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Phaseolus: A New World gift to mankind

Why common beans are so common?

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FOR AUTHORS

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EDITORIAL

I am proud to present this Legume Perspectives *issue dedicated to Common Bean, one of the most important grain legume crops for direct human consumption in the world. The origin and diversity of the crop is examined along with the current state of genetic information and the status of breeding programs worldwide. Articles describing beneficial associations with soilborne microbes leading to an increased productivity, biotic and abiotic stresses that affect this crop and breeding for beneficial traits to improve human health are also included. Hopefully, this collection of interesting articles will encourage and stimulate not only those knowledgeable readers, but to those who would like to become familiarized with this fascinating legume. I would like to take the opportunity to thank all colleagues who have made this issue possible: the authors of the articles, and the associated Phaseomics research teams, for their insightful contributions, and sincere acknowledgment to those people who contributed to produce this issue.*

Marta Santalla
Managing Editor of
Legume Perspectives *issue 2*

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Carte blanche to…

…Federico Sanchez

A new dawn in the bean horizon

Common bean is a major legume crop with over 25 MMT produced per year, and is the most important grain legume for direct human consumption in the world. It represents a major source of protein and calories, has high fiber content and is also a rich source of minerals, such as iron and zinc. Common bean originated in Mexico, but later diversifications and dispersals through the whole American Continent, and the rest of the World has made this grain legume to become rapidly popular for its nutritional qualities. Although, common bean was domesticated about 7000 years, predominantly in two diversity centers -Mexico and Central America, and the Andean region, new genetic pools are rapidly emerging in Europe, Asia and Africa. In developing countries bean varieties and landraces are cultivated, from sea level up to 9,000 feet above, mostly by small farmers in rain fed conditions without or minimum inputs. There are increasingly mapping populations established to study the inheritance of agronomic traits in different locations. Higher yields and other traits like improved symbiotic performance, drought tolerance; bacterial blight, rust and root rot resistances, have already been successfully transferred from wild accessions and closely related species, such as tepary bean (P. acutifolius) and the scarlet runner bean (P coccineus), to commercial varieties. P. vulgaris is true diploid species with an average genome size of ~600 Mb, and its nucleotide sequence from three different cultivars has been recently released. The sequence of wild accessions and species within the genus Phaseolus will follow. The synergy between genomic assets, vast genetic variability, and cutting edge genetic resources trigger exciting possibilities to increase yield and fitness in different environments, in hand of improved nutritional grain qualities. This new dawn brings opportunities to increase world production and consumption and to fulfill challenges among small holders in food insecure countries. ■

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Biodiversity and systematics of *Phaseolus* L. (Leguminosae)

by Alfonso DELGADO-SALINAS

Abstract: The New World genus *Phaseolus* comprises ca. 80 species and his center of diversity occurs primarily in the mountains of Mexico (ca. 60 species). Although most species are generally narrowly distributed, widespread species such as *P. lunatus* and *P. vulgaris* occur from northern Mexico to Argentina. Five of these wild species were brought by domestication into cultivation. Morphologically and ecologically distinctive within the NW Phaseolinae, *Phaseolus* phylogeny includes eight phylogenetic groups, where four of the five cultivated species are established in the *P. vulgaris* group. *Phaseolus* diversity is also expressed in floral morphology and floral presentation to pollinators (bees and hummingbirds). Ecological, geographical and reproductive studies are still needed.

Key words: New World *Phaseolinae*, *Phaseolus*, phylogeny, systematics

Distribution of the *Phaseolus* genus

The genus *Phaseolus* L. is a member of the economically important subtribe Phaseolinae (tribe Phaseoleae), and includes more than 80 wild species widely distributed throughout the Americas (except for Alaska, west and north Canada, western USA, Chile and southern Argentina), from south eastern Canada, southeastern and southwestern USA, Mexico, Central America, West Indies and to mostly eastern South America. Of these species, just over 60 are distributed mostly in the Mexican uplands (3). Five of these were domesticated and brought in cultivation, the "tepary" or "escumite" bean (*P. acutifolius* A. Gray), amazingly adapted to arid locations; the "ayocote" or "scarlet runner bean" (*P. coccineus* L.), splendid climber with beautiful blossoms, well-adapted to temperate cold environments; the "pallar" or "lima bean" (*P. lunatus* L.), a versatile vine occurring in seasonally dry or humid forests; the "gordo" or "year bean" (*P. dumosus* Macfadyen), well-adapted to temperate-humid sites, locally important to different human groups; however still neglected, and the world-wide cultivated "frijol", "poroto" or common bean (*P. vulgaris* L.). Because of this biodiverstity and human importance, the genus has been the focus of much taxonomic, monographic, phylogenetic, and molecular genetics studies (3, 5).

Morphological diversity in *Phaseolus* species

Morphologically, *Phaseolus* species are mostly perennial climbers with foliage bearing microscopic hooked hairs (Fig. 1.2); fleshy, sometimes yam-sized roots, associate to root- nodules; leaves trifoliolate, and inflorescences in rather long pseudoracemes. Stipules and stipels of leaves, as well as floral bracts carry patches of glandular hairs that function in some species as extrafloral nectaries to attract ants. The flowers are characterized by colorful petals, mostly puplish, but sometimes red, pink or white, with a noticeable standard, two prominent wing petals, the right-one disposed horizontally as landing platform, and a tightly and laterally coiled beak of the keel petals, where stamens and gynoecium are enclosed (Fig. 1.3 and Fig. 1.4). This conspicuous floral asymmetric arrangement is associate with the presence of secondary pollen presentation with a stylar or pollen brush, that brings an improved placement of pollen and pollen reception on highly designed style and stigmatic surfaces, and consequently attraction of specialized pollinators (bees and hummingbirds) endorsing reproductive isolation, and thus, certainly have impelled diversification in this group of legumes. The fruit is mostly an elastically-dehiscent pod, with 1 seed, like in *P. microcarpus* Mart. to 20 seeds in *P. pauciflorus* Sessé et Moc. ex G. Don.

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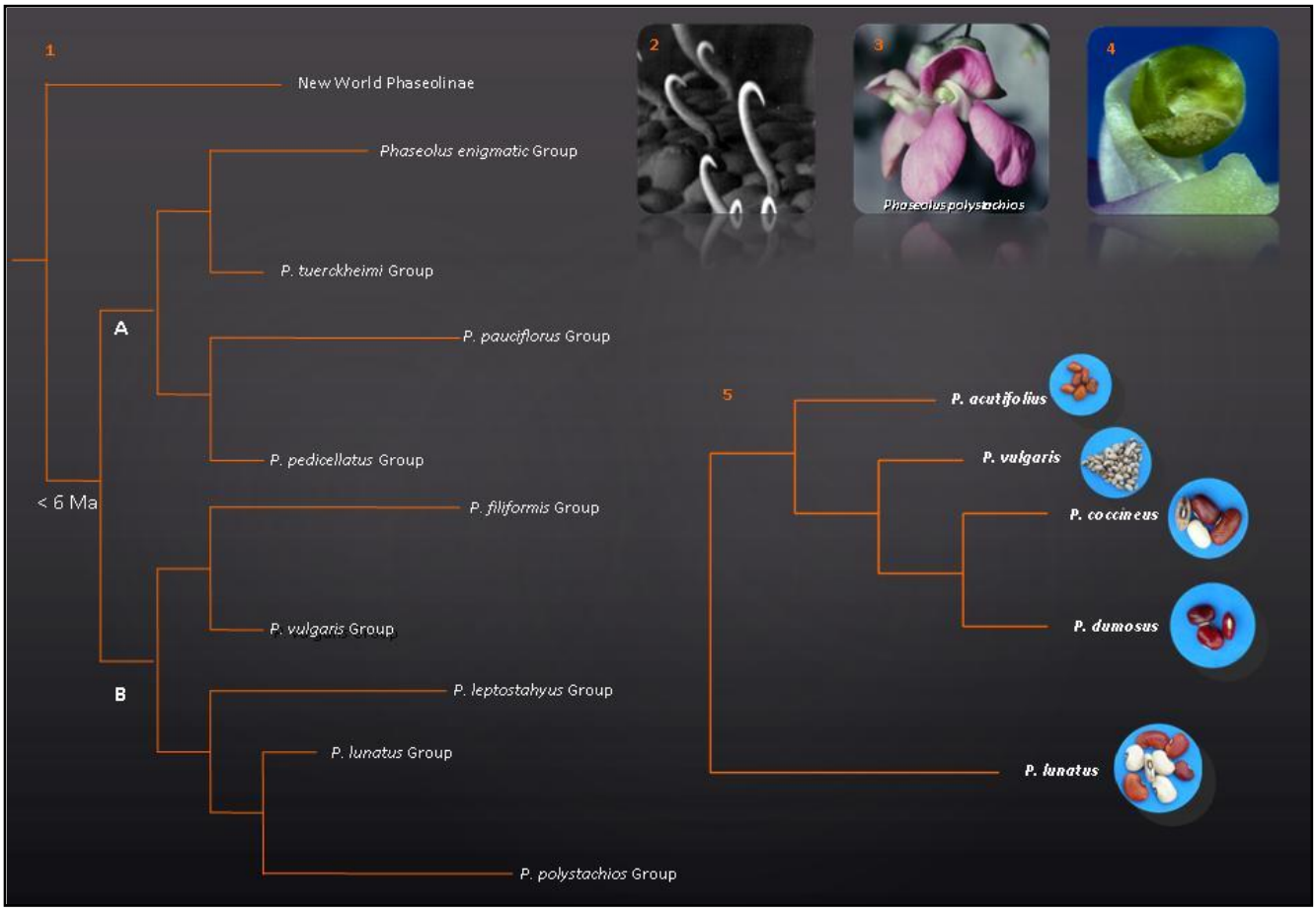

Figure 1. Phylogeny of *Phaseolus* species and diagnostic features. 1.1. Molecular phylogeny of *Phaseolus*. The monophyly of the genus is established as well as the interrelationships of the species; except for *P. glabellus*, *P. macrolepis*, *P. microcarpus*, *P. oaxacanus*, and *P. talamancensis* that grouped independently (enigmatic group), rest of species of *Phaseolus* are classified into one of the seven species clades (3). 1.2. Uncinate or hooked hairs. 1.3. Flower of *Phaseolus polystachios* (L.) B.S.P. 1.4. Keel petals, tightly and laterally coiled. 1.5. Portion of the phylogenetic tree showing the position of cultivated species in the *P. vulgaris* and *P. lunatus* Groups

Ecologically wild *Phaseolus* species appear to be distinct to other New World Phaseolinae species in being generally confined to oak, oak-pine, and pine forests; however there are few species that occur in seasonally dry tropical forests and related vegetations that are rich in succulent plants such as arid shrublands or even in sandy soils in coastlands. Some species in the *P. lunatus* phylogenetic group inhabit islands, such as *P. mollis* endemic to the Galapagos.

Phylogenetic clades of *Phaseolus*

Phylogenetically, *Phaseolus* is a monophyletic genus, diagnosed not only by molecular characters but also by morphological characteristics (i.e., synapomorphies). The latter include the tightly and laterally coiled beak of the keel petals (Fig. 1.3 and Fig. 1.4), inflorescences lacking swollen nodes (extrafloral nectaries), mostly persistent primary floral bracts, and foliage and reproductive parts bearing uncinate hairs (1) (Fig. 1.2). All known species of *Phaseolus* are established in eight phylogenetic clades (Fig. 1.1), where four of the cultivated species are included in the *P. vulgaris* group and only *P. lunatus* is positioned in a different group (3) (Fig. 1.5). *Phaseolus* is closely related to the rest of New World Phaseolinae but being morphologically, ecologically, and phylogenetically very distinct (Fig. 1).

Given the Mexican center of diversity for *Phaseolus*, and the estimated age of origin 6-8 million years ago (3), the extant diversification for the eight species clades within the genus reveals that most of this diversity came into existence well after the completion of tectonic activity in Mexico. The formation of such mountains perhaps facilitated the diversification of *Phaseolus* in upland regions, where *Phaseolus* species are today most abundant in oak and coniferous forests.

Taxonomic history of *Phaseolus* genus

Taxonomic activity in *Phaseolus* (and related genera) started perhaps when human groups in different parts of the world coincidently harvested, selected, and classified the *Phaseolinae* in their surroundings. Asian, African and American humans started at that point an important and never-ending selection process with different species of the actual *Phaseolus* and *Vigna* genera (Fig. 2). Domestication activities overcome traits and several crops came in cultivation by human in different regions of the world. Beans and other legumes nourished humans and no doubt contributed to the formation of important cultures. The name "phaselus" (for some authors, a Latin word for a plant with flowers that simulated a small boat) was coined for Old World plants, possibly to called cultivars of *Vigna unguiculata* (L.) Walp. The phaseolus name was adopted and made official by Linnaeus in 1753, and ever since more than 500 hundred plants were described as members of a not well-defined genus *Phaseolus*. In 1970, Verdcourt's seminal treatment of Phaseolinae (6) clarified the distinction between *Phaseolus* and *Vigna*, among others, and by doing this generated an intense interest in developing a more acceptable classification for this group of plants. Many botanists have confined their efforts since then to this diversified group of plants. Classification systems based in morphology, cytology, phytochemistry, and recently with the incorporation of molecular data have provided step by step gradually new meaning, knowledge and classification were not purely arbitrary (5). This *Phaseolus* monograph demonstrates how an intensive period of taxonomic activity has a significant effect on species discovery from a combination of old and recent collections. During the last twenty years, 25 species and the same amount of varieties have been described (2, 5).

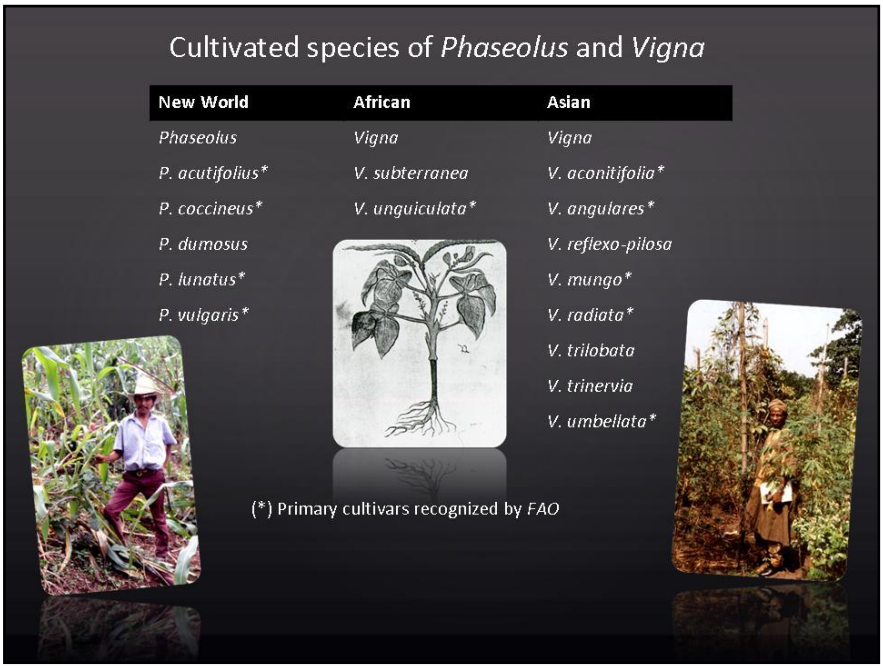

Figure 2. Cutivated beans of the subtribe Phaseolinae. Center of figure stands an early illustration of *Vigna unguiculata* (L.) Walp. with flowers similar to boats.

Despite all this accumulated information, there are still some basic aspects of habitat, ecology, and reproduction of *Phaseolus* species which are not understood. Work is still in progress, trying to ascertain species delimitation and in some cases settled complicate nomenclatures; as well as the use of geographical and ecological data of wild and cultivars in order to predict the effect of climate change to set exploration and regional conservation strategies. ■

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Domestications of *Phaseolus* beans and their importance for conservation and genetic improvement

by Paul GEPTS

Abstract: Domestication was initiated some 10,000 years ago. Its effects are pervasive and can be readily observed in the genetic structure of the wild and domesticated gene pools of crops in general and beans in particular. In turn, this genetic structure influences the way in which breeders utilize genetic diversity and germplasm specialists conserve this diversity. Interestingly, domestication is an ongoing process.
Key words: common bean, domestication, conservation, genetic improvement

Domestication is the process by which specific wild plants have been selected for adaptation to agricultural requirements and consumer preferences. Several thousands of years ago, hunter-gatherers in certain regions of the world started cultivating wild plants, possibly as a result of an imbalance between supply and demand for the wild plants they were gathering as a source of sustenance. As soon as these wild plants were sown and harvested, instead of merely gathered, they were subjected - in the cultivated environment - to quite different selection pressures than the ones they had encountered in the wild. This novel set of selection eventually led to strongly modified plants that were quite different from their wild ancestors and could not survive anymore without human intervention.

The traits that were strongly changed as a consequence of domestication selection pressures, as illustrated by *Phaseolus* beans, include seed dormancy (reduction), seed dispersal (reduction or elimination), growth habit (more compact, in bush as well as climbing types), photoperiod sensitivity (reduction or elimination), pod and seed size (increase), diversity of pod and seed shape and color (increase), concentration of toxic or anti-nutritional compounds (reduced), and susceptibility to pathogens or pests (increased) (4, 5, 20, 40). One of the consequences of this drastic change is that fully domesticated beans, similar to other crops, are incapable of autonomous survival without the assistance of humans.

Phaseolus: an example of the phenomenon of domestication

Among crops, *Phaseolus* beans provide a very interesting example of the phenomenon of domestication because of the multiple domestications to which they have been subjected. Indeed, among the approximately 75 to 100 *Phaseolus* species (10, 11), no less than five species have been domesticated. Two of these and perhaps a third one have been domesticated at least twice, leading to a total of seven and perhaps eight domestications (1, 14, 35), which can then be compared for a better understanding of the domestication phenomenon.

The five domesticated *Phaseolus* species are - in decreasing order of economic importance - *P. vulgaris* (common bean), *P. lunatus* (lima bean), *P. coccineus* (runner bean), *P. acutifolius* (tepary bean), and *P. dumosus* (year bean). Although the genus *Phaseolus* is a relatively young genus (4-5 million years; 10), the species giving rise to the five domesticates had acquired contrasting reproductive morphologies (from high levels of self-pollination to outcrossing via intermediate reproductive systems, including vegetative reproduction in runner bean), life histories (from short-lived annual plants to perennialism), climatic adaptation (including permutations of hot vs. cool and humid vs. dry environments), and interactions with microorganisms, such as Rhizobium or Bradyrhizobium, which engage in symbiotic nitrogen fixation with their bean host (13, 25).

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The multiple domestications observed today suggest that early farmers may have chosen to re-domesticate locally adapted species rather than - or in addition to - broadening the adaptation of the first *Phaseolus* species to have been cultivated. The five domesticated species - and other wild, as yet undomesticated, *Phaseolus* species may have conserved certain traits predisposing them to domestication. The broad geographic, ecological, and seasonal adaptation characterizing clade B in which these species are classified (10), may have facilitated their domestication and especially their subsequent spread. It was also suggested (12) that genetic linkage of several genes controlling domestication traits may have facilitated the maintenance of domestication traits in the face of re-occurring gene flow between the incipient domesticates and their wild progenitors. Such linkage has also been observed in crops as different as pearl millet, rice and tomato.

The region and timing of domestication of *Phaseolus*

Application of molecular markers has been instrumental in shedding light on the number and - in some cases - the potential region of domestication. Common bean was domesticated at least twice (14, 19), in Mesoamerica (a region encompassing Mexico, Central America, Colombia and Venezuela) and in the southern Andes (southern Peru, Bolivia, and Argentina). A recent analysis suggests that Mesoamerican common bean may have been domesticated in a more narrowly defined area in the Mexican states of Jalisco and western Guanajuato (21). Lima bean was also domesticated twice, in Ecuador and northern Peru (on the western slope of the Andes) and Mesoamerica (in an area partially overlapping with the presumed common bean domestication area) as suggested by recent analyses (35, 36). The runner bean was domesticated presumably in Mexico; recent data suggest, however, a potential second domestication in an unidentified area (1). Areas of domestication for tepary bean are located in northwestern Mexico, including the states of Jalisco, Sinaloa, and Sonora (28, 34). Finally, year bean was potentially domesticated in Guatemala, where wild types of this species have been identified (11).

The actual timing of these domestications is uncertain because of the limited number of archaeobotanical sites and remains, mostly consisting of seeds. Using accelerator mass spectrometry, a direct and, therefore, more reliable ^{14}C dating method (17), ages for seeds of ~5000 years were obtained. More recent data based on micro-remains, in this case *Phaseolus* starch grains from tooth calculus of human remains from Ñanchoc in Peru, reveal an age of ~ 8000 years in a pre-maize, domesticated context (31). Glottochronological data (timing based on language divergence) suggest the existence of words for beans in a proto-Mayan language some 3500 years ago (7).

Importance of genetic diversity for conservation and breeding

Few genes for morphological or physiological differences between wild and domesticated beans have been isolated so far. One exception is the gene for determinacy (main and lateral stems ending in a terminal inflorescence), which is a homologue of the *Arabidopsis thaliana TFL1* gene (23, 32). Candidate gene approaches as well population genetic methods can help identify other genes or genome regions involved in the domestication process (30).

Phaseolus beans provided one of the earliest examples that domestication has induced a more or less marked reduction in genetic diversity (14, 34). While this reduction is generally attributable to both genetic drift (across the genome) and selection (at or near genes for domestication), it is expected to be stronger in regions that are more conserved and for sequences with low mutation rates, accounting for the broad range of data. Results range from ~10% for SSRs (a high-mutation rate type of sequence) to some 3% for phaseolin electrophoretic data (14). These results have led to an increased emphasis on the conservation of landraces, not only in centers of origin, but also outside for example in Brazil (8, 9), Europe (2, 29, 33), and Africa (3). It also puts more emphasis on a more detailed characterization of adaptation of wild bean population to better understand the adaptation of domesticated beans and the extent to which the genetic and molecular basis of widening of the ecological amplitude of the domesticated gene pool.

The major split between Andean and Mesoamerican host gene pools in common bean is mirrored in a similar subdivision of strains among pathogens such as *Pseudocercospora griseola* (agent of Angular Leaf Spot; 16), *Colletotrichum lindemuthianum* (agent of Anthracnose; 18), and *Uromyces appendiculatus* (agent of Rust; 39). Geographic divergence between the Andes and Mesoamerica in the last 100,000 (24) to 500,000 (15) years has led to co-adaptation of the two host gene pools and their respective pathogens, such that Andean isolates are more virulent on average on Andean hosts, and vice-versa. As a consequence, breeders have sought to identify resistance for Andean host lines in the Mesoamerican host gene pool and vice-versa. They have also sought to pyramid resistance genes from the two gene pools in the hope, as yet uncontradicted, of achieving a more stable host resistance.

In some cases, bean breeders have had to incorporate resistances from sources outside the primary gene pool of common bean. In addition to being crops in their own right, tepary and runner beans are sources of genetic diversity for disease and stress tolerance in common bean. For example, tepary bean has been a major source of resistance to *Xanthomonas campestris* (causal agent of Common Bacterial Blight; 39). This resistance has been incorporated into advanced breeding lines or commercial cultivars (26, 38). Tepary bean could also provide tolerance to heat or drought. In runner bean, tolerance to low soil fertility, Bean Golden Mosaic Virus, Common Bacterial Blight and root rots has been identified and is being transferred to common bean (6, 27, 37, 39).

Current bean farmers in centers of origin and mostly in developing countries are both guardians and active managers of bean genetic diversity. They rely actively on this genetic diversity for their subsistence and have an intimate knowledge of its characteristics, including adaptation (41). They can also utilize pollen and seed gene flow among wild beans, landraces, and improved varieties to maintain level of genetic diversity in farmer's fields comparable to those in wild populations surrounding the fields, in contrast with the general reduction in genetic diversity observed between wild and domesticated beans (42). ■

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Common bean origin, evolution and spread from America

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Abstract: The common bean (*P. vulgaris* L.) has an evolutionary scenario that is almost unique, that makes it an important model among the crops. It is characterized by two independent domestication events that caused two clearly differentiated gene pools. This is seen by the co-existence of the wild and domesticated populations, and by the fertile and vital progeny that are obtained from crosses between these. Recent studies have also provided significant contributions towards the clarification of some of the issues regarding its origin and evolution, although some questions still remain unresolved and are a matter of intense debate.

Key words: origin, evolution, dispersal, gene pools, common bean

Origin and domestication of *Phaseolus vulgaris*

The genus *Phaseolus* that originated in Mesoamerica comprises five domesticated species: *P. vulgaris* (common bean), *P. dumosus*, *P. coccineus* (runner bean), *P. acutifolius* (tepary bean) and *P. lunatus* (lima bean). The common bean is the most economically important among these, as it is the main grain legume for direct human consumption, and it represents a rich source of protein, vitamins, minerals and fiber, especially in less-developed countries (6).

Eight principal crown clades were identified (9) from a study of the molecular phylogeny of *Phaseolus*, with the P. *vulgaris* group being the oldest, at *ca.* 4 Ma. The closest relatives to *P. vulgaris* are the Mesoamerican species *P. dumosus* and *P. coccineus*, which together form the so-called '*vulgaris* complex'. On the basis of sequence data of the α-amylase inhibitor gene, *P. vulgaris* diverged from *P. dumosus* and *P. coccineus ca.* 2 Ma ago (11).

P. vulgaris is a true autogamous diploid species, with 22 chromosomes and a haploid genome size that is estimated to be between 587 Mbp and 637 Mbp (4).

The wild common bean is widely distributed from northern Mexico to North-Western Argentina, and it is characterized by two major eco-geographical gene pools: the Mesoamerican and Andean. These two gene pools show parallel wild and domesticated geographical structures, as shown by several studies based on different datasets, which have included morphology and different types of molecular markers.

Until recently, the most credited hypothesis as to the origin of the common bean was a dispersion northwards (Colombia, Central America and Mexico) and southwards (southern Peru, Bolivia and Argentina) from the core area of the western slopes of the Andes in northern Peru and Ecuador, which resulted in the Mesoamerican and Andean gene pools, respectively (12). This hypothesis was supported by the identification of a new type of phaseolin (type I) in a wild *P. vulgaris* population discovered in northern Peru and Ecuador. This is a seed storage protein that is believed to be the ancestral form of the other phaseolin types.

However, recently, this hypothesis has been called into question by different studies (5, 16, 21). A Mesoamerican origin of the common bean was identified (5) through investigations into nucleotide diversity at five different gene fragments on a wide sample of wild *P. vulgaris* that is representative of its geographical distribution. This study highlighted the higher genetic diversity detected for the Mesoamerican gene pool, as compared to the Andean gene pool. This revealed a 90% loss of diversity for the Andean gene pool and confirmed the bottleneck in the Andes prior to domestication.

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The second novel outcome of this analysis (5) was the clear population structure identified in Mesoamerica, which up to now had not usually been considered as a single gene pool. The Mesoamerican accessions were split into four distinct genetic groups: one was represented by accessions distributed across all of the geographical area, from the north of Mexico down to Colombia, and the other three groups were composed of only Mexican accessions. Investigations into the relationships among these different groups showed that, remarkably, there is no clear distinction between the Mesoamerican and Andean wild gene pools, while there are different relationships between the Mesoamerican groups and the north Peru–Ecuador and Andean gene pools. So this study shows clear evidence of the Mesoamerican origin of the common bean, which was most likely to have occurred in Mexico. This outcome is consistent with the known distribution of most of the close relatives of *P. vulgaris*.

Thus, both of the gene pools from South America originated from the Mesoamerica populations of Central Mexico through different migration events, and the wild common bean from Northern Peru and Ecuador is presumed to be a relict population that only represents a fraction of the genetic diversity of the ancestral population.

The wild common bean underwent a complex process of domestication, which transforms a wild plant into a crop through structural and functional modifications (the 'domestication syndrome'). This makes the domesticated plant genetically different from its progenitors, and confers upon it better adaptation to different agro-ecosystems. In *P. vulgaris*, the process of domestication involved several morphological and physiological changes, such as differences in growth habit (indeterminate *vs* determinate), seed dormancy (present *vs* not present), photoperiod sensitivity (short-day *vs* insensitivity), shape, color and size of the plant and its harvested parts, and dissemination mechanisms (shattering *vs* non-opening pods).

Where did this domestication occur for the common bean? Several studies documented two independent domestication events: one in Mesoamerica and one in the Andes. These gave origin to two major gene pools that show partial reproductive barriers and marked phenotypic differences, according to morphology, seed-storage proteins, and nuclear and chloroplast markers (1, 2, 19). Following domestication, the domesticated gene pools of the common bean appear to have been organized into four Mesoamerican (Durango, Jalisco, Mesoamerica, Guatemala) and three Andean (Nueva Granada, Peru, Chile) races. These differed in ecological adaptation, geographical range, morpho-agronomic traits, allozyme alleles, and RAPD markers (3, 24), although their origins are still controversial today.

A further matter of debate is whether multiple domestications occurred within each gene pool, and the role of gene flow and introgression. For the Mesoamerican gene pool, different studies have suggested both single and multiple domestication events. Recently, for the first time, this question was studied by analyzing nucleotide data (16), and these strongly supported a single domestication event in Mesoamerica. The question could not be answered in the Andean gene pool because of the low level of diversity, which reduces the resolving power of such molecular studies. However, within the Andean gene pool, both single and multiple domestications have been suggested (3, 8, 21, 22). Using multilocus sequence data to test multiple demographic models in domesticated *P. vulgaris* landraces, a single domestication event in each gene pool was recently evidenced (15).

The identification of the presumed geographical center of domestication and its dating are other important aspects of common bean domestication that remain still under debate, Further efforts are clearly needed to investigate these puzzling issues more thoroughly.

The process of domestication leaves its signatures on the patterns of the genetic diversity in the genome of crop plants, and this generally consists of a reduction in the genetic diversity in crops, relative to their wild progenitors. Two major impacts on diversity result from domestication. First, the domestication syndrome, such as changes in traits selected for human use, leads to selection signatures at specific loci. The major traits that distinguish a wild bean from a domesticated bean have been localized on a genetic map (13). For those genes indicated, the high genetic diversity and selection intensity has indicated that the domestication process in the common bean proceeded rapidly, and through changes that involved only a few genes, but had large effects.

The genes for domestication are located in regions of high divergence between wild and domesticated *P. vulgaris* (18). Also, the regions linked to the domestication loci, where the highest diversity of the wild relatives is located, have probably been less exploited historically by farmers and breeders. This knowledge of the domestication loci is useful both for the identification of markers that are tightly linked to undesirable genes (e.g. shattering), and for the possibility of identifying the surrounding chromosomal regions that appear to contain the highest, and historically less exploited, diversity of the wild germplasm. Using a genome scan approach for the signature of domestication, it was estimated that a large fraction of the genome of the common bean (about 16%) was under the effects of selection during domestication. Interestingly, most of the amplified fragment length polymorphism markers that were putatively under the effects of selection due to the domestication loci were localized close to genes and quantitative trait loci that are linked to the domestication process (20).

The second major consequence of domestication is the reduction in the genetic diversity in crops relative to their wild progenitors, due to human selection and genetic drift through bottleneck effects. Contrary to selection, which only affects genetic diversity at target genes, bottleneck processes reduce neutral genetic diversity across the entire genome.

In analyzing the Mesoamerican and Andean wild and domesticated populations using amplified fragment length polymorphism markers, it was observed a strong reduction in the genetic diversity due to domestication (as wild *vs* domesticated samples) only in the Mesoamerica population (21). Very different patterns of molecular diversity can be highlighted by markers that differ substantially in their mutation rates, and indeed, simple sequence repeat (SSR) markers (14) showed a moderate reduction in the genetic diversity in Mesoamerica (*ca.* 10%). Data from wild and domesticated common beans (16) arose from an analysis of a genomic sequence similar to SHATTERPROOF 1 (*PvSHP1*), the gene involved in the control of fruit shattering in *Arabidopsis thaliana*. This offered the first estimates of the effects of domestication on the nucleotide variation in *P. vulgaris*. The loss of diversity in the domesticated accessions in the Andes was 54%, while in Mesoamerica this loss of diversity was 69%.

However, introgressive hybridization between domesticated forms and their wild relatives has often expanded genetic diversity, thus counteracting the effects of the initial domestication bottleneck. Through genetic analysis of Mexican *P. vulgaris* populations (17, 18) with different levels of simpatry, the wild and domesticated forms are not genetically isolated, as they show moderate and asymmetric gene flow (>3-fold higher from domesticated to wild, than *vice versa*). In the presence of gene flow, the marked phenotypic differences between the two forms growing in the same distribution area are explained by the selection that acts against the domesticated alleles in a wild context, and against the wild alleles in an agroecosystem.

The evolutionary history of the common bean was characterized by two independent domestication events that resulted in two clearly differentiated gene pools, with the co-existence of the wild and domesticated populations, and the production of fertile and vital progeny from crosses between its wild and domesticated forms. This has made *P. vulgaris* an important model among crops for the study of the genes and quantitative trait loci that were involved in the domestication process.

Evolution of *Phaseolus vulgaris* out of the Americas

The expansion and the pathways of distribution of the common bean out of the American domestication centers were very complex, and they involved several introductions from the Americas that were combined with exchanges between continents, and within continents among several countries. Outside of America, the common bean populations were more 'free' to pass through new evolutionary pathways that were not possible in the American center of origin, due to the spatial isolation between these two gene pools. Thus, several continents and countries have been proposed as secondary centers of diversification for *P. vulgaris*, including Europe (2, 23), Brazil (7), and central-eastern and southern Africa (10) and China (25).

Although the amount of available data is larger for Europe than for other secondary centers of diversification, it is clear that the proportions of the Mesoamerican and Andean gene pools can vary considerably across different continents, as also among countries within continents.

Within the Americas, the genetic diversity and the structure of a sample of 279 common bean landraces from Brazil using nuclear SSR markers and phaseolin, PvTFL1y, APA and SCAR markers was assessed (7). Surprisingly, despite the proximity to the Andes, they found that the Mesoamerican gene pools were 4-fold more frequent than the Andean gene pools. Similarities in climate and soil between the two areas might explain the success and diffusion of the Mesoamerican bean germplasm into Brazil. Moreover, multiple introductions of Mesoamerican germplasm in pre- and post-conquest times (10) might have had a considerable impact on the establishment of this pattern.

As highlighted by pioneering studies on the patterns of diversity of the phaseolins, both the Mesoamerican and Andean gene pools are present in Europe, with higher frequency seen for the Andean types. Recently, a study with chloroplast microsatellites (cpSSRs), nuclear markers (phaseolin and three indel-spanning markers of Pv*SHP1*), and morphological seed traits on a wide European collection (2) permitted to trace the distribution of the domesticated Mesoamerican and Andean gene pools in Europe. This study confirmed that the largest fraction of the European germplasm was of Andean origin (67%). However, in the eastern part of Europe the proportion of the Mesoamerican type tends to increase, with a maximum of 46% in Greece. Overall, this suggests that there was high gene flow among the different regions of Europe, and/or homogeneous selection.

In Africa, the Mesoamerican and Andean gene pools are approximately equal in frequency, even if there are striking differences between different countries due to different farmer selection preferences and the input of germplasm from national programs.

Even if China is a large producer of dry beans, and is the most important producer of the *P. vulgaris* snap beans in the World, an analysis of a 229-landrace collection revealed higher prevalence of the Mesoamerican type (25). At present, it is believed that there were only a limited number of introductions of the common bean into China, which were biased towards the Mesoamerican type.

Under the bottleneck model, it is expected that dissemination from the center of origin will lead to a reduction in genetic diversity, and theoretically, this reduction in genetic diversity should be proportional to the distance from the center of origin. The data obtained using nuclear SSR markers (14) demonstrated that contrary to expectations, and overall for the two gene pools, the reduction in genetic diversity has been strong for Brazil, intermediate for China, and low or nearly absent for Africa. This is probably due to the dissemination history of *Phaseolus* over the last few centuries, which has followed the routes of intense commercial activities and exchanges between different countries and continents.

For Europe, a direct comparison (2) of the levels of diversity between an American and a European collection using cpSSR markers was performed. They concluded that the intensity of the cytoplasmic bottleneck that resulted from the introduction of the common bean into Europe was very low (a loss of cpSSR diversity of *ca.* 2%). At the nuclear level, a much higher loss of diversity consequent to the introduction of the common bean into Europe (*ca.* 30%) was evidenced (19), and supported (2) by the hypothesis of a bottleneck at the nuclear level that was of greater intensity than for cpSSRs by studying the PvSHP1 nuclear markers.

Finally, to obtain more precise estimates of the bottleneck intensity at the nuclear level and to provide a clearer picture of the dissemination pathways of *P. vulgaris* all around the World, researcher efforts today are focused on sequence analyses and high-throughput sequencing technologies. ■

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Words denoting common bean in European languages

by Aleksandar MIKIĆ

Abstract: The well-known word *bean* may denote both common beans (*Phaseolus* spp.) and faba bean (*Vicia faba* L.) in most European languages. The ultimate origin of this word is the Proto-Indo-European root **bhabha-*, initially denoting faba bean, as one of the most ancient crops of the Old World and literally meaning *something swollen*.
Key words: etymology; faba bean; *Phaseolus* beans; Proto-Indo-European language

Today, *Phaseolus* beans, especially common bean (*P. vulgaris* L.) play a rather prominent role in the human diets across the Europe. In many European regions, most notably southeastern Europe, it gradually replaced other pulse crops, especially faba bean (*Vicia faba* L.). Along with this process, another one, of purely linguistic character, went in parallel. Its result is that in a large number of the modern European languages the words initially denoting faba bean shifted to denote common bean, leaving the latter with various additional adjectives to make it distinct from the former, such as in Baltic, Germanic and Uralic languages. (Table 1). On the other hand, the words denoting common bean in, for instance, Romance and Slavic languages are mostly derived from the Latin *phaseolus*, in its turn originating from the Old Greek φάσηλος, denoting a kind of bean. The word denoting faba bean in all Indo-European languages are descendant of the Proto-Indo-European **bhabha-*, meaning literally *something swollen* and being obviously descriptive (1). ■

Table 1. A comparative overview of the words denoting common bean (*Phaseolus vulgaris* L.) and faba bean (*Vicia faba* L.) in some modern European languages

Family	Branch	Language	*Phaseolus vulgaris*	*Vicia faba*
Afro-Asiatic	Semitic	Maltese	fazola; fula	fula
Altaic	Turkic	Turkish	fasulye	bakla
Déne-Caucasian	Basque	Basque	babarrun	baba
	Caucasian	Abkhaz	akezyd	akezyd
		Chechen	kho'	kho'
Indo-European	Albanian	Albanian	fasule	bathë
	Armenian	Armenian	lobi sovorakan	lobi
	Baltic	Latvian	parastas pupinas	lauka pupas
		Lithuanian	daržinė pupelė	pupa
	Celtic	Breton	favenn; hariko	favenn
		Irish	pónaire	pónaire
		Scottish Gaelic	pònair	pònair
		Welsh	ffa	ffa
	Germanic	Danish	buskbønne	hestebønne
		Dutch	boon	tuinboon
		English	common bean	faba bean
		German	Fisole; Bohne	Ackerbohne
		Icelandic	matbaun	bóndabaunir
		Norwegian	hagebønne	baunevikke
		Swedish	böna	åkerböna
	Hellenic	Greek	φασόλι	κουκιά
	Indo-Iranian	Ossetian	qædurhos	qædurhos
	Italic	Catalan	fesol	fava
		French	haricot	féverolle
		Galician	feixón	fava
		Italian	fagiolo	fava
		Occitan	fasòls	fava
		Portuguese	feijão	fava
		Romanian	fasole	bob
		Spanish	frijol	haba
	Slavic	Belarusian	pasulya	bob
		Bulgarian	fasul; bakla	bob
		Croatian	grah	bob
		Czech	fazole	bob
		Polish	fasola	bób
		Russian	fasol'	bob
		Serbian	pasulj; grah	bob
		Slovak	fazul'a	bob
		Slovenian	fižol	bob
		Ukrainian	kvasolya	bib
		Upper Sorbian	niski bob; buna	bob
Kartvelian	Georgian	Georgian	lobio	lobio
Uralic	Finno-Permic	Estonian	uba	põlduba
		Finish	papu	härkäpapu
	Ugric	Hungarian	veteménybab	bab

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Why we must preserve *Phaseolus* genetic diversity or the value of plant genetic resources in the legume world

by Molly WELSH

Abstract: Presently, the global *Phaseolus* germplasm is preserved in major collections, such as the one within the National Plant Germplasm Service of USDA, ARS, in Pullman, United States and eleven centers of CGIAR (Consultative Group on International Agricultural Research), as well as in many smaller national sites throughout the world. Although preserving genetic diversity of *Phaseolus* is expensive, methods to fund the process must continually be explored, with legume scientists, breeders, and consumers at the forefront of the drive to preserve these important resources.

Key words: Phaseolus beans; germplasm, genetic diversity, preservation

Step back in time; remember reading about the Irish Potato Famine in the mid 19th century, and in the 20th century the Southern Corn Blight emergency in the United States or the problem with Citrus Canker in the orange groves of Brazil?. The factor these divergent crops, all with serious disasters, had in common was lack of genetic diversity. These are but tiny examples showing the importance of genetic diversity for crop plants.

Legumes are one of the most important plant families, providing food, forage, and industrial crops to the world. As food for human consumption they are particularly important, providing up to 65% of protein in daily human diets. But many factors hinder improvement in legume crops: insect and disease predation, unfavorable environmental stresses and anti-nutritional factors. An emphasis on germplasm collection and evaluation as a source for solutions to these problems continues to be important (1).

But, easily we may combine these seemingly separate ideas into a discussion of the value for preservation of genetic diversity (germplasm). Inherently, its distinct monetary value is difficult to quantify as one of the prim directives to the preservation of the germplasm is that it remain identical to that which was originally collected and documented. Wild, weedy, and landrace accessions can't compete economically with highly developed cultivars, but have a chance of containing valuable traits not currently present in the developed lines. Threats to crops change and/or adapt at a relatively speedy rate, whether it is the developing climate change or insect and disease adaptations to the crop's resistance. It is important that there is a wide variety of places where breeders and researchers may search for answers to these threats.

Presently the *Phaseolus* germplasm is preserved throughout the world (Fig. 1). The NPGS (National Plant Germplasm Service, United States) and the 11 CGIAR centers (Consultative Group on International Agricultural Research) hold the largest collections, but there are many smaller national sites throughout the world (3). Preservation of genetic diversity is expensive and methods to fund the process must continually be explored (2). We, legume scientists, breeders, and consumers, should be at the forefront of the drive to preserve these important resources (Fig. 2). ■

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Figure 1. Diversity of *Phaseolus*

Figure 2. Molly Welsh collecting *Phaseolus* seeds

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Common bean gene mapping for molecular improvement

by Rosana PEREIRA VIANELLO* and Claudio BRONDANI

Abstract: As molecular genetics technology advances *Phaseolus vulgaris* has been benefited towards the knowledge and understanding of the entire genome. This progress has led to the development of a large and useful set of anonymous and gene-targeted molecular markers scattered throughout the entire genome, which has been mapped and resulting in a high-resolution linkage map, unavailable until recently for common bean. Several QTL mapping studies have provided baseline knowledge of genomic regions affecting traits of interest and suggested targets for candidate genes to be tested in association mapping. In this context, high-throughput genotyping based on new sequencing technologies will assist researchers to increase the effectiveness of the common bean breeding programs.

Key words: molecular marker, linkage map, QTL, MAS

Elucidating the hereditary basis of genetic variability for diverse and complex organisms is one of the major scientific challenges, even nowadays with the advent of high-throughput genomics technologies. For molecular breeding, genetic maps are particularly important because a gene related to an economically important trait can be located on the map, which leads to finding the precise chromosomal location, following the inheritance of a DNA marker in the phenotyped segregating individuals. For common bean, which has a genome size estimated at 450 to 550 million base pairs (Mbp) per haploid genome distributed on 11 chromosomes, the genetic maps became accessible since the 1990s. Thence, many molecular marker linkage studies have been performed mainly due to the advances in DNA techniques which have contributed to reducing costs and increasing accessibility to molecular approaches. Furthermore, these have made possible and affordable to construct genetic maps, allowing significant insights into the structure and organization of the *P. vulgaris* genome. In addition, to contribute to the identification and location of genes or loci governing a wide variety of traits including diseases resistance, components of yield, grain quality and the domestication process.

Over the past 20 years, significant advances have been made to understand the common bean genome, beginning with multiple efforts to develop genetic maps by integrating different classes of molecular markers, especially RFLPs and RAPDs. Remarkable scientific contributions were the development of permanent segregating populations (BAT93 x JaloEEP558 and DOR365 x G19833), of which accumulating data of segregating characters, allowed the alignment and growing information between the past and present maps (5). An important feature for genetic mapping as well as for comparative genetic studies has been the development of co-dominant microsatellite markers for *P. vulgaris*, initially allocated into a linkage map (15). From the year 2000, with the continuous scientific progress, a large set of microsatellite markers started to be integrated into common bean linkage maps, leading to a linkage map based exclusively on these markers (7). Subsequently expanded versions of core linkage maps have been released (8). The first report of SNP (Single Nucleotide Polymorphism) mapping in *P. vulgaris* (3) included a total of 118 new marker loci into an integrated molecular map for common bean.

In recent years, with increasing efforts to develop ESTs (Expressed Sequence Tags) resources for common bean, a new class of locus-specific DNA markers called 'functional molecular markers' have been developed. These approaches include the mining for repetitive sequences and for SNP markers. Increasing set of SNPs have been reported for common beans (9, 14), using genotypes of Andean and Mesoamerican common bean gene pools. Currently, SNP-based genetic markers have attracted great attention when creating dense genetic linkage maps, since they are abundant and can also provide gene-base and syntenic-marker relationships. These advances have been well documented for common bean, and recent reports have described increasing number of mapped markers allowing the establishment of a genome synteny study among important

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legumes (11). Recently, a high-density integrated genetic map was constructed, of which more than 1,000 markers were placed across the 11 linkage groups of common bean providing a framework for analysis of the entire genome, which has a high potential to be used for synteny and QTL analysis (4, 5).

It is expected that in common bean, progress in QTL mining and mapping will provide understanding of the genetic traits of interest and to unravel candidate genomic regions that can be deployed in crop improvement through molecular breeding. Most of the target traits are related to domestication and to agronomically relevant traits. In this regard, breeding programs can be carefully focused on useful genomic regions to search for candidate genes to be tested in association mapping and in the selection process to identify lines with desirable phenotypes. Undoubtedly, in order to contribute to an increase in the efficiency of the common bean genetic breeding programs. A number of reports highlighting locations of QTLs distributed throughout the genome in different environments, and associating traits linked to yield components, plant and root architecture (13), nutrient content and cooking time in the seeds, are cited (1, 6). Regarding biotic stress, remarkable and continuous efforts have been made for the search, identification and use of molecular markers that co-segregate with genes that conferring resistance to pests and diseases (12). Subsequently, markers linked to the major QTL regions for resistance were characterized and used for indirect selection (2) or converted into sequence-characterized amplified regions (SCAR) that, in a practical way, proved to be useful to develop common bean lines or cultivars with different genes pyramided to increase the diseases resistance (10).

However, despite all experimental QTL research that have been made over the years, only a few QTLs related to the same trait were mapped in common locations in the linkage maps. This is partly due to the reduced size of segregant populations, distinct genitors used, background genetic interaction, G x E interaction and the limitations due to the partial alignment of linkage groups among different studies. In view of the progress in genomic technologies approach, the high-resolution map densely composed by molecular markers, enabling with the identification of haplotypes for genome-wide selection, constitutes a promising strategy to locate QTLs that control significant and useful proportion of the phenotypic variation for a number of economically important traits. Thereafter for common bean, an autogamous plant, the implementation of marker-assisted selection will have its most effective use for the improvement of quantitative traits. ■

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Progress toward a draft sequence of the common bean genome

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Abstract: Common bean, the most consumed food legume in the world, is a societal crop important as an economic commodity and a principal protein source. For continued improvement, it is essential to develop modern breeding tools that scale in high- and low-throughput research environments. The development of these tools is best realized in the context of a whole genome sequence of common bean. The sequencing of G19833, an Andean genotype, was funded by the US Department of Agriculture and Department of Energy, Joint Genome Institute. The final draft assembly covers 88% of the 590 Mb genome and consists of ~26,000 full genes. Associated with the development of the genome sequence is a high density genetic map consisting of ~7,000 SNP loci. Low-coverage sequence data was mined, and ~2,800 indel markers with utility to distinguish variation with major US market classes were discovered. These indel markers can routinely be used in low-throughout, low-cost screening programs that aid the development of improved cultivars better adapted to local production systems.

Key words: common bean, genome, *Phaseolus vulgaris*, phaseomics, sequencing

Common bean (*Phaseolus vulgaris* L.; 2n = 2x = 22) is in important global crop that is an important dietary component for developed and developing countries. FAOSTAT (http://faostat.fao.org/) provides an important glimpse into the production and utility of common bean. From a single country perspective, Brazil leads the world in dry bean production with 3.3 million MT in 2010. Yet from a continental perspective, Africa leads the world with 4.0 million MT. From a food security perspective, half of the world production is in countries termed by FAO as "low income, food deficit countries". Since common bean is an important source of calories and protein in a number of these countries, world-wide efforts are underway to improve the nutritional value of common bean. To support these efforts, a common bean genome sequencing project was initiated with the goal of developing a draft assembly that could be initially mined for data to develop tools for modern crop improvement efforts. These tools can further be used to discover the genetic factors underlying the traits that define common bean as an important nutritional crop.

The United States common bean sequencing project

The United States common bean sequence project began with a white paper shared with multiple funding agencies (http://www.css.msu.edu/bic/pdf/Bean_Genomics_Status_2008.pdf). In early 2009, the United States Department of Agriculture (USDA) requested projects for sequencing of the genome, and funding was obtained for the project in September of that year. The investigators on the project were Scott Jackson (PI, Univ. of Georgia), Phillip McClean (North Dakota State Univ.), Jeremy Schmutz (HudsonAlpha Institute for Biotechnology), Daniel Rokhsar [Department of Energy, Joint Genome Institute, (DOE/JGI)], and Perry Cregan (USDA/ARS). In addition, funds were secured from the DOE/JGI.

The funded project followed many of the activities outlined in the original white paper. These included collecting the bulk of the sequencing data using ~400bp reads using the Roche 454 technology. Additional Sanger sequencing was included 1) to collect additional BAC-end sequence data, beyond what was already available for the target genotype, and 2) to generate fosmid (~35kb) sequence data. A new molecular map based on an F2 population (n = 247) was developed from the cross of Stampede (pinto) and Redhawk (red kidney). This consisted of nearly ~7,000 SNP loci. The SNP collection was primarily funded by this project with additional financial support from the USDA Common Bean Coordinated Agricultural Project (BeanCAP). Finally, RNA-seq data was collected from a collection of root, stem, leaf, flower, and pod tissues at different stages of development.

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The common bean genotype G19833 of Andean origin was sequenced for this project. This genotype was selected because of the availability of genomic sequence data at the onset of the project (5). The total genome coverage of the Roche 454 sequence data is 21.02X. The main assembly, obtained using Arachne (2), was interrogated and reoriented using the SNP genetic map and common bean/soybean synteny data (4) to obtain a scaffold-based assembled genome consisting of 520Mb. Based on a genome size of 590 Mb, derived from a genome estimate of 0.60 pg (http://data.kew.org/cvalues/) and a conversion factor of 1pg=978 Mb (3), approximately 88% of the genome is found in the assembly. To assess the degree to which the expression portion of the genome is represented in the final assembly, publicly available EST data was aligned to the assembly. 94% of those sequences aligned. A total of ~26,000 genes containing appropriate stop and start codons were discovered during the gene modeling and annotation steps. These were based on a total of ~31,500 primary and alternative transcripts. Of these ~21,000 have Pfam (1) annotations.

Applications of a modern common bean genome sequence to crop improvement

Modern crop improvement is relying more and more on the tools that are derived from sequencing projects. Principal among these are the abundant set of new markers. The BeanCAP project has evaluated the diversity of over 10,000 SNPs among greater than 500 genotypes of all major market classes in the US. These SNPs were identified using next generation sequencing of a set of 20 genotypes. A subset of ~6,000 SNPs has now been selected that is representative of the diversity among pinto, great northern, small white, black, kidney, and snap beans. These are currently being used by breeders and geneticists on the project to study diversity within their respective programs and to fine-map many traits of agronomic interest. The full set of 10,000 SNPs is also being used to decipher the genetic factors associated with many agronomic and nutritional traits. This is being accomplished using data collect from a four location, national trial of 300 modern breeding genotypes. All of the genotypes are from the Mesoamerican gene pool. Modern association mapping techniques will be applied to uncover significant associations between the 10,000 SNPs and the genes that control the traits.

Although SNP technology is powerful, many places in the world do not have the technology necessary to apply it to a breeding program. Rather, PCR-based diagnostic tools are preferred and more reasonably priced. Using the sequence data collected during the SNP discovery process, insertion/deletion (indel) events greater than eight base pairs were identified. Using a number of filtering steps a total of ~2,800 indels were identified. These have now been mapped onto the assembly. One obvious scenario can be envisioned that describes their effective use.

Once bi-parental and association mapping populations have been evaluated and significant SNP associations discovered, indel markers that are physically close to the SNP locus can be selected. Those indels should then be further tested to confirm their utility in breeding programs. Importantly, these indels were developed to represent variation found within specific US market classes. Given that the indel marker is site-specific and market class specific, the prospects of markers with a utility to track a gene of interest within those genetically narrow populations most often used by breeders are enhanced. ■

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Peculiar features of common bean subtelomeres

by Manon RICHARD[1], Stéphanie PFLIEGER[1], Artur FONSECA[2], Andrea PEDROSA-HARAND[2] and Valérie GEFFROY[1,3]*

Abstract: In the common bean (*Phaseolus vulgaris* L.) genome, most of the well-characterized large resistance (R) gene clusters are located at the ends (rather than the centers) of linkage groups, suggesting that the location at the end of a linkage group, and by inference a subtelomeric location, is favorable for *R* gene proliferation. In addition, terminal knobs (heterochromatic blocks) are present at most chromosome ends of *P. vulgaris*. These unusual features of common bean genome (subtelomeric localization of NBS-LRR sequences and presence of terminal knobs) will be better investigated with the availability of the complete common bean genome sequence.

Key words: common bean, *Phaseolus vulgaris*, resistance gene, NBS-LRR, subtelomere

In the human genome, extensive cytogenetic and sequence analyses have revealed that subtelomeres (regions adjacent to telomeres) are hotspots of interchromosomal recombination and segmental duplications (9). This peculiar dynamic activity of subtelomeres has been reported in such diverse organisms as yeast and the malaria parasite, *Plasmodium*. As expected for a plastic region of the genome subject to reshuffling through recombination events, subtelomeres exhibit unusually high levels of within-species structural and nucleotide polymorphism and they often contain fast-evolving genes involved in adaptative processes (9). In plants, this plasticity of subtelomeres has not been identified in *Arabidopsis thaliana*, and to our knowledge, has not yet been investigated at a large scale for other plant species with full genome sequences available. Recent data suggest that common bean subtelomeres present peculiar features.

In the common bean genome, most of the well-characterized large resistance (*R*) gene clusters are located at the ends (rather than the center) of linkage groups (LG). For example, the *Co-x*, *I*, *B4*, *Co-4* and *Co-2* loci have been mapped to the ends of LG B1, LG B2, LG B4, LG B8 and LG B11, respectively (2, 5, 6, 7, 13). Genetic complexity of these clusters is illustrated by the B4 *R* gene cluster where *R* specificities and *R* Quantitative Trait Loci (QTL) against a large selection of pathogens including the fungi *Colletotrichum lindemuthianum* and *Uromyces appendiculatus* and the bacteria *Pseudomonas syringae* have been mapped (5, 6, 11). Similarly, at the *Co-2* and *Co-4* *R* locus, *R* specificities against both the bacteria *P. syringae* and the fungus *C. lindemuthianum* have been mapped (2).

In plants, the most prevalent *R* genes encode proteins containing a nucleotide-binding site (NBS) and a C-terminal leucine rich-repeat (LRR) domain (NBS-LRR). *R* genes belonging to this class have been identified in various plant species, in monocots as well as in dicots, and correspond to *R* genes effective against all types of pathogens and pests, including fungi, bacteria, viruses, nematodes, oomycetes, and insects. In common bean, molecular analysis of the *Co-2* and *B4* clusters have revealed that these complex *R* clusters consist of a tandem array of more than 40 CC-NBS-LRR (CNL) sequences (3, 8) (Figure 1A). Fluorescence *in situ* hybridization (FISH) analysis revealed a subtelomeric location for these two complex *R* clusters (3) (Figure 1B, E). Furthermore, the *Co-4* anthracnose *R* locus was also confirmed to be in a subtelomeric position of a common bean chromosome, now named chromosome 8 (10). During the bioinformatic analysis of B4 R gene cluster, a new 528-bp satellite repeat, referred to as *khipu*, specific to the *Phaseolus* genus, was identified between the CNL sequences (3). In order to determine the pattern of *khipu* distribution at greater resolution, FISH was performed on meiotic pachytene chromosomes because they are less condensed than somatic chromosomes. Terminal knobs (heterochromatic blocks) of different sizes were visible at most chromosome ends of *P. vulgaris*, while *khipu* tandem repeats were present on 17 chromosome ends (Fig. 1C), mostly corresponding to cytologically visible terminal knobs (Fig. 1D, F), indicating the existence of frequent ectopic recombination events in *Phaseolus* subtelomeric regions.

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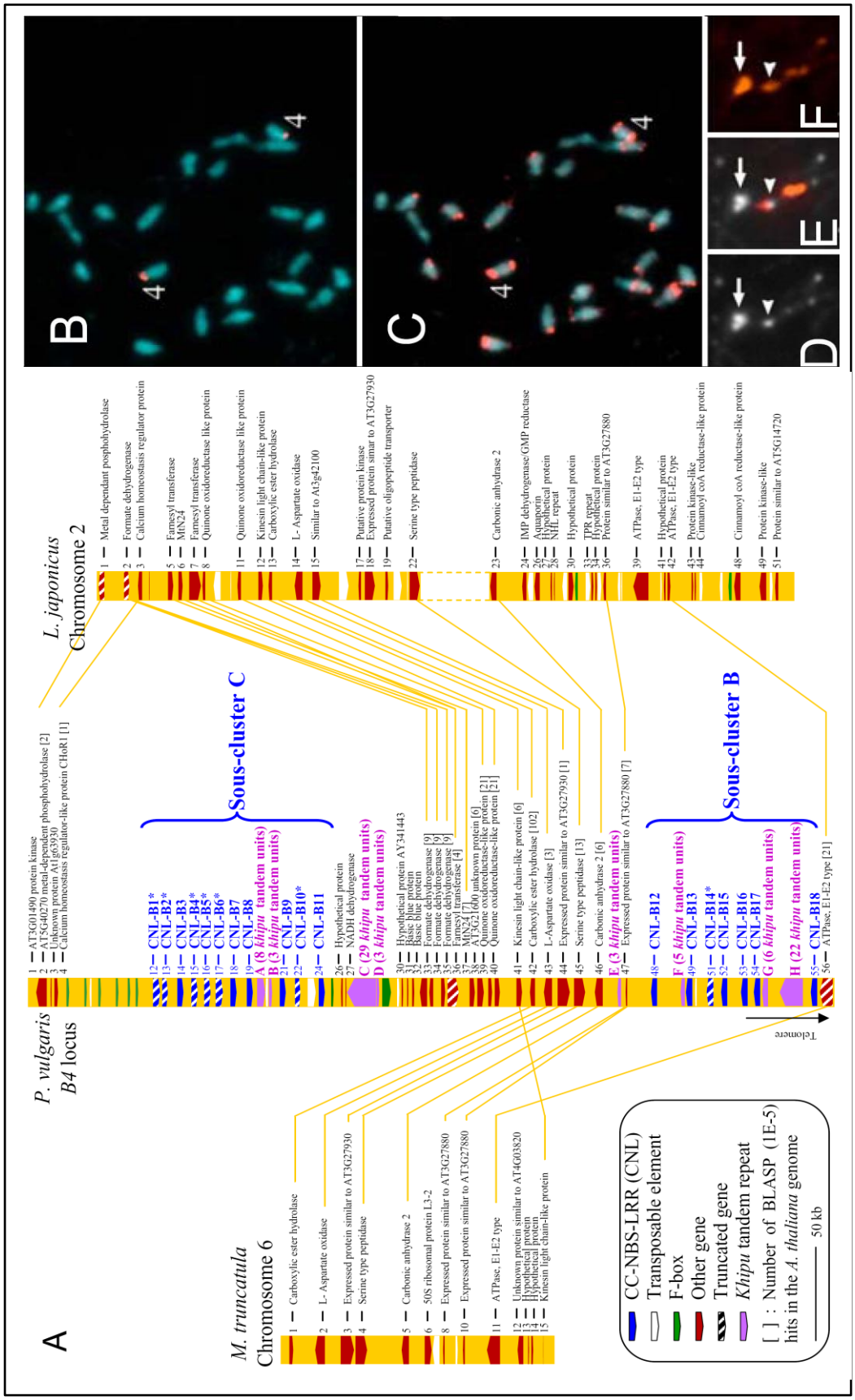

Figure 1. Sequence comparison between the *P. vulgaris* BAT93 B4 410-kb contig (center) and syntenic regions in *M. truncatula* chromosome 6 (left) and *L. japonicus* chromosome 2 (right). Yellow lines indicate significant homology matches between predicted genes. (A). B and C. FISH to mitotic *Pv* BAT93 chromosomes using B4-CNL (B) and *khipu* (C) as probe. D, E, F. FISH to pachytene chromosomes (D) using B4-CNL (E) and *khipu* as probe (F). The major knob and the minor knob are indicated with an arrow and an arrowhead, respectively. (3)

Unlike species with large genomes, heterochromatin is largely restricted to pericentromeric regions in small-genome plant species. For example, in the compact *Arabidopsis thaliana* genome (125 Mbp) only two knobs have been reported, while in the large maize genome (2,671 Mbp), numerous knobs have been reported (1). The relatively small genome of *P. vulgaris* does not seem to follow this tendency. Indeed, heterochromatic knobs have been detected in most *P. vulgaris* chromosome termini and *khipu* tandem repeats are components of most of them (3). This abundance of terminal knobs in *P. vulgaris* is in sharp contrast with results from other legume species such as *L. japonicus* and *M. truncatula*, where most of the heterochromatin is localised at pericentromeric regions and no terminal heterochromatic blocks have been reported, except for the 45S rDNA cluster on *Lj*2 (12). Thus, the complexity of bean subtelomeres does not seem to be obviously related to its genome size, because at 588 Mbp, *P. vulgaris* is not significantly larger than *L. japonicus* (472 Mbp) or *M. truncatula* (500 Mbp). In cereal, similar results were obtained for rye (*Secale cereale* L.) genome. Indeed, rye differs from phylogenetically related wheat (*Triticum*) and barley (*Hordeum*) in having large heterochromatin blocks in the subtelomeric regions of its chromosomes (4).

The availability of the complete genome sequence of *P. vulgaris* offers now a unique opportunity to investigate the peculiar distribution of NBS-LRR sequences in the common bean genome and their association with *khipu* satellite. ■

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Development of transgenic common bean with agronomic traits

by Thaís M. CIPRIANO[1,2], Abdulrazak B. IBRAHIM[1,2,3,4], Josias C. FARIA[5] and Francisco J.L. ARAGÃO[1,2,3,4]*

Abstract: The recent development of first transgenic germinivirus resistant common bean in the field is a direct benefit of advances in transgenic technologies and breeding techniques made possible by genome sequencing and application of high-throughput molecular tools as well as improved knowledge on morphological and cellular responses of different plant tissues and organs culture *in vitro*. Efforts from various research centers culminated in the employment of some of these techniques, resulting in the event Embrapa 5.1, generating the first commercially available transgenic common bean that met the Brazilian biosafety regulations. This represents an important phase in common bean biotechnology with the potential to boost productivity thereby improving economic returns to farmers all over the world.

Key words: common bean, transgenic bean, BGMV, biosafety

Common bean: production, utilization and challenges

Common bean (*Phaseolus vulgaris* L.) is one of the five domesticated species out of the 30 known members belonging to the genus *Phaseolus*. It is an important grain legume consumed in tropical and sub-tropical countries of Latin America, Africa and Asia. Cultivated by resource poor farmers, who are often unable to purchase and apply agrochemicals on a regular basis, the crop occupies more than 85% of the total area cropped with *Phaseolus* throughout the world.

Despite this nutritional importance and the ease with which it may be cultivated, its productivity has been declining in some regions due to such limiting factors as poor agronomic practices, diseases, insects, nutritional deficiencies, soil and climate constraints and lack of improved varieties and weed competition.

In order to address these problems, a number of common bean breeding programs initiated by different research centers all over the world, have led to the development and delivery of varieties with enhanced disease and insect resistance, greater drought resistance, and other important agronomic traits. This is in part, due to the identification of molecular markers for various traits and construction of genetic linkage maps in the crop, leading to the development of tools for marker-assisted selection-based breeding programs. In addition, map-based cloning of some genes identified from the crop assisted in the progress recorded so far in the development of improved varieties of common bean.

In spite of this, a lot needs to be done in order to, not only sustain this progress, but confront the mounting challenges faced by the crop in a rapidly changing environment. Although classical breeding will continue to benefit producers of beans, the full potentials of the crop are unlikely to be fully harnessed due to the obvious limitations of this approach. Thus it is unlikely to provide all the solutions for improving the crops to meet the needs of farmers and commercial production due, primarily to restrictions caused by limited genetic variability within the species and difficulties in assessing traits.

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Common bean genetic engineering

To confront this challenge, genetic engineering represents an important alternative that may accelerate the production of the crop with useful traits especially when applied along with classical breeding approach.

The last few decades saw the optimization of most of the systems developed for the regeneration and transformation of common bean. In parallel, the availability of characterized genes, including coding and regulatory sequences, has increased as a direct result of studies on structural and functional plant genomics. As in other crops, genetic engineering of common bean is focused on the introduction of foreign genes to promote tolerance to biotic and abiotic stresses, increase yield potential and reduce sensitivity to environmental adversity. The fact that this strategy allows for the development of elite crop lines in an efficient and timely manner-a feat which is often difficult to achieve through conventional breeding-, makes it a method of choice in many research centers. Success in the development of transgenic seeds of beans ensure increase yield and guarantee yield stability and production cost that maximizes economic returns to the farmers.

Methods employed in genetic transformation of common bean include *Agrobacterium*-mediated system, direct DNA uptake into protoplasts and particle bombardment. Although there have been advances in the methodologies for gene delivery and plant tissue culture, legumes such as common bean, only proved amenable to some of these systems after certain "unconventional" initiatives, such as the elimination of some steps involving tissue culture (4).

Although earlier researchers showed transient gene expression by electroporation and PEG-mediated protoplast transformation, no transgenic bean plants were obtained. One of the first reports was based on the use of *Agrobacterium* system. However, there was no molecular evidence for genetic transformation or progeny analysis. Later, another report appeared where *A. tumefaciens* was used to achieve expression of a *lea* gene that conferred abiotic tolerance in *P. vulgaris* in a protocol based on sonication and vacuum infiltration, although transformation efficiency was low (8).

Figure 1. Transgenic (GM) and non-transgenic (non-GM) plants under field conditions and natural infection by the *Bean golden mosaic virus*. Severe viral symptoms are observed in non-GM plants while GM plants do not show symptoms

The Brazilian experience

Perhaps the most reproducible system for common bean transformation is so far based on the biolistic process which had earlier led to the generation of some transgenic tissues and organs. A pioneer work that reported the introduction of genes of agronomic interest appeared in 1992 when *be2s1*, a gene coding for albumin, isolated from Brazilian nut, was introduced into cell lines of common bean in order to increase methionine level (1). Subsequent attempts using this approach led to an increase of 14 to 23% of methionine in the seeds of transgenic lines when compared to non-transgenic plants (3). A major setback to this remarkable success was the fact that 2S albumin from Brazilian nut was later iden tified as an allergen. Consequently, the development of transgenic common bean variety with improved methionine content was discontinued. In 1993 Russell et al. (9) introduced the *bar* gene, which encodes for phosphinothricin acetyl transferase (PAT), conferring resistance against the herbicide ammonium glufosinate with the coat protein gene from *Bean golden mosaic virus* (BGMV) in an attempt to develop virus-resistant plants. Although the resulting transgenic plants showed resistance against the herbicide under glasshouse conditions, there was no evidence of resistance against the virus.

This earlier work inspired further attempts that recorded some degree of success by cloning the genes *Rep-TrAP-REn* and *BC* from BGMV, under the control of CaMV 35S promoter in the plasmid p35SACBC. Following transformation, two transgenic lines exhibiting delayed and attenuated viral symptoms were obtained (2). In another report, transgenic plants were obtained using a vector containing a mutated *rep* (*AC1*) gene, which codes for a mutated AC1 (REP) protein bearing amino acid codon change in the putative NTP-binding motif (D262R). Resistance against the virus was observed in one line, which when studied over several generations, appeared to depend on inoculation level (7). As an improvement to the approach above, RNAi construct was used to silence *AC1* viral gene in an attempt to generate resistant transgenic common bean plants (6). Eighteen transgenic lines of common bean were obtained expressing intron-hairpin which led to post-transcriptional gene silencing of the *AC1* gene. Two of these lines exhibited high resistance and gave rise to progenies that were free from symptoms upon inoculation under high pressure of more than 300 viruliferous whiteflies per plant. This resistance was observed during the whole plant life cycle and at early stage of development. The plants were tested under field conditions in state of Goiás, Minas Gerais and Paraná (Brazil) (Fig. 1). Results from these trials confirmed that transgenic lines were immune to the BGMV (5). Biosafety evaluations, based on the guidelines of the Brazilian Biosafety Committee (CTNBio) and other regulatory authorities, were carried out with a view to obtaining authorization for commercial release of the first transgenic bean varieties. These studies comprised RNA characterization, food/feed safety analyses, molecular characterization, agronomic equivalence, environmental safety, composition and nutritional equivalence. In addition, the stability of foreign gene expression, gene flow and factors related to interaction of these plants exposed to natural stress under tropical environments were evaluated. Based on these studies, in 2011 the event Embrapa 5.1, resistant to BGMV, was approved for commercial release by CTNBio. A version of the proposal presented to CTNBio is available (in Portuguese) at the page http://www.ctnbio.gov.br/index.php/content/view/16501.html.

This marked an important milestone in Brazilian biotechnology because the transgenic beans arising there from, was the first of its kind to be approved for commercial release produced by a public national agricultural research center. This is especially important for small producers who stand to benefit through improved productivity, reduced use of pesticides (that could reach up to 40%) and thus reduce costs.

Conclusion

The remarkable success that led to the development of first commercially approved common bean underscores the great potentials attainable through application of transgenic technologies in farmer preferred varieties of the crop. The efficient and timely manner through which improved varieties of the crop can be obtained will guarantee yield stability and low production cost that maximizes economic returns to farmers while addressing the problem of food insecurity. ■

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MicroRNAs as post-transcriptional regulators in common bean (*Phaseolus vulgaris*)

by Georgina HERNÁNDEZ* and Bárbara NOVA-FRANCO

Abstract: Current knowledge on plant microRNAs (miRNAs) comes mostly from *Arabidopsis*. The majority of plant miRNAs targets are transcription factors. MiRNAs have been involved as relevant global regulator of plant developmental processes as well as response / adaptation to different types of biotic and abiotic stresses. Despite the agronomic importance of legume plants, the knowledge of roles of miRNAs in legumes, including common bean (*Phaseolus vulgaris*), is scant. MiRNAs are involved in biological processes like nutrient balance, development, reproduction and plant-microbe interactions; therefore we consider that research in *P. vulgaris* miRNAs is crucial for improvement of this staple crop. Here we will review recent information about miRNAs in common bean that has been derived from research groups of the National University of Mexico.

Key words: common bean, microRNAS, nitrogen fixation, abiotic stress, metal toxicty.

MiRNA biogenesis in plants

Complex biological processes such as plant development or plant adaptation to variable environmental conditions are finely and precisely controlled by multiple regulatory mechanisms. These include transcriptional and post-transcriptional regulation of gene expression where transcription factors and non-protein-coding RNAs play key roles.

The microRNAs (miRNAs) are small non-protein-coding RNAs that have emerged as ubiquitous post-transcriptional gene regulatory molecules in plants and animals. Plant miRNAs, approximately 21 nucleotides long, are derived from the processing of longer primary miRNA transcripts adopting hairpin-like structures. MiRNAs are negative regulators that suppress expression of their target mRNA mainly by inducing its degradation. The recognition of the miRNA target(s) is based in sequence complementarity (3) (Fig. 1).

Phaseolus vulgaris miRNAs

The first studies to identify miRNAs included the cloning and sequencing - employing traditional sequencing methods- of populations of small RNAs present in different plants. This strategy was used to identify miRNAs from common bean, both from different organs of plants grown in optimal conditions and from seedlings subjected to abiotic stresses such as drought, cold and salinity (1). Members from 16 conserved miRNAs families and eight novel miRNAs were identified in common bean. More recently, deep-sequencing technologies have allowed identifying larger numbers of plant miRNAs. High-throughput small RNA sequencing was applied to extend our knowledge of the common bean miRNA population (6). Small RNA libraries were prepared from common bean roots, seedling, flower buds, and leaves and these were sequenced using Illumina's platform. In this work 109 miRNAs belonging to 29 conserved families were identified for *P. vulgaris* and 29 novel miRNA candidates were predicted based on small RNA reads and precursor predictions (6). Bioinformatic analyses have been used to predict target genes for conserved and novel common bean RNAs (1, 6). As shown in Table 1, most of the predicted targets for conserved miRNAs that have been detected in *P. vulgaris* code for transcritpion factors belonging to different gene families. However other miRNAs recognize targets with variable functions *i.e.* pvu-miR2119 targets an alcohol dehydrogenase mRNA.

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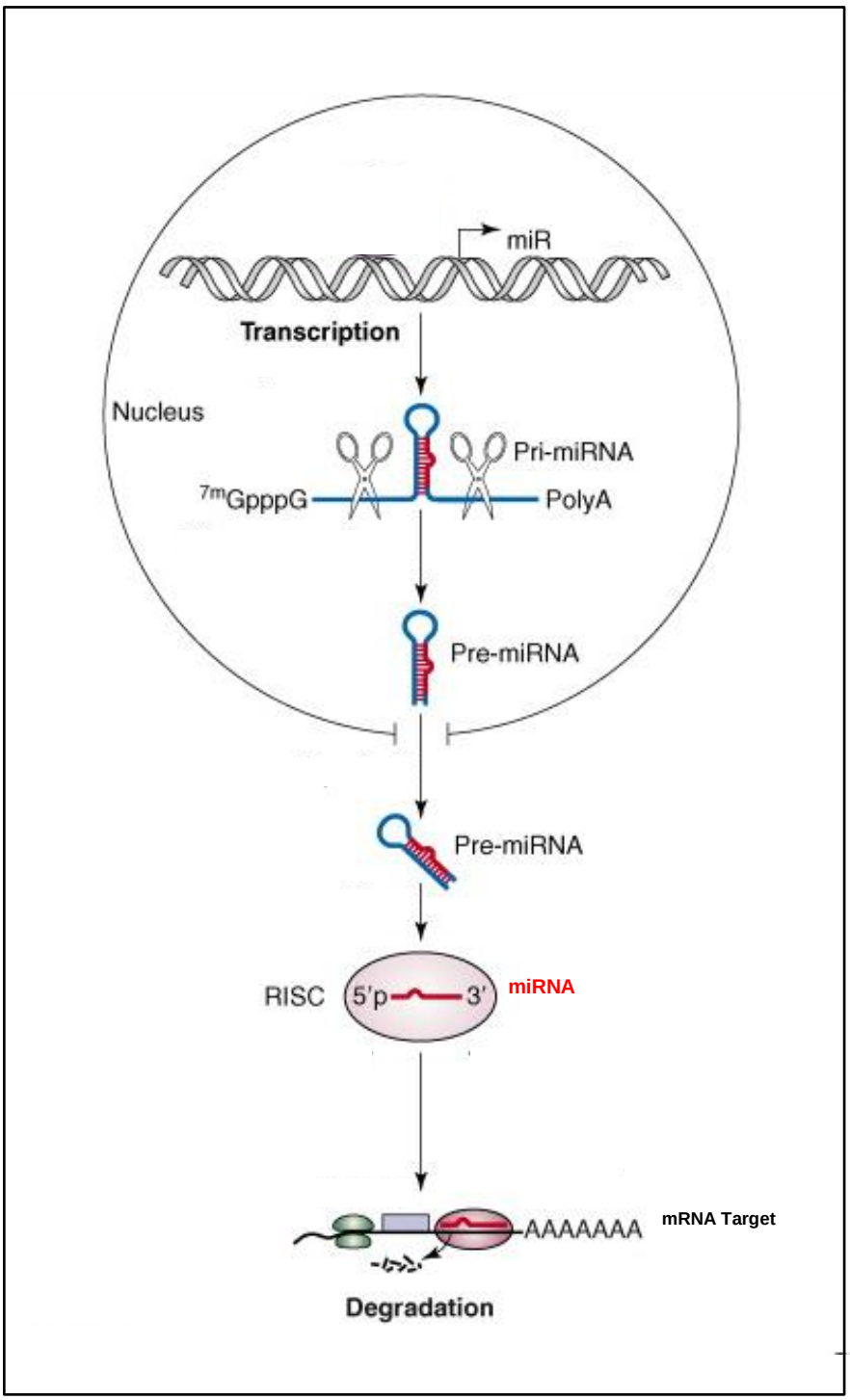

Figure 1. MiRNA biogenesis in plants. MIR genes are transcribed from their own locus. Precursors are processed to produce the mature miRNA that incorporates into RISC (RNA induced silencing complex), binds specifically to a target mRNA and induces its degradation. Modified from Zhao et al. (2007) Trends Biochem Sci 32

Published work about identification of miRNAs in common bean (1, 6) was done prior to having access to the genome sequence of this legume. However *P. vulgaris* genome was sequenced recently and it is now deposited in Phytozome database (*Phaseolus vulgaris* v0.9, DOE-JGI and USDA-NIFA, http::/www.phytozome.net/commonbean). This resource allows mapping the miRNA-coding genes in the *Phaseolus* genome and identifying the miRNAs precursors with predicted stem-loop structure.

Research in our group aims to define the roles of miRNAs in common bean nodule development/function and abiotic stress responses. The root nodules are novel plant organs that result from effective interactions between rhizobia, nitrogen (N)-fixing bacteria, and legumes. The differentiated N-fixing bacteroids establish in the nodules and fix atmospheric N that is assimilated, as ammonia, by the plant. Through this symbiotic interaction legumes produce their own fertilizer, and so it is relevant for sustainable agriculture. Common bean symbiotic N-fixation and crop yield is limited by abiotic stresses such as nutrient deficiency and metal toxicity that are common in acidic soils where beans are grown. Current knowledge indicates that legume miRNAs play important regulatory roles in processes like rhizobia-interaction, N-fixation and nodule development as well as in the mechanisms that allow common bean plants to cope with environmental stresses (7). We consider that the understanding of miRNAs roles in regulatory networks is relevant for common bean improvement.

We have analyzed the expression profile of miRNAs in leaves roots and nodules of nutrient-sufficient and nutrient-stressed (phosphorus, iron or nitrogen deficiency; and (aluminum, manganese or copper toxicity) common bean plants. For this we used a hybridization approach employing miRNA macroarrays printed with oligonucleotides complementary to known miRNAs from *P. vulgaris*, *Glycine max* (soybean) and miRNAs conserved in different plants (9). We have detected 42 miRNAs expressed in the different common bean organs and stress conditions. Recently we have identified the genes coding for these miRNAs in the *P. vulgaris* genome. Some miRNAs responded to nearly all stresses and in the three organs analyzed while others showed organ specific responses.

As mentioned before, our group is most interested in identifying relevant regulatory roles of miRNAs in the N-fixing nodules of common bean. Table 1 shows 19 miRNAs identified as nodule-expressed in *P. vulgaris,* via miRNA macroarray hybridization (9). These include conserved and *P.vulgaris* miRNAs. Analysis of the *P. vulgaris* genome sequence (http::/www.phytozome.net/commonbean) lead us to identify the number of loci that code for each of this miRNAs, which vary from 1 to 9, thus indicating the existence of different members of each miRNA family. Most of these miRNAs were also expressed in roots and/or in leaves, with the exception of miR172 that was detected only in the nodules. Two conserved miRNAs: miR319 and miR398 were detected only in stressed nodules (nutrient deficiency or metal toxicity) and not in nodules from plants in optimal growth conditions, thus indicating their role in stress responses.

We have demonstrated the participation of PvmiR399 in the PvPHR1 transcription factor signaling pathway for phosphorus (P)-deficiency in common bean (8). For this we used a functional genomics approach applying the RNAi technology in bean composite plants, with untransformed shoots and transformed roots resulting from *Agrobacterium rhizogenes* - mediated transformation (2, 8). We analyzed the transcript profile of genes that respond to P-deficiency in composite plants with low (silenced) transcript levels of PvPHR1 and PvmiR399 growing in P-deficiency as compared to optimal conditions. Our results demonstrated that, similar than in *Arabidopsis,* PvPHR1 controls P-deficiency signaling in common bean roots. Once P-deficiency is sensed -either locally or systemically- by unknown molecule(s) the PvPHR1 transcription factor positively regulates the expression of target P-responsive genes (for P transport, remobilization and homeostasis) and also of PvmiR399. The target of PvmiR399 is the ubiquitin E3 conjugase PvPHO2 that promotes degradation of some P-deficiency responsive genes through ubiquitination. In P-deficient conditions PvmiR399 will increase and so will exert a negative regulation upon PvPHO2 to prevent degradation of genes needed to cope with P-stress.

Table 1. MiRNAs in *Phaseolus vulgaris* nodules

miRNA	Target gene	miR loci in *P. vulgaris* genome [a]	miRNA expression in *P. vulgaris* [b]
miR156	Squamosa promotor binding-like protein (SPL)	5	N, R, L
miR157	Squamosa promotor binding-like protein (SPL)	6	N, R, L
miR159	MYB transcription factors	1	N, R, L
miR160	Auxin Response Factors (ARFs)	5	N, R, L
miR164	NAC, CUP Transcription factors	7	N, R, L
miR166	ATHBs	9	N, R, L
miR167	Auxin Response Factors (ARFs)	5	N, L
miR170	Scarecrow-like protein	6	N, R, L
miR172	APETALA 2 (AP2)	6	N
miR319	TCP Transcription factors	2	SN, L
miR390	Trans-Acting siRNA 3 (TAS3)	2	N, R, L
miR395	ATP Sulfurylase	4	N, R, L
miR396	GRL	2	N, R, L
miR398	Cu Superoxide Dismutase	2	SN, R, L
pvu-miR159.2	Chlatrin heavy chain	1	N, R, L
pvu-miR1511	SPIRAL1-like1	0	N, L
pvu-miR2118	U1 snRNP 70K	1	N, R, L
pvu-miR2119	Alcohol dehydrogenase	1	N, R, L
pvu-miR2199	ARF-GAP	1	N, R, L

Current research from our group aims to demonstrate the role of selected miRNAs in the nodulation and symbiotic process of common bean and also in the response of the plant to metal toxicity stress (5). There is still a lot to know about the crucial roles of miRNAs in common bean. We are confident that knowledge in this area will expand in the near future and it will contribute to improve yield and quality of the most important grain legume for human consumption in the world. ■

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Use of genetic diversity in common bean breeding

by James D. KELLY

Abstract: Common bean (*Phaseolus vulgaris* L.) is the most widely grown and consumed grain legume worldwide. Within the Americas a wide range of bean seed and growth types are grown from the Canadian Prairies to Salta in the northern Argentina. Beans are adapted from sea level conditions in the Caribbean to over 2000m in the Andean highlands. In many countries, beans are displaced by higher valued commodities and export crops to more marginal production areas with lower rainfall, shallow infertile soils, shorter growing seasons at higher elevations and latitudes. Here, I explore how genetic diversity and genomic tools can be used in breeding programs to improve common bean productivity and produce a higher value crop.

Key words: diversity, breeding, common bean, gene pools, market classes

Genetic diversity in common bean

Beans originated in Mesoamerica most likely in Mexico (1) but wild beans can be found from Chihuahua in northern Mexico to Salta in northern Argentina. Multiple domestication events occurred throughout this region and as a result beans are classified in the two major gene pools, Andean and Middle American (Table 1). The Andean gene pool is characterized by large-seeded types whereas the Middle America gene pool is genetically more diverse and consists of smaller to medium sized beans. There is free recombination between members of the same gene pool but breeders have confronted greater difficulties when crossing between gene pools as incompatibility barriers restrict free gene flow. Major genes can be moved across gene pools but more complex traits related to productivity and adaptation are not easy to transfer. Lack of breeding progress in larger seed types appears to be due in part to narrower genetic base in the Andean gene pool, as breeders have not successfully expanded the genetic diversity due to the inter-gene pool barriers to free genetic exchange. Breeders are now looking at members of wild *P. vulgaris* as more likely parental candidates to expand genetic diversity in Andean beans rather than members of the cultivated gene pool. Wild *P. vulgaris* has been a valuable source of genes that condition resistance to seed weevils. Eight major genes known as '*arc*' genes have been reported to provide varying levels of resistance to both the common and Mexican bean weevils. The trait is associated with a unique protein known as Arcelin that does not exist in cultivated beans. Four other members of the cultivated *Phaseolus* genus also offer opportunity to seek traits that are absent from common bean. Unique resistance to drought, common bacterial blight and rust are found in tepary bean (*P. acutifolius*) in the tertiary gene pool and some of these traits have been successfully transferred to common bean. Root rot and white mold resistance resides in the scarlet runner bean (*P. coccineus*) in the secondary gene pool and breeders continue to struggle to integrate these into useful common bean germplasm.

Useful traits from common bean

Bean breeders have to work within the major constraints of growth habit, maturity, and seed traits. Maturity and growth habit are constraints set by producers within specific production areas. Growers in the US Midwest want upright type II bean varieties that they can direct harvest and mature in 100 days ahead of the autumn frosts. In contrast producers in the semiarid highlands of Mexico prefer prostrate vine type III plant that they can sow at lower densities as these plant types are more responsive to the low and variable rainfall patterns in the region. Breeders in Central America want short season (70 days) varieties that they can plant in the short rainy 'postrero' season since the types evade drought and produce despite the limited and diminishing rainfall. Determinate type I bush beans are preferred by farmers in the highlands of Ecuador where they plant large-seeded Andean Calima types in contrast to the higher mountainous areas (> 1800m) where climbing beans such as Cargamanto are more productive as they are better adapted to those elevations and have a longer growing season. Transferring genetic gains achieved in one production area is not always possible as the growing conditions and growth habits preferred by farmers are very different and not universally suitable.

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Breeding for a specific seed market class within the wide array of commercial seed types (Table 1) that differ in culinary quality is equally challenging. Genetic progress in one seed type is not always transferable to contrasting seed types due to genetic incompatibility, dramatic differences in seed size, shape, color, patterns, and culinary quality including processing quality. Seed quality traits necessitate strict selection criteria that contribute to narrowing the genetic base of cultivated beans. Given the constraints imposed by producers and markets, breeders struggle to introduce genetic variability to meet production challenges, disease and insect pressure and the changing climatic conditions that will confront producers in the near future.

There is a clear recognition that intense selection for specific traits causes a narrowing of the genetic base of the resulting progeny. There is an equally compelling argument that to achieve high yields and rigid quality standards, breeders need to retain and combine desirable genetic components many of which are minor in effect. Breeders cross elite with elite lines to maintain and enhance those economic traits accumulated in elite lines. Introducing new genetic variability risks a breakup of these favorable genetic combinations and linkages. To meet these challenges, the use of 3-tier breeding pyramid (3) was proposed as the most effective way to introduce new variability while retaining the breeding structure needed to produce elite varieties. New variability is introduced at the bottom of the pyramid using breeding systems such as recurrent selection to introgress useful variability into germplasm that applied breeders can introduce into their breeding materials. Pre-breeding systems might also be utilized to introduce variability from wild and related species at this level. As new germplasm is 'tamed' it can be moved to the middle tier of the pyramid where it is more likely to be used in breeding future varieties. Different breeding methods can be deployed at different levels of the pyramid to optimize the specific goals. This strategy has resulted in development of upright type II plant habit in a wide array of seed types grown in the US using germplasm from Central America. Gains in larger-seeded Andean seed types have not been forthcoming and may require a different breeding approach.

Table 1. Gene pools, races, growth habits and seed size of different commercial bean market classes grown worldwide

Gene pool	Race	Growth Habit †	Seed size g/100 seed	Commercial class
Andean	Nueva Granada	Type I	50-95	Kidney, Calima, Alubia, Fabada, Canellini, Canadian Wonder, Mweze Moja, Kabanima, Kabulangeti
			45-60	Bush Cranberry, Sugar, Borlotti, Pompadour, Cargabello
		Type IV	55-100	Cargamanto, Sangre de Toro, Fabada,
	Chile	Type III	40-55	Vine Cranberry, Coscorron, Cacahuate, Araucano, Pompadour
	Peru	Type I	50-60	Yellow, Azufrado, Canario, Bayo, Mantequilla, Peruano, Jalo
		Type IV	35-55	Nuñas
Middle American	Mesoamerican	Type II	20-30	Black, Preto, Carioca
		Type II, I	20-25	Navy, Pea, Panamito
	Durango	Type III, II	35-45	Pinto, Ojo de Cabra
			32-42	Great Northern
	Jalisco	Type II, III	30-38	Small Red, Red Mexican, Rojo de Seda
			30-37	Pink, Flor de Mayo, Rosinha
	Guatemala	Type IV	20-30	Small Red and Blacks – Mexico, Central American only

† Type 1= Determinate bush; Type II = Indeterminate upright short vine; Type III = indeterminate prostrate vine; Type IV = Indeterminate climbing habit(s)

The Genomic era is finally reaching the *Phaseolus* community (2). The first two bean genotypes from each gene pool will be sequenced and the first SNP Chip platform will be available to bean breeders in 2012. Currently breeders have utilized a wide array of SCAR markers linked to many of the major disease resistance traits but the opportunity to expand marker-assisted selection to a wide array of traits is on the horizon. Additional information can be found on web sites below:
BeanCAP, www.beancap.org, and
BIC, www.css.msu.edu/bic. ■

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The symbiosis between *Phaseolus vulgaris* and rhizobia

by Carmen QUINTO*, Rosana SÁNCHEZ-LÓPEZ, Luis CÁRDENAS, Jesús MONTIEL, Manoj-Kumar ARTHIKALA, Noreide NAVA and Olivia SANTANA

Abstract: The symbiosis between legume and rhizobia, a highly specific and coordinated process, culminates with the formation of nitrogen-fixing nodules. This process is essential for a sustainable agriculture in food insecure countries. Here we investigated early events in the interaction *Phaseolus vulgaris*:*Rhizobium etli* by biochemical, cell imaging, molecular and genomic approaches. Functional analysis of *PvSymRK* (symbiosis receptor-like kinase) and several *PvRboh* (plant NADPH oxidases) genes suggests that they have key roles in nodule formation. The earliest (seconds) cellular responses in living root hair cells to NF were recorded by using specific fluorescent probes. Fast and transient intracellular reactive oxygen species (ROS) changes responding to NFs were found. These results will be briefly discussed.

Key words: *Phaseolus vulgaris*, nodulation, symbiosis, rhizobia, SymRK, Rboh, ROS, fluorescent probe, root hair, Nod factors

Plants establish beneficial associations (symbiosis) with soilborne microbes leading to an improved nutritional *status* of plants, therefore an increased productivity in nutrient-limiting conditions. Symbiotic relationship between legume and bacteria (known as rhizobia) is characterized by the development of a new root-derived organ, the nitrogen-fixing nodule. In the last 25 years, a remarkable progress has been made in the understanding on the molecular and cellular events involved in the organogenesis of the nodule (1).

The establishment of the nodulation process is accompanied by significant changes in the developmental programs of both symbionts. Once the rhizobia senses the flavonoids released by the legume root, a set of rhizobial nod genes are switched on driving the synthesis of lipochitin-oligosaccharides, known as Nod factors. In turn, perception of the Nod factors by specific receptors (LysM kinase-like receptors) present in the root hair triggers a series of cellular responses, including the activation of signal transduction pathways, changes in root hair morphology and gene expression. Cellular responses are accompanied by specific molecular responses such as ion exchanges (K^+, Cl^-, Ca^{2+}, H^+), calcium oscillations and rearrangements in actin cytoskeleton (3, 5). Altogether these changes promote rhizobial attachment to and the invagination of the root hair membrane resulting in the formation of an elongated tubular structure, known as infection thread (IT) that guides the rhizobia toward the infection site. In parallel cortical-cell divisions, underneath the IT, prompt the formation of the nodule primordium (6). As the IT reaches the central tissue of the young nodule, the bacteria are released into the intracellular environment of cortical cells. Finally, in a plant-derived membrane, the symbiosome, rhizobia differentiate into bacteroids that express the enzymatic machinery needed to convert atmospheric nitrogen into ammonia (11).

To gain insights into the molecular mechanisms activated at the initial steps of *Phaseolus vulgaris* nodule infection and organogenesis, our group has undertaken the functional characterization of legume genes recruited as part of the responsive signaling components. We have focused on three experimental strategies: RNA-interference-based reverse genetics, cell biology and in vivo microscopy of Nod factor/rhizobia responsive root hairs, and evaluation of ROS production.

As an initial approach we have investigated the relationship between the spatio-temporal distribution of *Pv*SymRK and the development of *P. vulgaris* nodules (6). SymRK, a leucine-rich repeat receptor-like kinase, is a signal transducer whose expression is required at different steps of the nodulation process: the rhizobia invasion of root hairs, the cortical cell division, the IT formation and growth, as well as the release of rhizobia to the intracellular space of cortical cells to form the symbiosome (7, 12). A novel function of *Pv*SymRK linked to a well-coordinated differentiation and/or development of the nodule vascular bundles in *P. vulgaris*, as we have recently documented (6). We got interested in the nodule vascular system when we were analyzing the immunolocalization of *Pv*SymRK along the nodulation process. We consistently observed an, unanticipated, immuno-fluorescence signal associated to both the root central cylinder and the nodule vascular system at stages as early as the provascular traces of the forming nodule primordium (3 days *post*-inoculation or dpi), as well as in nodule vascular bundles in mature nodules. Such a remarkable result drew our attention to the analysis of the development of nodule vasculature using an RNAi- mediated reverse

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genetics strategy. We have performed meticulous histological analysis of the scarce nodule-like structures harvested from *PvSymRK* down-regulated transgenic roots. In addition to the previously described deficiency in both root hair invasion and IT/symbiosome formation, we have found "defects" in the vascular system of these nodule-like structures. Some nodule-like structures presented immature vascular traces (lacking tracheary elements), an ectopic distribution of the vascular bundles or even the absence of vasculature (6). The diversity in the spatio-temporal functions of SymRK opens the question on the identity of the signaling molecules involved in the activation of the SymRK-mediated transduction cascade, an ambitious task.

In our group, special attention has been given to changes induced in the apical region of the root hair in response to Nod factors. As aforementioned, a dynamic actin cytoskeleton is one of the driving forces regulating growth in root hair cells in response to Nod factor treatment or rhizobia invasion. Calcium changes (increased calcium gradient) at the tip region of the root hair have been related to the actin cytoskeleton rearrangements induced in response to Nod factors perception (2). Growing root hairs are highly polarized cells that present a typical tip growth limited to the apical dome, where a continuous actin polymerization is taking place, similar to what it has been observed in growing pollen tubes and fungal hyphae (1). In this context, we were pioneers showing that the actin microfilaments, visualized by microinjecting fluorescent phalloidin, became fragmented in *P. vulgaris* root hairs upon exposure to Nod factors (2). We are currently exploring the advantages of expressing fluorescent-actin-binding proteins (i.e. fused to GFP; 13) in transgenic roots to visualize, *in vivo,* the dynamics of actin filaments at specific stages of the nodulation process, rather than using fixed tissues as reported in most studies. In the same direction, we have initiated a series of experiments using improved versions of fluorescent probes, such as calcium-, pH-, ROS (reactive oxygen species)- and hydrogen peroxide-sensitive GFP indicators that exhibit expanded dynamic range and more resistance to photobleaching when compared to other fluorescent indicators (8, 9).

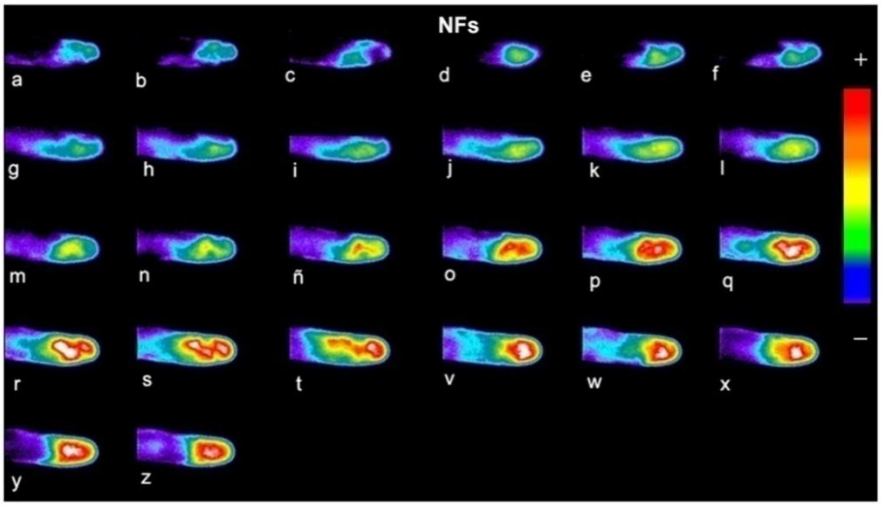

Figure 1. A fast and specific transient increase in the levels of tip-localized ROS signal is induced in *Phaseolus vulgaris* root hair in response to Nod factors. Serial images of a growing root hair cell loaded with CM-H2DCFDA (3.3 µM), a ROS-sensitive fluorescent dye. Upon addition of Nod factors (10^{-8} M; d), there is an increase in the tip-localized ROS signal. Images were captured every 10 sec. Exposition time was 10 msec.

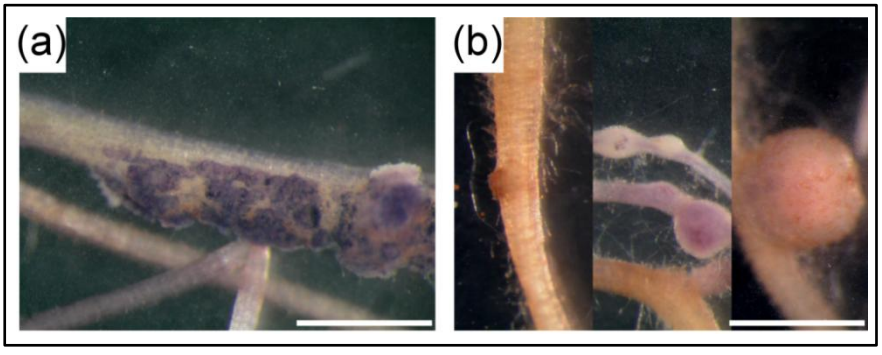

Figure 2. Superoxide accumulation in *P. vulgaris* nodule primordia (a) is inhibited by DPI (50 µM, 15 min) treatment (b). Superoxide accumulation (purple precipitates) was assessed by NBT staining. Scale bar= 1 mm.

In a previous report, we describe the use of ratio-imaging analysis in combination with a ROS-sensitive fluorescent dye (CM-H2DCFDA) to address ROS distribution in root hairs treated with Nod factors (4). We have observed Nod factors induce a rapid (15 sec to 1 min) and specific transient increase in the levels of tip-localized ROS signal (Fig. 1). Incubation with DPI (an inhibitor of RBOH/plant NADPH oxidases and flavin-containing enzymes, widely used to reduce intracellular levels of ROS) inhibits ROS changes induced by Nod factors. Fig. 2 shows that there is a superoxide accumulation during nodule primordia development, which is inhibited by DPI treatment. We have also demonstrated that extracellular ATP modulates intracellular levels of ROS. Localization of a large number of mitochondria coincides with the apical distribution of ROS signal, suggesting that these mitochondria may contribute to ROS production in this region of the root hair (4). In order to improve our understanding on ROS production and its functional relationship with the initial events of the symbiotic interaction of *P. vulgaris* with rhizobia, we have characterized the expression of *PvRboh* (respiratory burst oxidase homologs or plant NADPH oxidase) gene family, constituted by nine *PvRboh* genes (Montiel et al, manuscript in preparation). In particular, we have found that down-regulation of *PvRbohB* impairs ROS-production and IT growth, as observed in not-fully developed nodules produced in *PvRbohB*-RNAi roots (Montiel et al, manuscript in preparation).

In conclusion, our data generated using a combinatorial strategy based on reverse genetics and *in vivo* imaging of cells incubated with or expressing radiometric fluorescent probes are a contribution to the understanding of how the nodule infection and organogenesis take place. ■

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The response to water deficit in *Phaseolus vulgaris*

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Abstract: A major problem in common bean (*Phaseolus vulgaris* L.) agriculture is that most of the cultivated area is located in rainfed regions leading to low grain yields due to intermittent and terminal drought. To better understand the common bean response to water limitation, we have followed different approaches that include a physiological and molecular characterization of the response comparing cultivars differing in their drought resistance, the functional characterization of drought responsive proteins and of small non-coding RNAs (microRNAs). Some of the results are briefly described.

Key words: *Phaseolus vulgaris,* abiotic stress, water deficit, drought, gene expression, microRNAs, stress proteins.

A major problem in common bean (*Phaseolus vulgaris* L.) agriculture is that most of the cultivated area is located in rainfed regions leading to low yield (up to 80% of yield reduction) due to intermittent and terminal drought (3, 8). Common beans, as part of the basic food for people in Latino America, have been grown for a long time over a broad variety of environments, mostly under rainfed conditions, exposing them to recurrent droughts and to large changes in soil water availability (3); this has led to the selection of several genotypes associated with different mechanisms responsible of maintaining the plant functions needed not only for survival but also for enough seed production under drought. Hence, common bean could be a good crop model to identify different mechanisms to cope with water limitation.

Because the plant responses to water deficit differ depending on various factors, such as the plant developmental stage at the time of the stress impact, the severity of the adverse condition, or the combination of additional environmental stimuli (heat, light intensity, among others), it is relevant to identify and understand the physiological reactions that certain cultivars present upon particular water deficit conditions. This information, together with the identification and characterization of the different molecules participating in this response, will allow the establishment of functional links between these molecules and those physiological processes related to the plant adjustment or adaptive mechanisms operating under this environmental stress.

To obtain insights into the mechanisms involved in the plant response to water limiting conditions, we have focused on the analysis of physiological and molecular responses to water limitation in common bean (*Phaseolus vulgaris* L.). Three main approaches have been followed.

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Figure 1. *PvLea18* gene expression in transgenic roots of composite plants growing in optimal conditions (A) or after a salt treatment (75 mM NaCl, 48 hs) (B and C). In C, the lateral root meristems under stress conditions exhibit a similar *PvLea18* gene expression pattern than the meristem in the principal root (B).)

Because terminal drought is a major problem for common bean production as it occurs during the reproductive stage, importantly affecting seed yield in Mexico and other countries, we want to understand those mechanisms associated with the plant response to this stress condition. For this, we have characterized some physiological responses in cultivars belonging to the Durango race, considered a major drought resistance source for tropical environments (3). Particularly, we have used three cultivars Pinto Saltillo, Pinto Villa and Bayo Madero, with different drought susceptibility. The common bean cultivars were subjected to moderate and severe terminal drought treatments under greenhouse conditions, and we analyzed the following traits: relative growth rate, photosynthesis and transpiration rates, stomatal conductance, water-use efficiency, relative water content, proline accumulation, glycolate oxidase activity and their antioxidant response. The results from these experiments indicate that the resistance to terminal drought in Durango race and in particular in Pinto Saltillo cultivar is not only the result of an intrinsic high productivity but rather the consequence of the participation of various mechanisms: a fine control of stomatal conductance, an efficient CO_2 diffusion in leaf tissues and fixation, and an effective water use. These data strengthen the idea that in terminal drought resistant cultivars, response mechanisms that favor seed production over foliar growth prevail (1), and reinforce the pertinence of characterizing drought resistant genotypes selected for particular drought types, to build a better picture of those mechanisms involved in drought resistance during specific plant developmental stages and to particular environments (11).

We have also studied particular molecular mechanisms in the response to water deficit related to the function of proteins that accumulate in response to this stress condition. Those best characterized by our research are related to the role of the so-called LEA (Late Embryogenesis Abundant) proteins, which are ubiquitous in the plant kingdom and whose accumulation is closely associated to water deficit conditions imposed during development or by the environment (2). We have studied this group of proteins in common bean and Arabidopsis and found that they show similar accumulation patterns (2, 4). Even though we have been able to characterize the participation of some LEA proteins in response to water deficit in Arabidopsis, this task has been difficult in common bean, mainly because the limitation for transforming this species, among other constraints. Given this situation, we have worked in establishing the transformed 'hairy roots' system to characterize *P. vulgaris* water deficit responsive genes in an homologous system, where despite the analysis is only possible in these singular organs, it offers the opportunity to study their tissue and cellular localization and the effects of their deficiency and over-expression. This study has implied the establishment of conditions to impose water deficit for this particular experimental system to study some functional characteristics of these proteins. By using reporter genes fused to *LEA* gene promoters or by over-expressing some LEA proteins, we have been able to detect their localization in roots and root hairs, with a high accumulation in vascular tissues but also in cortex and epidermis. An interesting observation was their accumulation in meristematic regions, which correlates with the observation made in Arabidopsis regarding the impact of the AtLEA6 over-expression on the higher production of axilar and floral buds under water deficit (6). Inside the cell, these proteins are found in cytosol and nuclei (Fig. 1). Similar localization was previously found using specific antibodies (4). This validates the use of this system for such analyses.

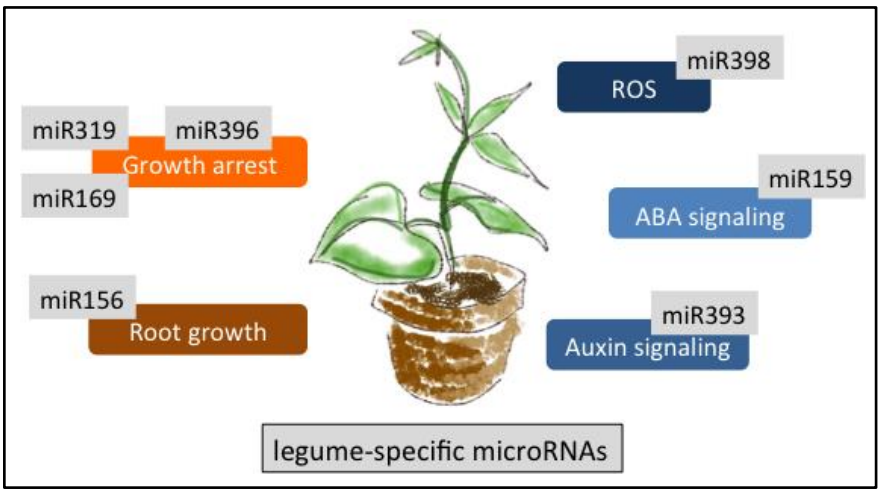

Figure 2. microRNAs participate in stress responses. Numerous microRNAs have been reported to participate during stress responses in different plant models, yet little is known about their roles in legumes and in particular in common bean. Here we depict only those miRNAs that have repeatedly appeared in different plant species along with cellular processes that may be affected based on the identity of characterized targets. Other less-conserved microRNAs can participate in legume-specific responses to stress, and their participation is under active scrutiny in our group. ROS: Reactive oxygen species; ABA: Abscisic Acid

A recently discovered mechanism of gene regulation involves the role of small RNA molecules known as microRNAs. microRNAs are small RNA molecules of 20-24 nts in length produced from longer precursors than are specifically processed by the type III RNAse DICER-LIKE 1 (DCL1) and associated protein factors. Within a multiprotein complex called RISC (RNA-Induced Silencing Complex), the microRNA is bound to the effector protein ARGONAUTE 1 (AGO1), and recognizes a target mRNA by RNA:RNA base-pairing. RISC subsequently directs mRNA down-regulation by AGO1-mediated mRNA endonucleolytic cleavage or by inhibiting mRNA translation. Hundreds of microRNAs have now been described in diverse plant species, where they have been recognized to regulate multiple processes including plant growth, root, leaf, and flower development, hormone signaling, as well as biotic and abiotic stress responses (6).

On this regard, we have focused on the analysis of microRNAs as regulators of this response in *P. vulgaris*. We have identified common bean microRNAs that are expressed under water deficit conditions, including several legume-specific microRNAs (1, 5). Their characterization, along with new data from high-throughput sequencing of small RNA populations from different organs of common bean has allowed us to obtain a global picture of microRNA dynamics (4). Their detailed study is revealing how common bean, and possibly other legumes as well, uses the repertoire of microRNAs to control plant processes in response to adverse conditions. In contrast with these microRNA studies, the identification of *P. vulgaris* target mRNAs has been hindered by the lack of sufficient genomic sequence data to obtain potential candidates. Thus, we have combined different strategies to identify miRNA targets: a bioinformatic prediction of targeted transcripts, a biochemical analysis of the AGO1 protein and of its interacting RNAs, and a high-throughput sequencing analysis of cleaved mRNAs to identify miRNA targets under water deficit conditions (Fig. 2).

The recent availability of the sequenced genome for *P. vulgaris* (www.phytozome.net) has allowed us to explore potential candidate targets at the genomic level. This comprehensive analysis will be invaluable to identify mRNAs subjected to microRNA regulation and it will provide us with novel tools to understand what cellular processes are being regulated by microRNAs during adverse conditions.

All together, our data will help to better understand strategies used by common bean and other legumes to cope with adverse environmental conditions. ■

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Molecular breeding for biotic stress resistance in common bean (*Phaseolus vulgaris*): Example of resistance to ascochyta blight, an intractable disease of tropical highlands

by Matthew BLAIR*, Luz N. GARZÓN, Oscar A. OLIVEROS and Gustavo A. LIGARRETO

Abstract: Biotic stresses cause the greatest reduction in yield potential of common beans (*Phaseolus vulgaris* L.) in most production areas devoted to the crop. Diseases are especially important in most years under most production systems. This is due to the multiple disease pathogens that attack common bean as well as the high prevalence of inoculum in most production regions. In addition, co-evolution of fungal strains and crop germplasm has led to diseases of high virulence especially in Latin America where the crop was domesticated and is still grown. Movement of pathogens from one continent to another and recombination in some fungal genomes has created new strains in Africa especially. The example that we will use in this mini-review is that of Ascochyta blight, an important disease of Latin America and some parts of Africa.

Key words: Ascochyta blight, breeding, common bean, fungal pathogen

The fungal pathogen: Ascochyta blight

Ascochyta blight (Fig. 1) is caused by the fungal pathogen *Phoma exigua* var. *diversispora* and generates losses of up to 84% in common beans grown in highland environments of the Andes, Central America or East Africa. Within the Andean region, the disease is especially prevalent in the Departments of Antioquia, Boyacá, Cauca, Cundinamarca and Nariño of Colombia as well as in many parts of Ecuador and Peru. The disease is also important in high elevation areas of Guatemala in Central America and in Tanzania in Eastern Africa. Few studies have analyzed resistance sources for levels of disease infection nor the inheritance of resistance to Ascochyta blight in beans. In some initial screening in Peru, some resistance was found in genebank accessions mostly from a relative species of common bean named scarlet runner bean (*P. coccineus* L.). Other research using only common bean genotypes, found that resistance was never very high and only a few accessions presented intermediate levels of resistance (3, 6).

Meanwhile, the existence of pathogenic races has not been confirmed, nor genotypes for the analysis of differential infection have been defined. Given this, we have recently made a collection of isolates for the pathogen in an attempt to differentiate strains from different regions of Colombia based on differential reactions with previously described resistance sources (4). In that study, we characterized the reaction of ten landraces and local cultivars through pathogenicity tests with four isolates of the disease agent as a first step towards creating a differential set of interactions. We found that cultivars varied in disease levels and that races of Ascochyta blight are likely to exist since both strong and weak pathogenicity strains were identified. Notable sources of resistance have been G4032, G6436, G20523, ICTA Hunapu and several ASC and ASR breeding lines (1, 4). Variability in resistance sources was mainly found within the Mesoamerican genepool (Table 1) while Colombian Andean landraces were all susceptible. The identification of potential races among the fungal strains was not surprising as races are well known and amply characterized for the pathogens causing anthracnose (*Colletotrichum lindemuthianum* Sacc. and Magn.) and rust (*Uromyces appendiculatus* pv. *phaseolicola* Pers.) diseases in common bean.

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Table 1. Sources of resistance evaluated against four Colombian isolates of the disease Ascochyta blight caused by *Phoma exigua* var. *diversisporum*.

Genotype	Gene Pool (*P. vulgaris*)	Infection level with corresponding strains[a]			
		Popayán ASC1	Antioquia ASC3	Cundinamarca ASC35	Santander ASC 236
Agrario	Andean	S	I	I	I
Cabrera	Andean	S	I	I	I
Cargamanto	Andean	I	I	I	I
D. Moreno	Andean	S	S	S	I
G3367	Mesoamerican	S	I	S	S
G3991	Mesoamerican	S	S	S	I
G9603	NA	S	S	S	S
G4032	Mesoamerican	I	I	I	I
G6436	Mesoamerican	I	I	I	I
G10747	Mesoamerican	I	I	S	I
G19833	Andean	S	S	S	I
DOR364	Mesoamerican	S	S	S	S
G2333	Mesoamerican	S	S	S	S
G19839	Andean	S	S	S	I
G35182	*P. coccineus*	R	R	R	R

Figure 1. Symptoms of Ascochyta blight in field essays

Molecular breeding for Ascochyta blight resistance

A major research effort has gone into tagging resistance genes and identifying linked molecular markers (7). Initial studies were almost all performed with small segregating populations and bulked-segregant analysis. Later studies included quantitative trait locus (QTL) analysis, to screen for levels of resistance to various pathogens where inheritance was not limited to single gene resistance factors. Many of these QTL analysis were performed with recombinant inbred lines (RIL) made from contrasting mapping parents and in some cases the same RIL population was used to screen for resistance to one or more disease resistances. Inheritance studies to fungal disease resistance have identified a large set of major resistance genes and a number of QTLs for reduced symptoms. More studies of this sort are needed especially for less-well studied diseases in this crop such as Ascochyta blight.

In the case of our focus pathogen, no inheritance studies have yet been performed. It seems likely that inheritance to Ascochyta blight is mostly quantitative as resistance is only intermediate and reflects differences in disease development on different varieties. Leaf lesion size is an important trait that is quantitatively inherited as is the infection of pods. A set of genetic mapping parents were found to vary for resistance to some Asocochyt blight strains (4) with the resistant allele coming from the Andean genotypes (G19833 and G19839) and the susceptible allele coming from the Mesoamerican genotypes (DOR364 and G2333). This seems to be conterintuitive since disease resistance sources have tended to be lacking in the Andean genepool for this disease, while most resistance has been observed in Mesoamerican bush and climbing beans. Full QTL studies with the derived RIL population would be of interest but would require better testing conditions as Ascochyta blight requires cool temperatures for optimum development and therefore is not adapted to greenhouse screening. Association of resistance with genotyping of derived lines might be an interesting way to

discover markers associated with resistance QTLs. This would be especially useful for progeny derived from G2333 as this genotype has been used for crosses to improve anthracnose resistance and it is important to monitor that susceptibility to Ascochyta blight should not be co-inherited with Anthracnose resistance.

A novel method of screening for QTL associations will be with candidate genes based on the full suite of resistance gene homologues (RGH) genes in common bean. We have carried out a project to identify over 400 RGH genes from the whole genome of the Andean genotype G19833 in an attempt to determine which function as disease resistance genes and against which pathogens. Many anthracnose resistance genes now appear to belong to this gene family and it is highly likely that NBS-LRR genes play a role in defense against various fungal pathogens.

Inter-specific crosses: a potential source of resistance to be handled with care

We have seen that common beans of both the Andean and Mesoamerican genepools are mostly susceptible to Ascochyta blight with some exceptions among Guatemalan black beans and other germplasm entries. Meanwhile high levels of resistance are found in the secondary genepool species scarlet runner bean (*P. coccineus*) and year-long bean (*P. dumosus*) (2). Inter-specific crosses are possible between these species although most segregants are more similar to one or the other species and repeated backcrossing is needed to return to the self-pollinating common bean type. Inter-specific crosses have not been made specifically for Asochyta resistance but crosses made for other purposes have been suitable for screening this trait.

Several populations derived from interspecific *P. vulgaris* x *P. coccineus* and *P. vulgaris* x *P. dumosus* crosses with the genotypes G35575 and G35999 have been screened for Ascochyta blight resistance so far (5). Both types of crosses have been found to result in lines that are intermediate but not high in tolerance to the disease.

Unlike previous interspecific lines with a Mesoamerican genepool background the lines from (5) were of Andean background. Results of that study confirmed the variability in the pathogenic capacity of different isolates of the causal agent and the need for differential varieties for a more accurate description of the isolates and categorization into races. Lines derived from G35575 were more resistant than those from G35999 but only to some of the Ascochyta strains. Most interspecific lines have been developed from a single or two backcrosses, something important to consider when breeding with interspecific crosses.

When making interspecific crosses, one must consider the characteristics of the donor species as well. For example, negative attributes of scarlet runner bean are its outcrossing nature, its poor seed set and its thick seed coat. Year-long bean has a thick seed coat but is less of an outcrosser. Large seededness, high mineral concentration as well as adaptation to cool rainy conditions are desirable traits of scarlet runner bean, however almost all scarlet runner beans have a vine architecture or are photoperiod sensitive and are difficult to cross or manage agronomically. Although some scarlet runner beans are type III bush beans with slightly less photoperiod sensitivity these generally do not carry as many desirable traits for resistance to diseases.

Conclusion

In conclusion, the breeding for Ascochyta blight resistance cannot rely on inter-specific crosses alone for variety development and it may advisable to use gene tagging and more precise marker-assisted backcrossing to transfer resistance genes within the primary genepool for Andean or Mesoamerican beans or from the secondary genepool to common bean. Therefore, QTL and gene-tagging studies should be extended to crosses within each species and inter-specific crosses to find the most important genes controlling resistance. The complete understanding and comparative analysis of LRR-NBS genes in the *Phaseolus* genus will aid in the transfer of resistance gene specificities between species. This is expected to result in durable resistance for intractable diseases such as Ascochyta blight. ■

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Resistance in common bean to fungal disease improved by plant-to-plant signalling

by Elizabeth QUINTANA-RODRÍGUEZ and Martin HEIL*

Abstract: Fungal infections are a common threat in the cultivation of common bean (*Phaseolus vulgaris*). However, the resistance to the fungal pathogen, *Colletotrichum lindemuthianum*, in high-yield cultivars of *P. vulgaris* can be enhanced via plant-to-plant signalling. Volatile compounds such as limonene, nonanal and methyl salicylate contribute to the antifungal properties of resistant bean cultivars and enhance also the resistance in neighbouring plants, even when these belong to susceptible cultivars. Plant volatiles represent an attractive alternative to the use of pesticides in the fight against fungal infections of common bean.

Key words: Volatile organic compound, plant-plant signalling, priming, resistance inducers

Plants have to cope with pathogens and herbivores during their entire life. For that reason the plants have developed elaborate mechanisms to respond to attack by detrimental organisms. In addition to pre-existing defence barriers, such as cuticles and cell walls, plants possess an inducible immune system that controls the activation of defence mechanisms after the recognition of an attacker. These responses are systemic, that is, the defence mechanisms are expressed both in the affected and in distant, yet undamaged organs. This spread of resistance throughout plant tissues is termed systemic acquired resistance (SAR) (4).

However, resistance on the one hand is costly in terms of energy and resource consumption (6). Highly resistant plants are therefore characterized by low growth rates and low yield. Thus, crop plants, including common bean (*Phaseolus vulgaris*), have lost a large part of the inducible resistance traits during the breeding for high yield and result much less protected from infections than their wild ancestors (1). On the other hand, resistance- (R-) gene mediated resistance is commonly highly specific and active only against selected strains of a given species of pathogen. In conclusion, we face an urgent need to search for new means of resistance induction.

Anthracnose, caused by *Colletotrichum lindemuthianum*, is one of the most important diseases in common bean, causing losses of up to 100% of the harvest when the climatic conditions favour the pathogen. Main strategies in the control of this disease include the use of sterile seeds, the application of fungicides, and the development of resistant cultivars. However, due to the high genetic variability among the strains of *C. lindemuthianum*, all these strategies lack broad efficiency.

We explored the potential that is represented by broadly resistant wild genotypes and landraces to search for inducible resistance traits. Wild genotypes of common bean and resistant cultivars such as Pinto Villa respond to challenging with *C. lindemuthianum* strain 1088 with the release of multiple volatile organic compounds (VOCs). Interestingly, plants of the susceptible cultivar Negro San Luis exhibit phenotypic resistance when growing close to challenged Pinto Villa plants (Fig. 1A).

We speculated that the expression of this resistance is caused by plant-to-plant signalling, that is, a resistance activation in as yet undamaged plants, or parts of a plant, that are exposed to VOCs emitted from challenged, resistance-expressing plants. This phenomenon has been broadly described for wild lima bean (*Phaseolus lunatus*). Lima bean plants exhibited a resistance to the bacterial pathogen, *Pseudomonas syringae pv syringae* when being located close to conspecific neighbours in which SAR to pathogens had been chemically induced (7). Because VOCs that are emitted from bean have anti-fungal effects, among others (2), the phenomenon of plant-to-plant signalling was likely to exist also in common bean.

Our recent observations showed that VOCs emitted from challenged common bean plants lead to resistance to anthracnose in plants of cultivars that are commonly considered susceptible to the fungus. The resistance was quantitatively similar to the resistant plants at the phenotypic level and was as strong as the resistance induced by the chemical resistance inducer, benzothiadiazole, BTH (Fig. 1B).

The volatile profiles were characterized in the headspace of challenged plants (of both susceptible and resistant cultivars) and also in plants induced chemically with BTH. Based on the identification of differentially emitted VOCs, we selected nonanal, limonene, and methyl salicylate to investigate their effect on the germination of fungal conidia, an important phase in the early steps of the infection of a plant with the fungus. All VOCs exerted an inhibitory effect on the germination of fungal spores, with limonene having the strongest effects (Fig. 2).

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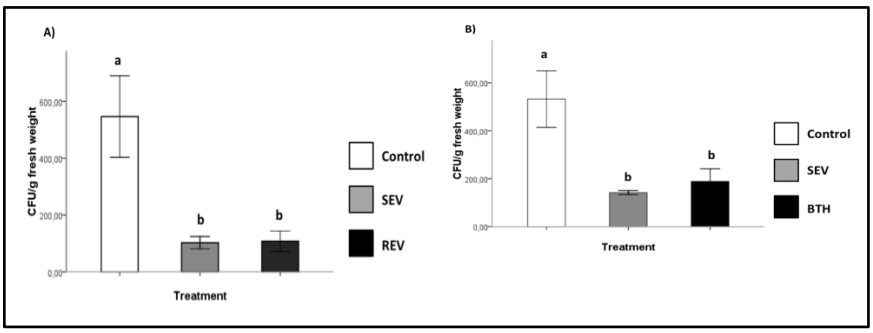

Figure 1. Airborne resistance induction in common bean. A) Quantitative evaluation of disease in controls (no treatment), susceptible Negro San Luis plants exposed to volatiles (SEV) coming from an infected resistant (Pinto Villa) plant and resistant emitter plant, Pinto Villa (REV). B) Quantitative evaluation of disease on susceptible Negro San Luis plants treated directly with 0.5 mM BTH (BTH) or exposed to the air coming from infected resistant plants (SEV). Control plants were sprayed with distilled water. Pathogen population was recovered in all the treatments and is represented as numbers of colony forming units (CFU) per gram leaf fresh mass. Sample size was n=6. Different letters indicate significant differences among treatments (P < 0.001 according to LSD).

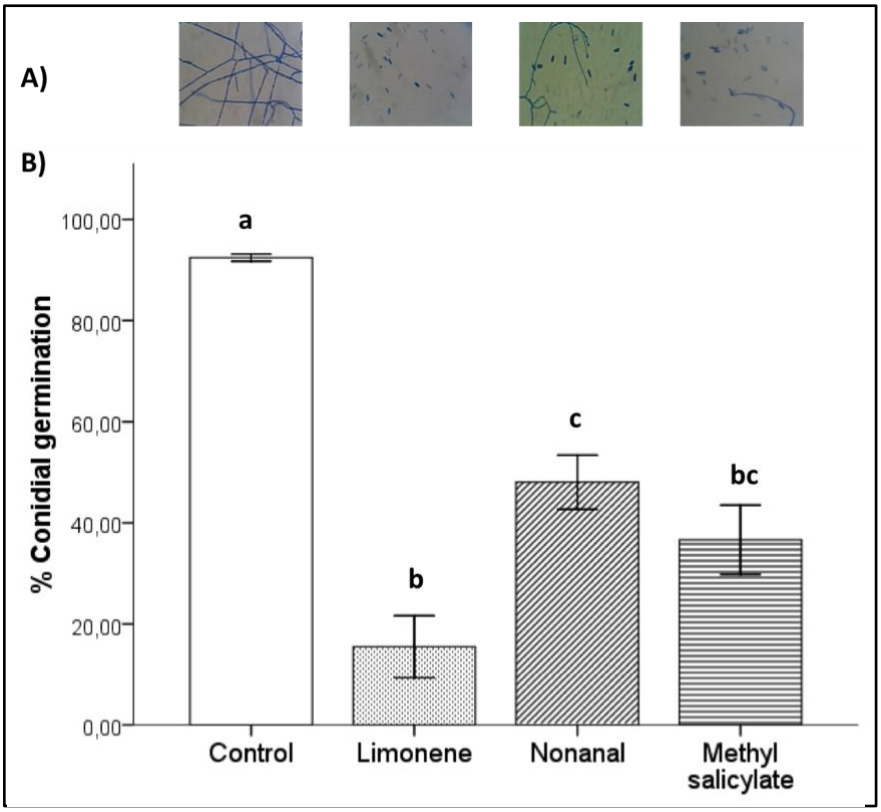

Figure 2. Effects of volatiles on conidial germination. The fungal pathogen *C. lindemuthianum* was exposed to different volatiles to analyze the effects on fungal development. A) Visual effect on the conidia exposed to volatiles; B) Percentage of germinated conidia after the exposure to volatiles. The bars represent the percentage of conidial germination, sample size n=5. Different letters indicate significant differences among treatments (P < 0.001 according to LSD)

Limonene was already reported to have inhibitory effects on the growth of *Fusarium verticilloides* (3). Nonanal presented also a negative effect on the growth of this pathogen. Interestingly, this VOC as well as methyl salicylate induced the resistance *to P. syringae* pv *syringae* in Lima bean (5, 7).

In conclusion, the VOCs that are produced by resistant bean cultivars in response to challenging them with a fungal pathogen enhance the phenotypic resistance in neighbouring plants, and this effect is likely to be caused by two potential and perhaps additive mechanisms: (1) associational resistance caused by direct inhibitory effects on spore germination and (2) the inducing effects of these VOCs on resistance-related genes. VOCs represent a highly attractive new strategy to enhance the resistance in common bean to anthracnose. ■

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Nuña popping bean: a kind of witness of the first steps of common bean domestication

by Ana M. GONZÁLEZ[1,#], Fernando J. YUSTE-LISBONA[2,#], María LORES[1], A. Paula RODIÑO[1], Antonio M. DE RON[1], Rafael LOZANO[2] and Marta SANTALLA[1]*

Abstract: Nuñas are native pole beans from South America that possesses the unique property of popping. Nuñas could be a new, nutritious and healthy snack food with potential for North America, Europe, Japan and other industrialized areas, but they are unsuitable for commercial production in temperate zones because they are photoperiod sensitive. Popping ability is quantitatively inherited and controlled mainly by dominant and epistatic effects. Eight QTLs were identified for popping ability on linkage groups 3, 5, 6, 7 and 9 and accounted together for 31% of the phenotypic variance. These QTLs would be good candidates for marker assisted selection to improve popping in nuña bean cultivars for their production as healthy snacks.

Key words: QTLs, marker assisted selection, evolution, *Phaseolus vulgaris*

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Origin and diversity of nuña pop beans

Popping bean or nuña bean (*Phaseolus vulgaris* L., Fabaceae) is traditionally grown in the Andean highlands of South America at 2,000-3,000 m asl, where they are occasionally sold in local markets or consumed at home, thus far known and thought to be an ancient and pre-ceramic landrace (1). It seems probable that nuña beans originated in the Andes, where in some locations from Peru and Bolivia are sympatric with wild and primitive common bean populations, and perhaps at an early stage in the development of Andean agriculture (2). The first selection pressures leading to domestication of common bean could have resulted in the development of popping beans, and it appears that toasting grains was a well-established tradition in the Andes and possibly in Mesoamerica, where early maize races have also been used for popping. However, no evidence of nuña beans in Mesoamerica has been found most likely due to genetic differences between the Mesoamerican and Andean gene pools, which could be responsible, among others, for their contrasting popping ability and photoperiod response (1).

Nuña bean is tropical in appearance, with vigorous climbing growth habit and day-length sensitivity, and is consumed after a quick toasting process. The foremost trait that distinguishes popping bean from all other types of bean is the ability to expand the cotyledons after grains explode in response to heating, which is referred to as popping expansion, similar to popcorn, although the popping mechanism is different. Variation in popping ability, seed size, and color has been observed among ñuña landraces (Fig. 1). Nuñas have a higher content of starch and copper than dry bean varieties and a lower content of protein, phosphorous, iron, and boron. Antinutritional factors such as lectins were higher in raw and boiled nuña samples than in toasted nuñas, while tannin levels did not change from raw to toasted treatments. Overall in-vitro digestibility was slightly lower for toasted nuñas than boiled dry bean (3).

Breeding for popping traits is highly valuable for healthy snacks

Patterns of genetic variability in popping bean germplasm have been studied by using morphologic and molecular data (1, 3, 5, 6, and 7). Expansion coefficient is the most important quality parameter for popping beans. In popcorn, seed moisture content above or below an optimum range will dramatically reduce popping percentage (4). Previous studies also indicated that popping performance of nuña beans was related to the moisture content of seeds (5). Except moisture, little is known on the factors influencing popping ability in bean unlike in maize, where several physic-chemical properties of the grain are well studied (8).

Popping ability should be combined with bush growth habit, early maturity, and photoperiod insensitivity for commercial production in temperate zones. Our studies indicated that additive effects have only minor importance in the total variation of popping performance, and few genes in a mainly dominant fashion and epistasis could be interacting to confer popping ability in common bean. In consequence, more rapid advance will be made in the improvement of popping performance in nuña bean by using a breeding procedure which emphasizes the dominant and epistatic gene effects. Studies evidenced that the backcross with the nuña as the recurrent parent enhanced popping ability among progeny (6). Transgressive segregation was also observed for popping traits (Table 1), suggesting that extreme popping phenotypes resulted from complementary effects of alleles from two parents. Since transgressive segregation relies on additive genetic variation, the extreme phenotypes can be maintained and fixed through artificial selection, providing the potential for improvement of popping ability.

Figure 1. Unpopped (left) and popped (right) seeds of nuña bean landraces

Table 1. Single–locus QTLs, mean values and range for two popping traits measured in 185 recombinant inbred lines (RILs)

QTL	LG[a]	Marker interval	$h^2(a)$[b]	Add[c]	PMB0225	PHA1037[d]	Range RILs
Expansion coeficiente (EC)							
EC3	3 (67.8-71.0)	BMc259-IAC24	4.3	3.7	8.14	57.26	-53.33 – 383.33
EC5	5 (40.6-46.1)	E32M60-BM175	4.6	4.1			
EC7	7 (24.2-36.6)	BM185-BMc294	2.8	-6.1			
EC9	9 (60.9-70.2)	PV-at007-BMc184	3.4	3.4			
Popping dimensión index (PDI)							
PDI3	3 (71.0-84.9)	IAC24-BM287	7.0	3.7	-0.36	25.05	-26.57 – 45.08
PDI5	5 (35.8-37.8)	E32M60-Bmc321	6.9	1.3			
PDI6	6 (2.3-5.0)	Bmc238-E40M60-91	2.2	1.5			
PDI7	7 (24.2-36.6)	BM185-BMc294	6.1	-1.7			

Phenotypic selection for popping ability is laborious and time-consuming. Marker-assisted selection (MAS) approaches have been difficult to apply in the case of complex traits as popping ability, because individual QTLs have small genetic effects which in many cases are also environmentally modulated; hence, the identification of potential candidate QTLs for MAS is crucial. Eight QTLs for expansion coefficient and popping dimension index were detected (Table 1) in an intra-gene pool mapping population generated from the cross PMB0225 (unpopped parent) x PHA1037 (popped parent). These QTLs were located on several linkage groups (LGs 3, 5, 6, 7 and 9) and they together explained 31% of the phenotypic variation; interestingly four of these QTLs co-localized on LG3 and LG7), which explained 21% of the phenotypic variation. These QTLs not only showed stability across significantly correlated traits, in separate and combined environments, but also they are shared QTLs for more than one trait which could be managed simultaneously in a breeding program.

The inheritance of popping ability was shown to be complex. Dominant gene action and additive *x* additive and dominant *x* dominant genetic effects were shown to be important in the genetic regulation of popping. The complexity of the inheritance of popping expansion shows that more complex breeding strategies could be more successful. The discovery of different QTLs with significant genetic effects for popping traits provides ample scope for an effective pyramiding approach, in which candidate QTLs could be simultaneously selected using PCR-based cost-effective marker systems. Therefore, by means of a QTL pyramiding approach, it could be possible to combine QTL alleles with positive effects for popping ability on a day-length-insensitive genotype through molecular breeding; it would allow overcoming the main drawback that has restricted the production and commercialization of nuña beans in temperate regions. ■

Acknowledgements

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Nutritional quality of common bean: more nutrients or less antinutrients?

by Francesca SPARVOLI* and Roberto BOLLINI

Abstract: Common bean production accounts to about 23 million metric tons and represents one third of the total world production of pulses. Due to its energy, protein, dietary fibre and minerals content, common bean is the major staple food for about one billion people living in Eastern Africa and South America and its consumption is also encouraged in developed countries. Nevertheless, beans have several disadvantages: they require long cooking-time and seed proteins are poorly digested and low in sulphur amino acids. Moreover, seeds accumulate anti-nutrients, such as lectins, phytic acid, tannins and raffinosaccharides, that hamper its nutritional value, reduce micronutrients bioavailability and cause stomach discomfort and flatulence.

Key words: α-amylase inhibitor, antinutrients, lectins, phytic acid, galacto-oligosaccharides, storage proteins

The good and bad of bean seed storage proteins

Protein content of common bean seed is about of 20% of the dry matter. The largest fraction of these proteins is made up by storage proteins, which contribute to up to 80% of the total protein. Storage proteins are represented by phaseolin and legumin (7S and 11S globulins, respectively) and the APA proteins (α-Amylase inhibitor, Phytohemagglutinin, Arcelin), a group of lectin-related polypeptides. All but legumin are glycoproteins. Contrary to other legumes, phaseolin is very abundant and normally represents about 50% of total seed proteins, while legumin is only 10%. APA proteins account for another 15% of total seed proteins, although in some wild genotypes arcelin may reach up to 50% of total proteins with a proportional decrease of phaseolin. APA proteins are considered protective factors against seed eating predators. These proteins are coded by a small multigene family that evolved through duplication and diversification of a common lectin ancestor (1). APA proteins may be very toxic to monogastric animals and/or insects, thus contributing to the low nutritional. Phytohemagglutinin has sugar binding property and is the true lectin. Its presence constitutes a possible risk, since in raw or incorrectly cooked bean seeds it has been shown to cause outbreaks of gastroenteritis, nausea and diarrhoea.

α-Amylase inhibitor (αAI) and arcelin may be considered truncated forms of PHA, in which one or two loops involved in sugar binding function are missing, respectively (Fig. 1). In the case of αAI, which is a potent inhibitor of both mammalian and insect (but not plant) α-amylases, the precursor polypeptide is cleaved into two smaller subunits and this structural change is required to its biological activity. The presence of αAI in the seed negatively affects starch utilisation. Arcelin has not a clearly understood biological activity, however its presence is associated to the highest levels of resistance against the two major bean pests, the bean weevil (*Acanthoscelides obtectus*) and the Mexican been weevil (*Zabrotes subfasciatus*).

Phaseolin and APA proteins have been described as highly resistant to proteolysis in the digestive tract of humans and monogastric animals. This, at least in part, has been attributed to their conformation which is characterised by a high content of beta-sheet structures and by the presence of glycan chains at the surface of these proteins. Both these structural constrains may limit the access of the proteolytic enzymes, making these proteins resistant to hydrolysis. APA proteins are even more resistant to proteolysis than phaseolin and this, in the case of arcelin, has been regarded as one of the mechanism by which this class of proteins protects the seed from the attack of bruchids beetles. α-Amylase inhibitor is also a very stable protein, in fact in its native form it is highly resistant to trypsin proteolysis for several hours at 37°C.

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Although incomplete digestibility of phaseolin may limit the nutritional value of bean seeds, the presence of this protein may confer health properties to the seeds. In fact, there are evidences in soybean and lupine that peptides derived from digestion of 7S globulins have hypocholesterolemic activities (2). Since legume globulins are highly conserved and common bean is closely related to soybean, similar properties may also hold true for phaseolin. Healthy properties have been reported also for αAI which presence in the diet helps in controlling obesity and diabetes. Much attention has been given to αAI as a basic ingredient in several commercial anti-obesity and anti-diabetes products, referred to as "starch blockers", due to their ability to interfere with the breakdown of complex carbohydrates leading to reduction of glucose derived energy intake. The efficacy and security of "starch blockers" is frequently debated since many of the commercial preparations display very low αAI activity and are rich in PHA.

Exploiting natural variability in bean species (*P. vulgaris* and P. *coccineus*) has proven to be useful to identify genotypes having different combinations of storage proteins. For example, most of bean genotypes accumulate PHA and αAI, while arcelin is found only in wild accessions collected in central Mexico; APA-null genotypes or some containing only αAI have also been reported while genotypes devoid of phaseolin may be found in *P. coccineus*. Thanks to this variability, breeding lines and varieties have been obtained with the aim to improve the nutritionally quality of the seed, to be more suitable for the production of starch blockers and to be resistant to bruchid beetles (Table 1).

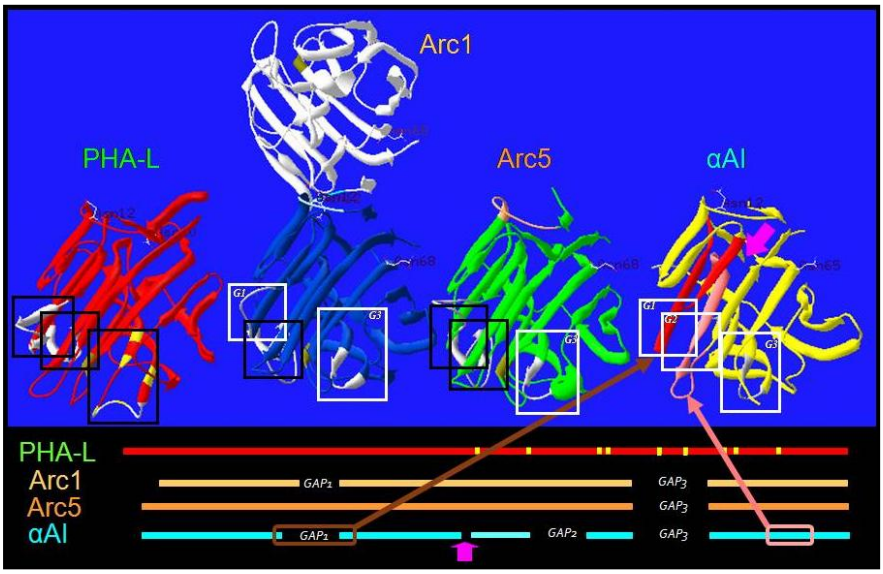

Figure 1. Comparison of linear (lower panel) and 3D structures (upper panel) of representative APA proteins. Yellow bars indicate the position of amino acid residues involved in sugar binding activity in PHA-L. White boxes (*G1, G2, G3*) indicate deletions (*GAP1, GAP2, GAP3*) occurred in arcelins (Arc1 and Arc5) and αAI compared to PHA-L (corresponding regions are indicated with black boxes); brown and pink arrows indicate αAI regions involved in α-amylase recognition; green arrows indicate the site at which the precursor is processed to produce mature and active αAI.

Bean seed biofortification: strategies to improve micronutrient bioavailability

Iron deficiency anaemia (IDA) is the most prevalent micronutrient deficiency affecting more than two billion people throughout of the world. Bio-fortification, through the development of micronutrient rich crops, is one of the strategies adopted to fight against IDA and more generally against micronutrient deficiencies. Due to its worldwide consumption and nutritional qualities, common bean has been identified as a strategic target crop to increase dietary iron for human beings (http://www.harvestplus.org/content/iron-beans).

Bean seeds have an iron content that vary between 35 and 90 μg/g and breeding to increase iron concentration by 60-80% in cultivated varieties has been achieved by researchers at CIAT (Centro International de Agricoltura Tropical). The iron content in these bio-fortified bean varieties has been improved up to 100 μg/g. However, increase in iron content not necessarily translates into increased iron bioavailability, that may be strongly reduced by the presence in the seeds of iron absorption inhibitors, such as phytic acid (PA), polyphenols (PP) in coloured varieties, and seed 7S globulins. Thus, any strategic approach for bean bio-fortification, based either on increasing iron content or reducing iron absorption inhibitors, should consider nutritional trials to be performed to verify the success and extent of the bio-fortification strategy.

Table 1. Literature resources related to various aspects of common bean seed quality

storage protein composition	purpose	reference
phaseolin variants	improve phaseolin digestibilty	Montoya et al. 2010, Food Res Int 43:443-449
phaseolin null; PHA, arcelin null	improve sulphur amino acid content; increase αAI content	Marsolais et al. 2010, J Proteomics 73:1587 – 1600 Bollini, unpublished
arcelin	improve resistance against *Z. subfasciatus*	Cardona et al. 1990, Entomol Exp Appl 56:197–206
PHA, arcelin null	increase αAI content	Confalonieri et al. 1992, Plant Breed 109:329-334

Experiments to assess iron bio-availability in high iron bean (HIB) lines, performed using different *in vivo* (human and animal feeding) and *in vitro* (Caco-2 cells) systems, have shown that the benefit of increased iron content may be vanished by the presence of high levels of PA and PP in the seeds. In fact, improved iron bio-availability was gained when iron content was increased in beans with low PP content. Furthermore, if the PA content of the HIB lines is too high, then normal iron (52 mg/g) bean lines provide significantly more bioavailable iron (3).

Since PP accumulate in seed coat and bean has a wide array of variability in seed colours, ranging from white to black, low PP genotypes can be easily identified. Conversely, natural variability in PA content is not very high and the best way to gain significant PA reductions is by obtaining low phytic acid (*lpa*) mutants, in which PA may decrease between 40 and 90%. Although such type of mutants have been identified in several grain crops, often PA reduction was associated with negative agronomic traits, such as lower seed viability and emergence, reduced plant growth rate and grain yield. These findings may limit the use of *lpa* mutants for iron bio-fortification, since acceptable agronomic performance should be guaranteed to small farmers and poor populations that would benefit from bio-fortified crops.

In common bean a single *lpa* mutant was identified and shown to carry a defective MRP type ABC transporter (*Pvmrp1*) necessary for phytic acid transport to the vacuole (4). Compared to wild type genotypes, bean *lpa* seeds have a 90% PA reduction, seven fold higher free iron extractability in mild acid conditions and 25% less raffinosaccharides (this reduction is expected to diminish flatulence). Agronomic analyses of the original *lpa* mutant and of derived *lpa* lines have shown that, despite the strong PA reduction in the seed, seedling emergence, seed yield and plant growth were not affected and not statistically different from those of wt and parental genotypes (5). A recent work aimed to evaluate iron bioavailability in *lpa* lines, using an *in vitro*/digestion Caco-2 cell culture model, has shown that the amount of ferritin produced in Caco-2 cells fed with *lpa* and low PP seed digestate is about twice that found in cells fed with the normal PA and low PP genotype (6).

In conclusion, available data indicate that the future direction for an effective iron biofortificaton in common bean will require a breeding approach to combine HIB lines, low PP genotypes and *lpa* mutant(s). ■

Snap bean (*Phaseolus vulgaris* L.) quality profile by sensory descriptive analysis

by Mar VILANOVA, Paula RODIÑO, Ana GONZÁLEZ, Pilar CANOSA, Iría RODRÍGUEZ-VEGA, Manuel RIVEIRO and Marta SANTALLA*

Abstract: Sensory quality of snap beans (*Phaseolus vulgaris* L.) influence consumer preferences. The application of sensory descriptive analysis (SDA) for snap bean quality is shown in this work. SDA has allowed generating descriptors for appearance, aroma, flavor and texture, which could be used to characterize snap bean varieties.

Key words: sensory quality, common bean, quality, *Phaseolus vulgaris*.

Importance of sensory descriptive analysis in food quality

Sensory quality is the ultimate measure of product quality. Sensory analysis comprises a variety of powerful and sensitive tools to measure human responses to foods. Specific scientific methods have been developed to accurately, reproducibly and objectively measure or estimate human responses to stimuli. All descriptive analysis methods involve the detection and the description of both qualitative and quantitative sensory aspects of a product by a trained panel. Panelist must be able to detect and describe the perceived sensory attributes of a sample, appearance, aroma, flavor, texture or sound properties and they must learn to differentiate and rate the intensity of these descriptors.

The flavor profile method (FMP) was the first reported descriptive method, developed in the 1940s to complement existing sensory techniques. FMP is a consensus technique, and vocabulary development and rating sessions are carried out during group discussions, with members considering aspects of the detectable flavor components (5). Quantitative descriptive analysis (QDA) was developed during the 1970s and it has gained acceptance for sensory evaluation (8). QDA is one of the most comprehensive and informative tools used in sensory analysis for evaluating food and dairy products (8). The principle of QDA is based on the ability to panelists to measure specific attributes of a product in a reproducible manner to yield a comprehensive quantitative product description amenable to statistical analyses (2).

Snap bean quality by sensory descriptive analysis

Sensory Descriptive Analysis has been applied in the last years to evaluate bean quality (4, 7). QDA was applied to know the gamma radiation effect on sensory attributes of the common bean (*Phaseolus vulgaris L.)* variety Carioca Tybatã from Brazil (1). Other authors have also applied SDA to correlate the sensory descriptors with volatile compounds in rehydrated French beans (9), and to evaluate the sensory characteristics of local varieties of common bean from Cuba (4) and Spain (7).

A study of 10 snap bean varieties by sensory descriptive analysis, including both fresh and cooked immature pods, was carried out in the MBG-CSIC. A selection of descriptors to evaluate the snap bean quality profile was identified by a panel (Figure 1), according to the motivation (all volunteers) and availability (attendance at all sessions). Sessions were carried out by following the NORME ISO 11035 (6). The Geometric Mean (GM) permitted to take into account descriptors which were rarely mentioned, albeit very important in terms of the perceived intensity, and those descriptors perceived with a low intensity but nevertheless often mentioned in the analysis (3).

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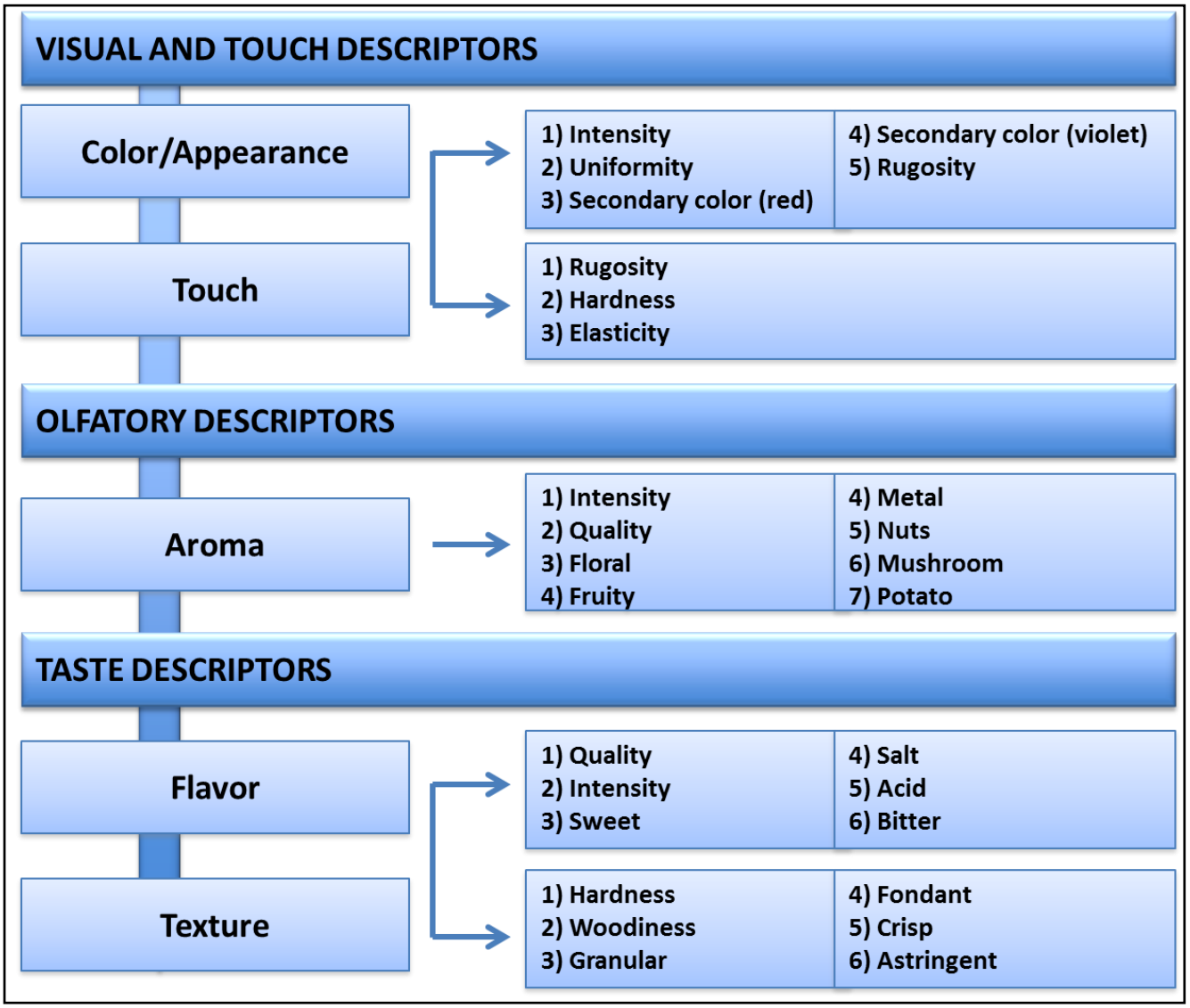

Figure 1. Sensory descriptors for snap bean quality profile, according to a scale from 0 to 9

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Current state of knowledge of the Lima bean (*Phaseolus lunatus* L.) in Mexico

by Jaime MARTÍNEZ-CASTILLO*, Rubén H. ANDUEZA-NOH, Luciana CAMACHO-PÉREZ, Félix DZUL-TEJERO, Julián COELLO-COELLO, Matilde M. ORTÍZ-GARCÍA and M. Guadalupe CARRILLO-GALVÁN

Abstract: Lima beans are an important crop in Mexico. Within Mexico, a potential center of domestication of this species, the Yucatan peninsula has the greatest richness of landraces. These landraces have high levels of genetic diversity, but many are at high risk of genetic erosion from a variety of factors. In this region, there are wild populations, which favor wild to crop introgression and the resultant genetic variation may undergo selection by the Mayan farmers of this region.

Key words: domestication, genetic diversity, Lima bean, Mexico, wild–crop introgression

Importance of Lima bean in Mexico

Mexico is one of the main centers of origin of agriculture and domestication of plants. Of the plants domesticated in this country, beans (*Phaseolus* sp.) are a representative food of the Mexican culture. The genus *Phaseolus* comprises over 50 species, most of which are wild species. Only five species have been domesticated, and all five are present in Mexico: the common bean (*P. vulgaris*), runner bean (*P. coccineus*), tepary bean (*P. acutifolius*), piyola bean (*P. polyanthus*) and Lima bean (*P. lunatus*). Although each of these species is particularly important to Mexican culture, in this review we only present significant results from molecular studies obtained over the past 12 years for Lima bean from Mexico.

In Mexico, the Lima bean is cultivated by a large number of ethnic groups living in the country. This crop is known by different names, among the most commonly used are "ibes" in the Yucatan Peninsula, "patashetes" in Chiapas, "pataxtles" in Veracruz and "combas" in Guerrero. Because of the large variation in the type of seed produced, the landraces also have numerous names, as is the case of "ibes" in Mayan agriculture of the Yucatan Peninsula. This region has the largest number of Lima bean landraces, with more than 25 landraces (Fig. 1), which have been named on the basis of shape, color and seed production cycle (4). Examples are the landraces known by the Mayan names of *chak-putsica-sutsuy* (red heart of the Dove) and *madza-Kitam* (boar eyebrows). In Mexico, Lima bean is not cultivated intensively as in some other countries (i.e., Peru, United States, Madagascar), which has prevented it from acquiring greater economic importance. Lima bean is grown mainly in the Mesoamerican traditional farming system of slash and burn known as milpa. For example, in the Yucatan peninsula, this crop is the fourth most important species of the Mayan milpa.

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Figure 1. Intraespecific diversity of Lima bean from the Yucatan peninsula, México. Bottom row are wild and weedy populations, middle and higher rows are landraces

Lima bean domestication in Mexico

Beans (*Phaseolus* sp.) were domesticated in America in pre-Columbian times. Lima bean is now recognized to have been domesticated in at least two different sites (8). One site is in South America, in the mid-altitude valleys located between the countries of Peru and Ecuador. A second site is located in Mesoamerica; ribosomal DNA-based evidence shows that it could be somewhere in west-central Mexico (8). Recent results based on chloroplast DNA, support to the West-Central Mexico as a domestication site for Lima bean, but also indicate the possible existence of another site in Mesoamerica, possibly in Guatemala or Honduras (Andueza-Noh, personal communication) in the area known as the Mayan Highlands. Although previous studies based on protein analysis had already indicated this second possibility, improved and increased sampling of wild *P. lunatus* in Central and South America is needed to clarify this hypothesis.

Diversity and genetic erosion in Lima bean in Mexico

There are few studies in Mexico on diversity and genetic erosion in Lima bean using molecular data, and these have been done only in the Yucatan Peninsula. In a phytogeographical study that encompassed all of Mexico, it was found the Yucatan peninsula presents the greatest richness of Lima bean landraces across the country. This high diversity (4) is at risk of being lost because nearly 70% of the area planted with Lima beans in this region was dominated by three landraces, while another 12 landraces were planted by a few farmers in small plots. Molecular studies have reported that these 12 landraces have high levels of genetic diversity compared to the other three more abundant landraces and compared to landraces in other Mesoamerican regions (1, 6). The high risk of genetic erosion of the landraces is due to various factors including (3) the intensification of traditional agriculture Maya, (1) the incorporation of small producers into the market and, (3) destruction from natural phenomena such as hurricanes and droughts. This genetic risk has increased over the past 30 years (6, 7).

Gene flow and genetic introgression in Lima bean

In areas where landraces and wild populations of *P. lunatus* still grow sympatrically (Fig. 1), genetic exchange between them can be found. Such is the case in the Yucatan peninsula which was reported as having high levels of genetic diversity for wild populations (5). A regional analysis showed the existence of low levels of wild-crop gene flow, but the flow from landraces to wild populations was three times greater (Fig. 2) (2). In a recent study at the plot level (*milpa*), genetic introgression was found between these populations, with the introgression levels depending mainly on positive or negative selection on the introgressed forms by Mayan farmers (3). We have detected weedy forms (Fig. 1) that could be acting as a genetic bridge between landraces and wild populations. In some cultivated species, as could be the case for Lima bean in the Yucatan peninsula, the existence of weedy forms has produced an asymmetry in gene flow, favoring the highest gene flow from landraces toward wild populations. All these studies will help in assessing the risks of a possible release of transgenic varieties of this species in Mexico.

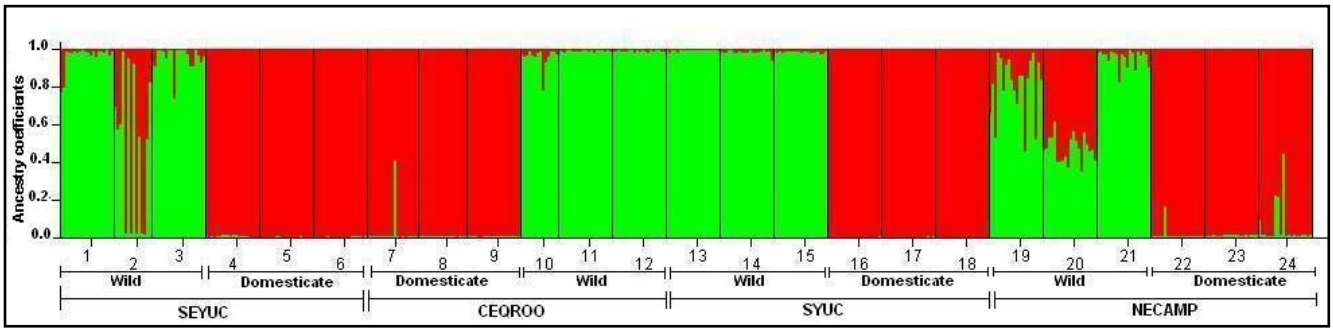

Figure 2. Bayesian analysis of the wild-crop gene flow in Lima bean from the Yucatan peninsula, México. Each column is a wild (green) or domesticated (red) populations. SEYUC (southeastern Yucatan), CEQROO (Central Eastern Quintana Roo), SYUC (Southern Yucatan) and NECAMP (northeastern Campeche) are the main agricultural zones from the Yucatan peninsula. In this case, wild populations (2, 19, 20) have the highest levels of gene flow from the domesticate populations

Perspectives on the study of Lima bean in Mexico

Much remains to be investigated in the Lima bean from Mexico. We are currently using microsatellite molecular markers to understand the structure and genetic relationships of this species throughout Mexico to obtain basic information on the domestication of the species. We are also conducting *in situ* and *ex situ* conservation programs of the landraces that are at risk of being lost in the traditional agriculture Maya of southeastern Mexico, and deepening our knowledge about the role of traditional farmers in the process of wild-crop genetic introgression and in selection of the genetic variation generated for this important micro-evolutionary process. Only when the contributions of the farmer are properly taken into account we could achieve an accurate understanding of the dynamics, generation and maintenance of genetic diversity of the landraces. ■

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The Common Bean Museum in Smilyan

by Svetlana ANTANASOVIĆ[1], Mariya SABEVA[2], Siyka ANGELOVA[2], Branko ĆUPINA[1], Đorđe KRSTIĆ[1], Sanja VASILJEVIĆ[3] and Aleksandar MIKIĆ[3]

Abstract: Sensory quality of snap beans (*Phaseolus vulgaris* L.) influence consumer preferences. The application of sensory descriptive analysis (SDA) for snap bean quality is shown in this work. SDA has allowed generating descriptors for appearance, aroma, flavor and texture, which could be used to characterize snap bean varieties.

Key words: Bulgaria, common bean, folklore, museum, *Phaseolus vulgaris*

In the southern Bulgaria, in the Smolyan Province, quite close to the border with Greece, there is a small village of Smilyan. There, like in many other regions of the Balkan Peninsula, *Phaseolus* beans have been grown for at least 250 years. Similarly to the local landraces of other pulse crops, such as faba bean (*Vicia faba* L.) or chickpea (*Cicer arietinum* L.), each household cultivates, uses and maintains its own one, sufficiently enough to satisfy its needs. It is well-known that common bean is the most important pulse crop in human diets across the Balkans and it is no wonder that it deserved a kind of sacred place devoted especially to it: a common bean museum. Though not large, it contains a large number of home-made artwork of the common bean seeds and welcomes thousands of visitors from Bulgaria and other countries each year. Welcome and *добър апетит!* (enjoy your meal)! ■

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Figure 1. The interior of the Common Bean museum in the village of Smilyan, Bulgaria

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