procedures, such as BLAST analysis, they do not represent experimental data. In contrast, GenBank has a large collection of Fabaceae coding sequences (CDSs) that were characterized using genetic or biochemical procedures to confirm the nature of the gene product. If the sequence extends from the start to a stop codon, it is labeled a complete CDS. About one - third of the Fabaceae CDS are complete CDS. Of all the Fabaceae species, G. *max* and *Pisum sativum* L contain the most completely described sequences.

With 119 genes, *Phaseolus* contains more experimentally defined complete genes than the recently studied model legumes *M. truncatula* (74 genes) and *L. corniculatus* (45 genes). This is certainly indicative of the long history of *Phaseolus* as an experimental organism. For example, the *P. vulgaris* phaseolin storage protein gene was the first plant gene shown to contain an intron (29). The extensive analysis of this gene family has provided important information regarding gene expression in plants (30). Early studies on such *Phaseolus* genes as phenylalanine ammonia lyase (31), chitinase (32), chalcone synthase (33), and chalcone isomerase (34) provided important insight into the physiological response to disease attack in plants.

A number of experiments have positioned genes on the *P. vulgaris* linkage map. Murray *et al.* (28) obtained end sequences of the Bng RFLP clones. Subsequent annotation using the sequences as query for Blast searches of GenBank (Blast E-value < 1 x 10-5) identified 87 genes. Because the Bng clones have previously been mapped (5), the genetic location of these genes is known. Yu *et al.* (35-37) and Blair *et al.* (38) used deposited GenBank sequence data to discover simple repeats that were used to develop microsatellite makers. The mapping of more than 50 of the microsatellite markers has genetically defined the position of these genes. In addition, Rivkin *et al.* (8) defined the genetic location of nine disease-resistance-related sequences, whereas Ferrier-Cana *et al.* (39) placed eight similar genes at a single gene cluster. Collectively, the genetic location of nearly ISO genes is now available in *Phaseolus.*

For a species without a major current genomic program, other approaches are necessary for gene discovery. One of the most useful is homology-based PCR cloning procedures, which can provide insights into the evolutionary relationship of *Phaseolus* genes to other plant genes. Rivkin *et al.* (8) used this procedure to clone nine *Phaseolus* resistance gene analogs (RGA) homologous to previously cloned resistance genes. Each RGA exhibited a higher degree of homology with a sequence from soybean than another *Phaseolus* RGA (8), demonstrating that ancestor legumes contained lineage specific sequences that were transmitted. Because many other Fabaceae RGA sequences recently have been reported, we reanalyzed the data by including those additional sequences. The *Phaseolus* RGA sequences are found in five highly supported different lineages (Fig. 4.1), and each lineage consists of multiple legume species. This confirms previous observations regarding the evolution of this important gene family in legumes.

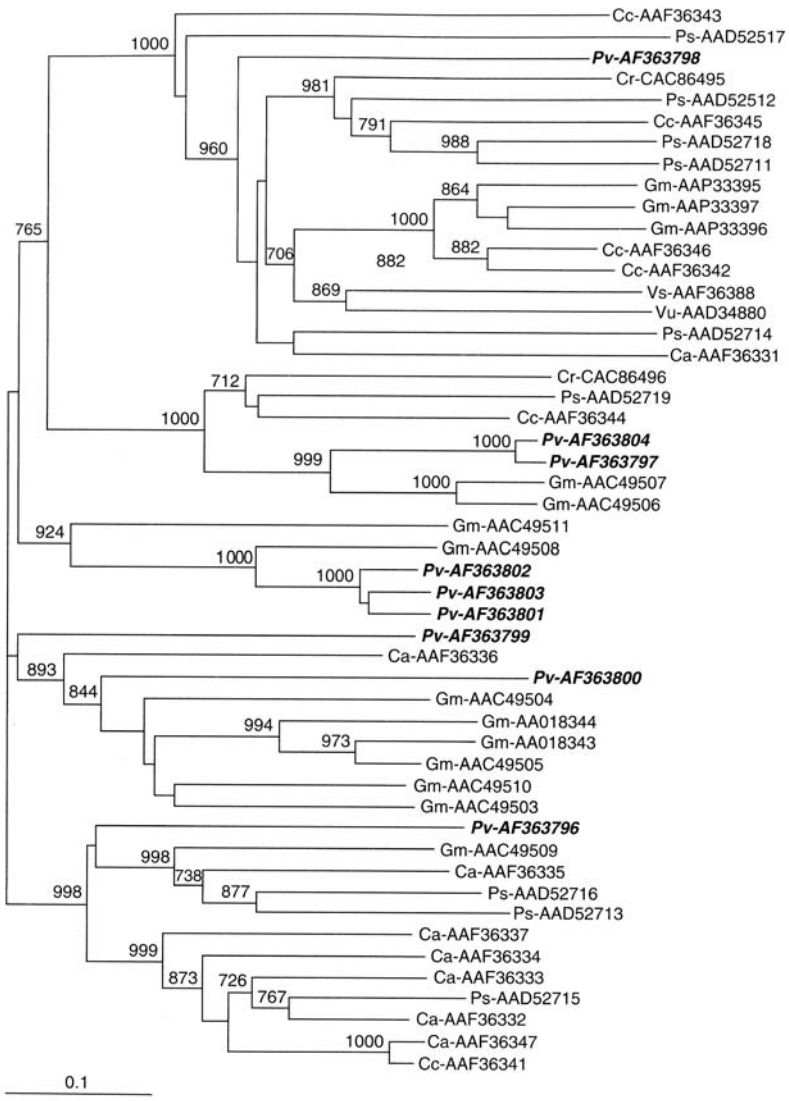


**Figure 4.1.** Phylogenetic analysis of resistance gene analog sequences in the Fabaceae. RGA amino acid sequences for Fabaceae species that ran from the P-loop to the kinase 2a and 3 domains were selected from GenSank. These were aligned, and neighbor-joining analysis was performed. Those nodes supported with bootstrap values great than 700/1000 are noted. Each RGA is designated by its species abbreviation followed by the GenBank accession number. The abbreviations are: Ca *(Cicer arietinum),* Cc *(Cajanus cajan),* Gm *(Glycine max),* Ps *(Pisum sativum),* Pv *(Phaseolus vulgaris*), Vs (*Vigna subterranea*), and Vu (*Vigna unguiculata*). The *Phaseolus* sequences are in bold italics.



A similar approach was used to study various kinases in *Phaseolus.* By carefully designing primers to the catalytic domain, Vallad *et al.* (9) cloned 25 sequences related to the *Pto* resistance gene from tomato, the only resistance gene known to exclusively contain kinase functionality. This approach successfully cloned *Pto-like* kinase sequences that were distinct from the many kinase known to exist in eukaryotic genomes. Phylogenetic analysis also determined that the *Pto-like* proteins form a distinct class of kinases. **In** addition, this research revealed a highly conserved kinase subdomain unique only to plant species.

These PCR-based cloning experiments focused on the relationship of *Phaseolus* to other legumes. Recently, McClean *et al.* (40) used intron sequences to study intraspecies and intergenic relationships with *Phaseolus.* Unique insertion/deletion events and nucleotide polymorphisms defined 20 *P. vulgaris* haplotypes, and small red Middle American beans formed a distinct group not seen when other sequences are used to study relationships within the species. Collectively, these experiments describe the overall utility of PCR-based gene cloning to extend the number of genes cloned in *Phaseolus.*

### *Genetic Markers*

Allozymes, seed proteins, restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPDs), amplified fragment length polymorphic DNA (AFLPs), microsatellites, and inter-simple sequence repeats (ISSRs) have been used to locate genes in common bean. The major applications have been the elucidation of geographic patterns of genetic diversity, molecular linkage mapping, marker-assisted selection, the development of contigs, and positional cloning of genes or gene clusters.

Allozymes were first described in common bean by Kami *et al.* (16,41-42). Further analyses were conducted by Koenig and Gepts (13), Singh *et al.* (43), and Debouck *et al.* (15). The major contribution of allozymes was to clarify the geographic boundaries of major gene pools in wild common bean. They helped define that wild beans of Colombia actually belong to the Mesoamerican gene pool, whereas the wild beans of Ecuador and northern Peru belong to an intermediate gene pool distinct from the Mesoamerican and Andean gene pools (15).

Allozymes also have been useful to identify eco-morpho-geographical races in common bean, particularly in the Mesoamerican gene pool. When specific allozymes were used as prior classification criteria for canonical analyses of phenotypic data, distinct groups were revealed that had characteristic morphological and agronomic traits and were distributed in different ecogeographica1 areas (44). These groups have been formalized as races, with three races each in both the Mesoamerican and Andean gene pools (17).

Isozymes have also been used to characterize genetic diversity in other *Phaseolus* species, notably runner bean *(P. coccineus)* (45-47), tepary bean *(P. acutifolius)* (48,49), and lima bean *(P. lunatus)* (50-54). In spite of their usefulness in identifying overall patterns of genetic diversity, isozymes have largely been superseded by markers that are more numerous, polymorphic, and better distributed in the genome.

Seed protein analyses have considered primarily the two largest seed protein fractions: phaseolin and the APA proteins (arcelin, phytohaemagglutinin, and

α-amylase inhibitor). Both are coded by a single, although complex, locus, consisting of multiple genes in tandem (6,55-58). Phaseolin electrophoretic diversity has been particularly useful to demonstrate the existence of multiple domestications in common and lima beans (10,59) and of a single domestication in tepary bean (60). The value of these markers is that different electrophoretic types can reflect multiple changes at the molecular level. Therefore, independent mutations leading to the same electrophoretic type are unlikely, and each type may have a single origin (61).

Both the phaseolin and APA loci code for important agronomic traits. Phaseolin is the major seed storage protein in common bean. The phaseolin locus is not only a structural locus for phaseolin (62,63) but also a quantitative trait locus (QTL) for phaseolin levels in the seed and for seed weight (64). Based on Sax's description of bean seed color phenotypes, it is likely that the seed weight QTLs actually correspond to the phaseolin locus, given its linkage with the *P* locus, which controls flavonoid pigments in the bean plant (64,65). The APA locus contains sequences that provide resistance against seed weevils (56,66,67). Both the protein and the corresponding DNA sequence have been used as markers for direct selection in breeding either for increased phaseolin or to transfer bruchid resistance from wild to domesticated beans (62,63,68). **In** spite of their advantages as a biochemical marker, seed proteins suffer the same disadvantage as allozymes in that their genome coverage is limited.

Molecular markers, based on direct or indirect DNA sequence analyses, have increasingly become the marker of choice in beans. **In** common bean, RFLPs have been used to confirm patterns of genetic diversity identified previously with biochemical markers. They confirmed the multiple domestication scenario for this species (12). RFLPs, however, were principally used as framework markers to develop molecular linkage maps in common bean. The maps of Vallejos *et al.* (5), Gepts *et al.* (69), Nodari *et al.* (70), and Adam-Blondon *et al.* (71) were based primarily on RFLPs, although they also contained isozyme and seed protein markers. Recently, these maps have been correlated into a consensus map based on the recombinant inbred population BAT93 x lalo EEP558 (Fig. 4.2) (6,72). Five hundred RFLP markers have been mapped in common bean, RFLP maps were instrumental in gaining insight into the inheritance of resistance to common bacterial blight (70,73,74). **In** addition, common bean was the first dicot in which the inheritance of the domestication syndrome was analyzed by **QTL** analysis (65). Recently, 150 RFLP markers have been transformed into sequence-tagged sites or SSRs (28).

RAPDs have been used extensively to develop molecular linkage maps but also to characterize genetic diversity. Although this type of marker suffers from lack of reproducibility among laboratories, it can be used within one laboratory with more consistent results. Numerous maps based primarily on RAPDs have been developed. primarily to map genes controlling disease resistances and to tag specific disease resistance genes (75,76). Some of the RAPD markers tagging disease resistance genes have been transformed in sequence-tagged sites (35,77-81).

RAPD markers have also been used to investigate genetic diversity and relationships in the common bean gene pool, not only in the centers of origin (81-84) but also outside the centers (83,85). One of the more salient findings is the identifi



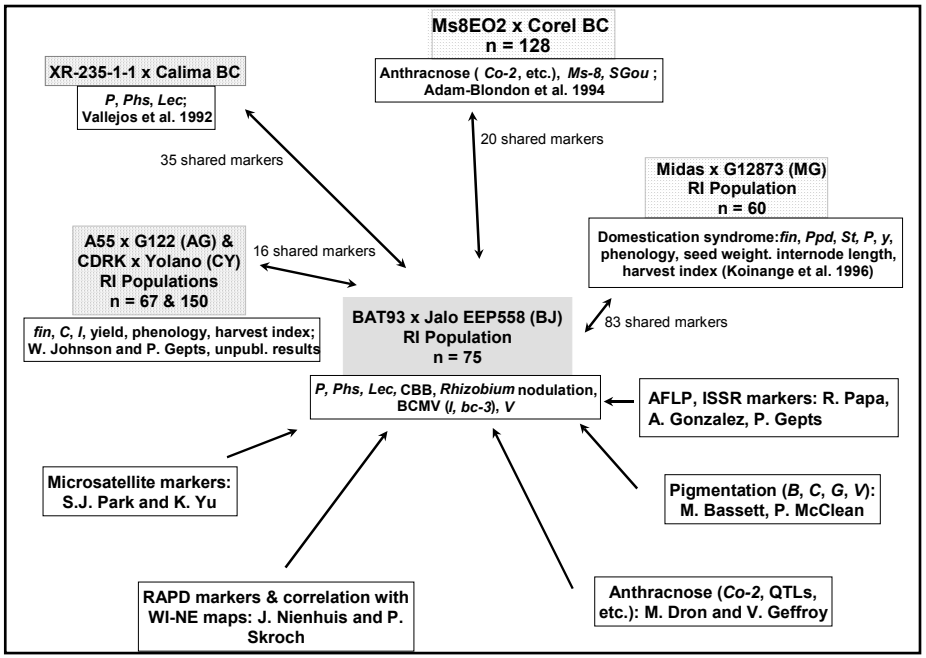


**Figure 4.2.** The central role of the BAT93 x Jalo EEP558 recombinant inbred population in the integration of molecular linkage maps of common bean (146).

cation of a fourth Middle American race, Guatemala (82). Freyre *et al.* (86) used RAPDs to determine the genetic relationships among wild common bean spanning the distribution range from northern Mexico to northwestern Argentina. They showed that although the Mesoamerican gene pool is geographically structured with Mexican, Central American, and Colombian components, the southern Andean gene pool was apparently unstructured. The intermediate gene pool (Ecuador and northern Peru) was distinct from the Mesoamerican and southern Andean gene pools.

One of the solutions to the lack or reproducibility of RAPDs has been the use of other PCR-based markers with longer primers. AFLPs have proven very useful to characterize wild common bean and lima bean germplasm (19,87) in order to infer the predominant direction of gene flow between domesticated and wild beans as well as to compare the spatial differentiation of domesticated and wild beans (88), and to document the high level of polymorphism and the ecogeographical differentiation among farmer-grown varieties in central Italy (89). **In** addition, they have been useful to distinguish closely related common bean genotypes for DNA fingerprinting (R. Papa and P. Gepts, unpublished data). Because AFLPs quickly generate a high number of markers, they have been used to develop low-density linkage maps, as well (90,91).

Microsatellite or SSR markers have been developed in recent years from published sequences (37,38,92) and from microsatellite-enriched libraries (38,93,94). These molecular markers (about 150) have been used principally to increase the density of existing

maps, especially the core linkage map established in the BAT93 x Jalo EEP558 (36,38). Additional microsatellite markers are needed, however, to increase their density on the linkage map. In addition, targeted identification of microsatellites tagging genes of agronomic importance should be conducted, and the codominance of these markers should be utilized to further characterize genetic diversity in common bean. One the questions raised is the extent of homozygosity in a predominantly selfmg species such as common bean in contrast with a predominantly outcrossing species such as runner bean, *P coccineus.*

About 15 molecular linkage maps have been established in different mapping populations in common bean (reviewed in 75,76). Genes, whether major genes or QTLs, for a wide variety of traits have been located or tagged on these maps. Traits include, but are not limited to: disease resistance (common bacterial blight (5,70,95-98), halo blight (95), white mold (99-102), anthracnose (71, 103-113), rust (77,96,114-118), bean common mosaic virus (5,70,95,119), *Fusarium* root rot (78,120)); seed bruchid resistance (56,70); growth habit (65,91,121); seed weight (65,122); seed color (7,123-128); yield (90); canning quality (129); and seed composition (62,63,130).

Generally these are low-density maps, but they have allowed breeders to gain an understanding of the inheritance of disease resistance-for example, in different tissues or genetic backgrounds or when challenged with different strains. Mapping analyses have shown that the major genes for domestication are concentrated on three to four linkage groups (65). As in other species, genes for disease resistance appear to be clustered, and some IS putative clusters have been identified (76). Resistance gene analogs (RGAs) have been identified, but so far they overlap only partially with the disease resistance gene clusters (8), suggesting that additional disease resistance genes and RGAs need to be mapped.

Molecular markers have also been used to characterize the genetic diversity of organisms associated with the bean host. A parallel distribution of genetic diversity in the host and the organism suggest a possible coevolution as shown for the angular leaf spot pathogen (131,132). Indeed, there are two major gene pools for this pathogen, which are generally more virulent on the host from the same geographical origin, i.e.. Mesoamerica and the Andes. Similar observations have been made for other pathogens. such as anthracnose, rust, and common bacterial blight. This coevolution provides excellent opportunities to study coevolution at the molecular level and to follow the evolution of disease resistance genes and their diversification process. For anthracnose, a cluster on linkage group B4 includes both Mesoamerican and Andean resistance specificities, suggesting that this cluster preexisted the divergence from the ancestral gene pool in Ecuador and northern Peru (109,110).

## Bacterial Artificial Chromosome Libraries

BAC libraries are large-insert libraries that have been very useful in cloning large portions of genomes in common bean (133), pearl millet (134), sugarbeet (135), cotton (136,137), sunflower (138), and maize (137-139). Insert sizes are generally from 100 to 150 kb. Availability of such libraries allows researchers to develop a physical map of the genome of choice by developing contigs of overlapping clones (140). In turn, this physical map can be related to the genetic map by locating existing genetic



markers on the contigs and, conversely, locating markers obtained from analysis of the contigs onto the genetic map (141). Contigs become a starting point for positional cloning of specific genes (142,143).

A correlation of physical and genetic maps can accelerate the discovery of genes underlying phenotypes of agronomic interest (142). It can also help in identifying more closely linked markers for marker-assisted selection (MAS). Furthermore, BAC clones can be used as probes in in *situ* hybridizations of chromosomes (144). In common bean (P. *vulgaris),* BAC libraries have been developed previously by Vanhouten and Mackenzie (133) for an Andean cultivar Sprite FR, and by Kami and Gepts (145) for the Mesoamerican breeding line BAT 93. In common bean, initial *in situ* hybridizations have been performed to kayotype the common bean genome and relate the cytological, genetic, and physical maps (146).

A compelling aspect of the biology of common bean is the existence of information regarding its ancestry and phylogeny (Fig. 4.3) (24,147). Domesticated common bean consists of two major geographic gene pools, Andean and Mesoamerican,

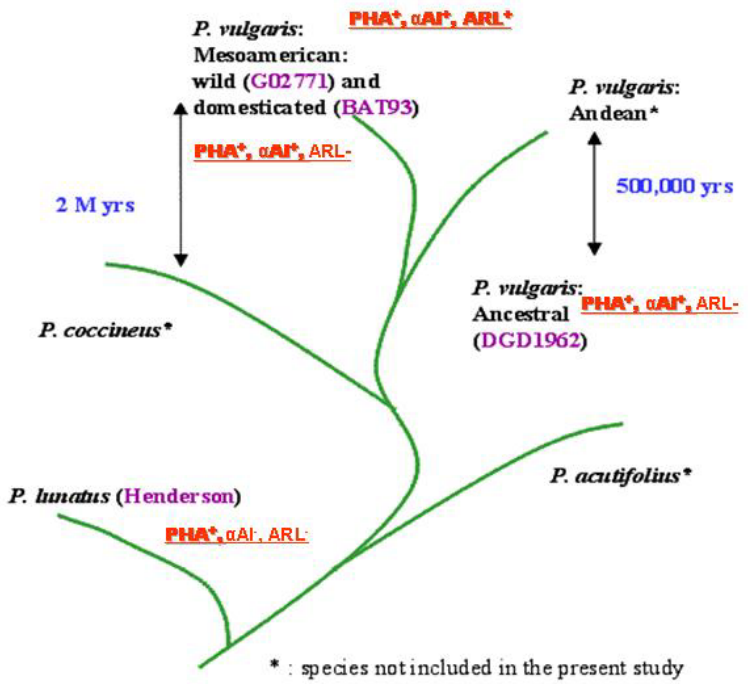


**Figure 4.3.** Phylogenetic and genealogical relationships among domesticatec Phaseolus species (summarized from 161,162). BAC libraries have been developed ir P. *vulgaris* genotypes GO2771, BAT93, and DGD1962 and in P. *lunatus* Henderson +PHA: phytohaemagglutinin; αAI: a-amylase inhibitor; ARL: arcelin. *Libraries in these taxa exist or should be developed to complete the model.

originating from independent domestications in the southern Andes and Mesoamerica. In turn, the wild common bean gene pools in these two areas are derived from a Common ancestor located in the Andes Mountains from Ecuador and northern Peru (147). Several *Phaseolus* species, some of which have also been domesticated, are closely related to common bean (Fig. 4.1). These include the runner bean *(P. coccineus),* the year bean *(P. polyanthus,* a hybrid between a proto-P. *vulgaris* and *P. coccineus),* and the tepary bean *(P. acutifolius)*. All the preceding species belong to the *P. vulgaris* clade or lineage within the genus *Phaseolus*. A more distantly related species is lima bean *(P. lllnatus),* which belongs to a different clade altogether (24).

To analyze microevolutionary changes in genome structure, four BAC libraries are currently being developed in four different *Phaseolus* genotypes. Specifically, we want to follow the evolution of the APA complex locus on linkage group B4 (75). APA is a family of closely related seed proteins: α-amylase inhibitor, phytohaemagglutinin, and arcelin. Phytohaemagglutinin (PHA) proteins are widespread among all legumes, including *Phaseolus.* In the *P. vulgaris* lineage, the α-amylase inhibitor subfamily (αAI) appeared by duplication and divergence. Finally, in the Mesoamerican branch of *P. vulgaris,* some wild beans show a third subfamily, that of the arcelins (ARL). Consequently, the four BAC libraries are being developed in selected genotypes that encompass the entire evolution of the APA proteins in the *Phaseolus* genus. The four genotypes include *P. lunatus* cv. Henderson ($PHA^+\alpha AI^-ARL^-$), *P. vulgaris* wild DGD I 962 ($PHA^+aAI^+ARL^-$), *P. vulgaris* Mesoamerican domesticated BAT93 ($PHA^+\alpha AI^+ARL^-$), and *P. vulgaris* Mesoamerican wild G02771 ($PHA^+\alpha AI^+ARL^+$). These complement the BAC library developed in cv. Sprite of Andean origin (133).

For each of the libraries, high molecular weight DNA was obtained after nuclei isolation (148). Following partial digestion with *Hin*dIII and electroelution (149), the DNA was ligated into the pIndigoBAC5 vector (Epicenter Technologies) and transformed into electrocompetent *E. coli* cells (Strain DHlOB ElectroMax cells, Life Technologies). Clones have been distributed in 384-well plates and arrayed on high-density membranes. The characteristics of the libraries are presented in Table 4.2. Based on a conservatively large genome size of 637 Mb

**TABLE 4.2**
Main Characteristics of P. *vulgaris* BAC Libraries Developed at UC-Davis

| Library | Number of clones | Size | Genome equivalents | Empties % | Chloroplast # | Chloroplast % | APA # | Phaseolin # |
|---|---|---|---|---|---|---|---|---|
| | *Phaseolus vulgaris* | | | | | | | |
| BAT93-A | 36,864 | ~110 | 5.7 | 11 | 21 | 0.05 | 5 | 6 |
| BAT93/*Hin*dIII | 110,592 | 125 | 20.8 | <0.5 | 50 | 0.04 | 16 | 13 |
| DGD1962 | 52,608 | 105 | 8.7 | 1.40 | 200 | 0.4 | 10 | 11 |
| G02771 | 55,296 | 139.4 | 12.1 | <0.5 | 49 | 0.08 | 38 | 14 |
| | *Phaseolus lunatus* | | | | | | | |
| "Henderson" | 55,296 | ~130 | 11.3 | <0.5 | 16 | 0.03 | ND | ND |



(150,151), the genome coverage ranges from 6x to 12x. There was generally a low frequency of empty and chloroplast DNA clones.

## Transformation

Common bean has been transformed by several methods. These include electric particle discharge (152), *Agrobacterium* (153-155), and particle bombardment (156,157). Recent improvements in the transformation efficiency in *P. acutifolius* (153) suggest that improvements in *P. vulgaris* can be expected as well either by direct improvements in the method as applied to *P. vulgaris* or by sexual transfer of the transform ability trait to *P. vulgaris* (A. Mejia, CIAT, Cali, Colombia, personal communication). An alternative is to use heterologous systems. Examples include phaseolin and arcelin regulatory sequences in tobacco and Arabidopsis (158,159).

## Summary

An international consortium was formed during a meeting held at Cuernavaca, Mexico, 3-4 March, 2001. The consortium is called *Phaseomics* (160). The long term goal of the consortium is to coordinate the development of genomic resources that can be used in an applied setting to develop the next generation of beans that are higher yielding while also providing tolerance to disease or environmental stresses. The resources will include gene sequence data, protein profiles, global expression patterns, large insert libraries, transformation protocols, and information management.

Some of the earliest *Phaseomics* efforts are EST library sequencing. Hernandez *et al.* (161) are analyzing over 3,000 ESTs developed from *P. vulgaris* root nodules induced by Rhizobium infection. An anthracnose resistant *P. vulgaris* genotype was used to generate EST clones that were later sequenced. Currently, 735 unique sequences have been identified (162). The largest number

(21,000) of ESTs was generated from a project to study embryonic development in *P. coccineus* (25).

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