

Genetic Diversity of Lima bean in Relation with Phytoecography in the New World

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Abstract: *Phaseolus lunatus* L., known as Lima bean, is a Neotropical food legume adapted to a wide range of ecologies, including temperate, humid and semi-arid tropical conditions. This adaptation is the consequence of the large distribution of the wild Lima bean populations in the various natural areas of the New World, involving both lowland and highland areas, as well as distinct vegetation types and climates. These wild materials have given rise to landraces of specific ecological properties, according to the origins of the ancestral wild populations in the two main centers of diversity: Mesoamerican and Andean regions. In order to exploit the potential of this food legume, it is essential, first, to know the genetic organization of the diversity of this cultigen at the intra- and inter-specific levels, and, second, to examine the phytoecography of the wild forms and the landraces in the New World. A state of knowledge is given on the taxonomy and the phylogeny of the genus *Phaseolus* and Lima bean. Special attention is devoted to the primary and the alien gene pools of *P. lunatus*. Data on phytoecography on both wild populations and landraces throughout Latin America help in determining the ecological requirements and potentials of this food legume in the traditional farming systems of the tropics. Current results or progress in these areas are useful to preserve the gene pools of this promising, but neglected, species through *in situ* and *ex situ* approach, and to develop, on the basis of this diversity, improved and productive cultivars of Lima bean.

Key words: *Phaseolus lunatus*, Lima bean, genetic diversity, phytoecography, *in situ* conservation.

For plant genetic resources, more attention is now given to the ancestral wild populations and landraces of the cultigens. This interest is due to their poor representation in germplasm collections, their high values as a reservoir of genetic variation, and as a source of novel variants for plant breeding (Frankel, 1974; Harlan, 1976; Brown, 1978; Delgado-Salinas *et al.*, 1988; Marshall, 1990; Bianchi-Hall *et al.*, 1993; Debouck, 2000a; Baudoin, 2001). However, these materials (particularly the wild populations) are not easy to maintain in gene banks. The limited

information available on the ecogeographic distribution of these populations and the necessity of assembling genetic stocks sufficiently representative of the total genetic variability displayed by such materials are among the major reasons for this difficulty. Further-more, seed dispersal and variation in both seed ripening and dormancy among individual plants and populations are also additional constraints for an adequate *ex situ* storage of wild populations (Debouck *et al.*, 1993; Ehrman and Cocks, 1990; Marshall, 1990).

In order to circumvent such obstacles, *in situ* conservation, e.g., in the environment in which wild populations or landraces evolve, is regarded as a dynamic conservation system which ensures the preservation of representative populations throughout their natural geographic range and facilitates the continuing evolution of the crop gene pool. It maintains the genetic integrity and the potential of adaptation of each population and complements also *ex situ* conservation avoiding the inadequate field sampling of ecotypes for storage in gene banks (Ingram and Williams, 1984; Altieri and Merrick, 1987; Maxted *et al.*, 1987; Brush, 1991; Astley, 1992).

In order to preserve plant genetic diversity on the basis of the two *in situ* and *ex situ* conservation approaches, a prerequisite is a good knowledge of the genetic diversity and the phylogeny of the target species, but also of the phylogeography of the ancestral wild forms and landraces of the cultigen. In particular, phylogeography provides valuable information on the diversity of ecological niches and, consequently, on the genetic variability resulting from any change in the environment, on the evolution and damage of vegetation structure and on the risk of genetic erosion (Maquet and Baudoin, 1997). This discipline gives an estimation of the different ecologies where the species is naturally distributed, on its plasticity and, therefore, on the plant breeding potentialities.

Purpose of this paper is to examine the genetic organization and the

as Lima bean, on the basis of data gathered mainly in the New World.

Phylogeny and Taxonomy of *Phaseolus*

A good understanding of the phylogenetic relationships among species within crop gene pools is of great importance both to germplasm curators and plant breeders. Phylogenetic studies are useful in identifying the wild progenitors and other relatives of domesticated species (Pages and Holmes, 1998), and in clarifying the composition of their secondary and tertiary gene pools. This will help to define both sampling strategies for *ex situ* collections and management approaches for *in situ* conservation, as well to prioritize material for utilization. Gepts (1996) suggests that determining the closest relatives of cultivated beans, for example, has important implications for crop breeding because domestication has caused a genetic bottleneck in all *Phaseolus* cultigens. Furthermore, breeding programs that involve inter-specific crosses are particularly important in *Phaseolus* (Huel and Scoles, 1985).

The genus *Phaseolus* (subtribe Phaseolinae, tribe Phaseoleae, family Leguminosae) has a complex taxonomic and nomenclature history, which is well illustrated by *P. lunatus*, the Lima bean (Maquet, 1995). Recent phylogenetic investigations strongly support a monophyletic *Phaseolus*. In this modern circumscription, *Phaseolus* is strictly of New World origin, concentrated in tropical and warm temperate America, between

upon by most legume taxonomists, the exact number of species within the genus is still debated (Debouck, 1999). However, there are probably around 50 species in the genus, of which five are domesticated: common bean (*P. vulgaris* L.) which is the most economically important cultigen of the genus, Lima bean (*P. lunatus* L.) which ranks second in economic importance, scarlet runner bean (*P. coccineus* L.), tepary bean (*P. acutifolius* A. Gray), and year bean (*P. polyanthus* Greenm.). In the five cultigens, wild ancestral forms have been identified; they all had a wide distribution before domestication. In the case of common bean and Lima bean, wild forms are distributed in both northern part of America (Mexico and Central America) and southern part of the continent. This situation is reflected by the presence of two groups of material for each of these two cultigens: the Mesoamerican and Andean groups (Debouck, 2000b).

The five *Phaseolus* domesticated species belong to two distinct lineages. The *P. vulgaris* group includes *P. vulgaris*, *P. coccineus*, *P. polyanthus* and *P. acutifolius*. The fifth cultivated species, *P. lunatus*, is part of a very well-defined clade that includes the South American and oceanic island diversification of *Phaseolus*, i.e., *P. augusti* Harms, *P. bolivianus* Piper, *P. lignosus* Britton, *P. mollis* Hook, *P. pachyrrhizoides* Harms, *P. rosei* Piper, and *P. viridis* Piper (Caicedo *et al.*, 1999; Delgado Salinas *et al.*, 1999).

A clade containing *P. viridis* (from Oaxaca, Mexico) and *P. lignosus* (from Bermuda) is sister to the rest of the primarily South American group. The Andean *P.*

pachyrrhizoides, *P. augusti* and *P. bolivianus* form a monophyletic group that is sister to a lineage containing both Mesoamerican and Andean accessions of *P. lunatus*, as well as one accession of *P. mollis*, a species endemic to the Galapagos Islands. A Peruvian accession of *P. lunatus* and *P. mollis* are resolved as a sister group, suggesting a mainland origin for the Galapagos species. *P. rosei* collected at the type locality (Chimborazo and Ecuador) falls within the Andean group of wild Lima beans; *P. rosei* could be an Andean wild form of Lima bean (which would make its correct name *P. lunatus*) (Toro Chica *et al.*, 1993; Caicedo *et al.*, 1999).

Taxonomy of *P. lunatus*

The latest available taxonomic treatment of *P. lunatus* is by Baudet (1977). A botanical variety, *P. lunatus* var. *lunatus*, was created for all the cultivated genotypes and a var. *silvester* for the wild form. The var. *lunatus* includes the three cultigroups defined by Mackie (1943): cv-gr. Sieva with medium-sized, flat seeds; cv-gr. Potato with small, globular seeds; and cv-gr. Big Lima bean with large, flat seeds. This classification, however, is far from reflecting the whole range of seed diversity and genetic variability among the cultivated materials due to free gene flow occurring between the three cultigroups (Baudoin, 1988). The primary gene pool of *P. lunatus* comprises the wild populations and the landraces of Lima bean, which can be grouped into two main races: the Andean and the Mesoamerican. Each race is characterized by distinctive morphological characters (Maquet, 1995; Debouck *et al.*, 1987), ecological adaptation (discussed later), seed

storage proteins (Debouck, 1989; Lioi, 1994; Gutierrez Salgado *et al.*, 1995; Maquet, 1995), allozymes (Maquet *et al.*, 1997; Lioi *et al.*, 1998) and molecular markers (Nienhuis *et al.*, 1995; Fofana *et al.*, 1997; Caicedo *et al.*, 1999; Lioi *et al.*, 1999; Fofana *et al.*, 2001). Escaped forms and weedy forms (natural hybrids between the wild form and a landrace) are observed throughout Latin America. In addition, several collections from northern Peru have shown that either more cultigroups of native varieties should be defined, or the concept of cultigroup is becoming obsolete because of the great diversity found in the field (Debouck *et al.*, 1987). Hawkes (1986) suggested avoiding Latin names at the intra-specific level for cultivated species. The best taxonomic treatment may be to use the binary combination, i.e., *P. lunatus* L., with some additional indication of the biological status of the material, e.g., wild, weedy, hybrid, landrace, etc. (Debouck, 1991; Maquet, 1995).

Origin, Domestication and Genetic Diversity of *P. lunatus*

Central America, where approximately 40-50 *Phaseolus* species occur today, is considered the centre of origin of the genus (Sousa and Delgado Salinas, 1993). *Phaseolus* species are numerous along the mountainous systems of the Sierra Madre Occidental and the Eje Volcánico Transversal of Mexico, which were formed during the Oligocene-Miocene and Later Tertiary or Pliocene, respectively. Therefore, current *Phaseolus* diversity may stem from the Oligocene or later (Delgado Salinas, 1985).

With regard to *P. lunatus*, the existence of a secondary gene pool in the Andes,

with taxa such as *P. pachyrhizoides*, would indicate a centre of speciation in this region, more recent in comparison to that of Central America. This is supported by evidence from seed protein and isozymes studies presented by Maquet (1995), Maquet and Baudoín (1996) and Maquet *et al.* (1999). An Andean origin was also suggested by Fofana *et al.* (1999), Caicedo *et al.* (1999) and Sparvoli *et al.* (2001) using RAPD, AFLP and lectins and lectin-related proteins, respectively. A wild Lima bean with small seeds has long been reported from several parts of Central America (Standley and Steyermark, 1946). However, another wild type with slightly larger seeds was more recently discovered in north-western Peru (Debouck *et al.*, 1987). Two separate domestication events have since been demonstrated, from two different wild forms, with different distribution ranges and distinct ecologies (Gutierrez Salgado *et al.*, 1995; Maquet, 1995; Fofana *et al.*, 1997). Due to the restricted distribution of the large-seeded wild type, it is assumed that one domestication event occurred within the southern Andes of Ecuador and the north-western Andes of Peru. By contrast, the range of the small-seeded wild Lima bean is huge, and a precise location for the domestication of the small-seeded cultivated Lima beans has still to be determined.

Lima beans seem to be an ancient crop, although some dates have been revised downward (Kaplan and Lynch, 1999), and more ancient in South America than in Central America. The earliest records are from the Central Andes of Peru and date to 3,000 years BP (revised date), although a pod from the coast of Peru has been

dated to 5,600 years BP (Kaplan, 1994). The evolution of the cultivated species under domestication has been reviewed recently (Debouck and Smartt, 1995; Debouck, 1999). As in most crops, increases in the size of pods and seeds are the most striking differences between the cultivated forms and their wild ancestors. Reduction in hardness, dormancy and anti-nutritional factors (i.e., antitrypsin factors, cyanide glycoside) has also occurred. Most landraces contain low levels of linamarin (Baudoin *et al.*, 1991).

White-seeded cultivars have been selected. This mutation appears from time to time in wild populations, for example in Yucatan, Mexico (Debouck, 1999). The loss of seed dispersal mechanism is also a common feature of crop domestication. Major evolutionary changes have occurred in growth habit. The original climbing habit with profuse branching, observed in the wild form, was selected for a quick harvest, leading to the bushy determinate growth habit and annual landraces. In addition to these changes in plant habit, some selection has been undertaken for photoperiod insensitivity. As pointed out by Koinange *et al.* (1996), most of the evolutionary changes in beans are due to mutations of just a few genes, the increase in seed size being one noteworthy exception.

Domestication has taken place from a limited number of wild populations and is generally associated with an overall reduction of genetic diversity, contrasting with an increased diversity in morphological traits (mostly ones under direct, conscious selection by farmers). The reduction of

demonstrated with molecular markers, which are thought to be neutral to selection. Using seed-protein markers, Maquet *et al.*, (1990), Gutierrez Salgado *et al.* (1995) and Maquet (1995) showed reduced genetic diversity among the small-seeded Mesoamerican landraces, and also among large-seeded Andean landraces though not to the same extent and in different markers. As a consequence of this "founder effect", much of the genetic diversity has been left out of the domesticated stocks; possibilities are to be explored in terms of unrealized potentials from the wild forms.

Allozyme studies complement the data obtained from seed-proteins. The total gene diversity of the Lima bean at the species level ($H_{es} = 0.26$) is similar to that of *P. acutifolius* (Schinkel and Gepts, 1989) and *P. coccineus* (Escalante *et al.*, 1994), but higher than that of wild *P. vulgaris* forms ($H_{es} = 0.13$) (Koenig and Gepts, 1989). On the basis of allozyme data, the genetic diversity of the Lima bean is evenly distributed between the Mesoamerican and the Andean gene pools (Maquet *et al.*, 1997). This confirms the results obtained by Nienhuis *et al.* (1995) using RAPD markers. Allozyme data also showed that wild and cultivated forms of the Lima bean are characterized by similar levels of gene diversity and are not differentiated within each gene pool. However, Fofana *et al.* (1997) showed using RAPDs that the wild form and landraces of each gene pool were indeed genetically differentiated. Such discrepancies between studies could result either from the different nature of the markers or from the sample used.

Internacional de Agricultural Tropical (Cali and Colombia): the cultivated forms represent around 90% of this germplasm bank, the remnant being wild and weedy forms (Maquet and Baudoin, 1997). Although some disparities are observed in the geographic distribution of the accessions, more landraces are present in South America than in Central America while the opposite situation occurs with the wild forms. Seed multiplication and rejuvenation of this base collection on the field have to take into account not only the ecological requirements of each accession, but also the reproductive biology of the plant. *P. lunatus* is a self-compatible annual or short-lived perennial species with a mixed mating system, i.e., predominantly self-pollinating, but with a fair amount of outcrossing (Baudoin, 1991). Within the primary genetic reservoir of Lima bean, gene diversity is mainly distributed among rather than within accessions ($GST = 0.755$) (Maquet *et al.*, 1997). Lima bean germplasm is characterized by a high inbreeding coefficient ($f = 0.891$). In spite of this, and a low rate of gene flow, the intrapopulation gene diversity estimated from the CIAT collection ($Hs = 0.032$) is significantly different from zero and higher than that of other selfing species, such as *P. acutifolius* ($Hs = 0.0004$) (Schinkel and Gepts, 1989) and *P. vulgaris* (Koenig and Gepts, 1989).

Because of the wide distribution of their wild ancestors and multiple domestication events, cultivated common bean and Lima bean probably have a wider genetic base than the other cultigens of the genus, *P. acutifolius*, *P. coccineus* and *P. polyanthus* (Debouck, 1999). This can perhaps partly

explain the vast ecological expansion of these two beans, in comparison to the limited success of the other three species.

Currently, no natural inter-specific hybrids involving *P. lunatus* have been reported. Nevertheless, the Lima bean, *P. lunatus*, has a high genetic diversity beyond the intra-specific level (Baudoin, 1991; Maquet *et al.*, 1997). Studies of inter-specific hybridization (reviewed by Debouck, 1999) were carried out to investigate relationships between Lima bean and its wild allies from Mesoamerica in the context of the gene pool concept of Harlan and de Wet (1971), as modified by Smartt (1990).

The secondary gene pool of the Lima bean probably consists of the South American species (i.e., *P. augusti*, *P. bolivianus*, *P. pachyrrhizoides*). These species differ very little (Caicedo *et al.*, 1999; Delgado Salinas *et al.*, 1999) and may in fact constitute a single species with geographic variants.

The tertiary gene pool of *P. lunatus* includes the following species from the USA and/or Mexico with varying levels of relationships and compatibility (Baudoin, 1991; Debouck, 1999; Delgado Salinas *et al.*, 1999; Delgado Salinas, 2000): *P. jaliscanus* Pipet, *P. juguilensis* Delgado, *P. maculatus* Scheele, *P. marechalii* Delgado, *P. polystachyus* Britt., Stern & Pogg, *P. ritensis* Jones, *P. salicifolius* Pipet, *P. sonorensis* Standley, *P. xolocotzii* Delgado. Two variants of *P. polystachyus* are usually recognized: *P. sinuatus* Nutt. and *P. smilacifolius* Pollard.

All attempts to cross *P. lunatus* with *P. vulgaris* have failed to produce a viable

and fertile hybrid (Al-Yasiri and Coyne, 1966; Mok *et al.*, 1978; Leonard *et al.*, 1987; Kuboyama *et al.*, 1991). Early success claimed by Honma and Heeckt (1959) with the use of heterozygous parents is problematic, as mentioned by Hucl and Scoles (1985). The reciprocal cross *P. lunatus* x *P. vulgaris* is even more difficult to obtain (Rabakoarihanta *et al.*, 1979). The difficulty in obtaining fertile viable hybrids between common and Lima beans confirms their distinct taxonomic positions in the genus. Some species could fall between these two extremes, but most have not been studied in sufficient detail so far.

In conclusion, *P. lunatus* shows high levels of intra-specific genetic diversity, distributed between Mesoamerican and Andean gene pools and also between wild and cultivated forms. In addition, the cultigen is characterized by a wide alien gene pool, covering at least 12 species present in both Mesoamerican and Andean regions of the New World.

Wild Lima bean populations can still be found in the centers of diversity, from Mexico to Ecuador, and in different ecologies. *P. lunatus* thus gives us the possibility of observing the mechanism contributing to the maintenance of genetic diversity over many generations, and to obtain reliable data as a model system of relevance not just to the more critically threatened *Phaseolus* species, but also for other species with similar botanical characteristics.

Phytogeography

Phytogeography in the New World

While *Phaseolus* species are found in

the rainfall pattern coincides with favorable conditions of temperature and photoperiod for growth and reproduction; a dry season follows that allows for seed dispersal and conservation in the soil during period of less favorable temperatures. Survival is mainly in the form of seeds, although tuberos roots can also play a role in the *in situ* preservation of some *Phaseolus* species (Debouck, 2000b). As mentioned above, wild forms of common bean and Lima bean are present in both Central and South America. Both are floristic elements of climax forests: subhumid premontane and lower dry tropical forests in the case of the wild Lima bean, subhumid montane forests in the case of wild common bean. Their ecological success and adaptation in disturbed habitats has been a positive factor in their domestication.

The wild form of *P. lunatus* is only found in the Americas, while the cultivated form is widely distributed all over the tropical world and in some temperate regions (i.e., Bulgaria, Italy, USA, Canada, etc.). In particular, some short-cycle breeding lines are cultivated in southern Canada and northern USA. Weedy forms or escapes have been reported in the Democratic Republic of Congo. Despite this extensive distribution, we restrict our discussion to the New World, the centre of origin and of primary diversity of *P. lunatus*. With regard to the cultivated form, only the landraces are considered here due to their major importance in current improvement program and on-farm conservation projects (Altieri and Merrick, 1987; Brush, 1995).

The geographic distribution of wild and cultivated Lima beans extends from the

to the tropical humid climate of the Amazonian region in Ecuador and Peru, to the temperate climates of highland areas 2,500 m above sea level (masl).

Wild forms

The studies of Maquet and Baudoin (1997) and Debouck (2000b) provide a good knowledge on the distribution and ecologies of the wild Lima bean populations in the New World. Tables 1, 2 and 3 (adapted from these studies) indicate, respectively,

and drier regions. In this zone, rainfall varies from 800 mm to 1,500 mm per year, with a well-defined dry season of 3-5 months. At mid-altitudes (800-2,000 msl), the *Tierra templada* is covered with evergreen forests with a T_m varying from 17° to 22°C. Temperature is a critical factor and excessively low temperatures (temperature of the coldest month <14°C) restrict the geographical distribution of wild *P. lunatus* populations in altitude and latitude.

Table 1. Ecological habitats colonized by wild *Phaseolus lunatus* in the Neotropics

Geographic area	Vegetation types
Mexico	Lowland tropical deciduous forests and scrubs
Central America	Lower tropical dry and subhumid forests
South America	Colombia, Venezuela: lower tropical dry forest; South Ecuador, Peru: western humid lower mountain forest

Vegetation types according to Rzedowski, 1978 (for Mexico); Holdridge, 1967 (for Central America); Cabrera and Willink, 1973 (for South America).

the ecological habitats of the wild *P. lunatus* in the New World, and characteristics of natural distribution areas in Meso-America and in the Andes for these wild materials.

The small-seeded wild form of *P. lunatus* extends at low altitudes from Sinaloa, Mexico (23°-26°N) to Salta, Argentina (25° S°) and through the Caribbean Islands (Maquet and Baudoin, 1997). Wild populations are mainly distributed along the Pacific slopes of the mountains of Mexico and Guatemala, but are found exclusively on these slopes further south in Meso-America. Caducifoliate and semi-deciduous forests characterize the region at low altitudes (<800 msl), often referred to as *Tierra caliente*, with an annual average temperature (T_m) higher than 22°C. These forests are the transition between very humid

Both small- and large-seeded wild populations are present in South America, but not in the same ecologies. Small-seeded populations are distributed along the east slope of the Andean cordillera in central-northern Colombia, central-southern Peru and northern Argentina. The large-seeded wild form of *P. lunatus* extends in the Andean region from Cajamarca, Peru to Imbabura, Ecuador, and possibly into Colombia (Toro Chica *et al.*, 1993). In Ecuador and northern Peru, caducifoliate forests become steppic (*Matorral*) due to low rainfall. This could suggest that large-seeded wild populations are best adapted to drier conditions. In addition, these populations are tolerant of lower temperature (T_m around 15°C). No wild populations have been collected in the

Table 2. Some characteristics of natural distribution areas of wild *P. lunatus* forms in Meso-America

Country	Habitat	Elevation (masl)	Annual mean temp. (°C)	Annual mean rainfall (mm)	Length of dry season (months)
Mexico	Pacific and Atlantic slopes, warm valleys, at the border or inside dense semi-deciduous or deciduous forest, clearing of evergreen or semi-evergreen forests	0-1,500	16-27	750-1,500	5-7
Guatemala	Pacific and Atlantic slopes, warm valleys, clearing of warm and humid subtropical forests	50-1,800	20-26	500-3,000	1-6
Salvador	Dry forests, but forests almost completely destroyed and replaced by cropping systems	10-1,000	22-28	1,700	4-6 (severe)
Nicaragua	Semi-arid deciduous forests and montane sub-humid forests (with pines and oaks)		25	1,000-1,500	4-6
Honduras	Mountains with woodlands made of pines and oaks	50-1,000	22-28	1,000-2,000	2-5
Costa Rica	Pacific slopes, clearing of dense evergreen and deciduous forests	100-1,800	22-27	1,400-3,000	1-5

eastern part of South America. Although Piper (1926) reported the species in Brazil, he did not provide geographical data. Wild populations are also not found in Chile (probably due to excessive dryness in northern Chile) and in Bolivia, despite a similar climate in nearby Peru and Argentina.

Landraces

Small-seeded landraces are grown in the semi-arid subtropical region of the south-western USA, mainly in Arizona, home of the Hopi Amerindians (Kuhnlein,

Appalachian Mountains when the first bush type appeared in eastern USA at the end of the 19th century, showing adaptation to subtropical climate. Landraces in Meso-America are also characterized by small seeds and are distributed from Mexico to southern Panama, an important part of the "milpa" subsistence agricultural system (S.A.R.H. *et al.*, 1984; Maquet and Baudoin, 1997). A similar situation exists through the Caribbean Islands, where Lima beans are present at low and mid-altitudes in the traditional homegardens known as "conucos" in Cuba (Esquivel and Hammer, 1988). According to Hazlett (1986),

Table 3. Some characteristics of natural distribution areas of wild *P. luteatus* forms in the Andes

Country	Habitat	Elevations (masl)	Annual mean temp. °C	Annual mean rainfall (mm)	Length of dry season (months)
Colombia	Atlantic coast	50-600	25	1,000	1-2
	East slope of the eastern Andean Belt	1,600	20		
Ecuador	Dry forests of lower mountain; very humid forests of low mountain; thorn sub-desert of low mountain; damaged vegetation, valleys	300-1,900	14-20	500-1,500	1-4
Peru	Eastern slope of the Southern Andean belt;	1,000-1,400	24	1,300-2,000	1-4
	In the North (Cajamarca), thorn dry forest with <i>Cereus</i> and graminaceae	1,100-1,800	14	700	3
Argentina	Province of Salta	650	17-23	700-1,300	1-6

Indians at the frontier between Costa Rica and Panama show a very good drought tolerance.

Small-seeded landraces are also distributed throughout South America (Maquet and Baudoin, 1997). In the eastern part, they are particularly common in north-eastern Brazil. The frequency of large-seeded landraces increases in central and southern Brazil (Erickson, 1982). Consequently a hybrid zone has developed, with landraces having intermediate seed sizes. Landraces are cultivated in the Andean region from Venezuela to Argentina, with small-seeded landraces found at lower altitudes (700 masl on average) than large-seeded landraces (1,880 masl) (Maquet, 1995). It is also essential to mention the occurrence of large-seeded landraces in

valleys of the arid coastal region in Peru. According to Mackie (1943), these genotypes are the final products of an evolutive branch, i.e., the Inca Branch, following trade routes of pre-Colombian residents and giving rise to the cv-gr Big Lima bean. In these coastal valleys of Peru, annual rainfall averages only 2.2 mm; temperatures average 13°-19°C due to the cooling effects of cloudy skies and ocean currents; dense fog also provides additional moisture for growth (Baudoin, 1991).

There is scarce information from Chile, but large-seeded landraces have been collected in the dry north (Maquet, 1995).

Weedy types resulting from gene flow between wild and cultivated forms have been observed where these grow

sympatrically (Maquet, 1995). For example, hybridization between the small-seeded wild form and the cultigen is known from Mexico, Guatemala, Costa Rica, the Bahamas, Cuba, Puerto Rico, and Jamaica (Correll and Correll, 1982; Liogier, Martorell, 1982; Maquet, 1991; Esquivel *et al.*, 1993). The weedy type is also present in the Andean region and particularly in Ecuador and Peru (Debouck, 1990). It is distributed from 1,800 to 2,000 masl in Peru and even up to 2,400 masl in Ecuador.

Discussions

The wild Andean form has a very limited distribution from north of Ecuador to north of Peru, which reflect a reduced adaptability to low and highland environment. On the contrary, the wild Meso-American form is geographically widely distributed from Mexico to the north of Argentina, extending on the eastern slopes of the Andes and in the Caribbean Islands. This wild form is found in a wider array of elevations, from 3 to 1,820 masl, and therefore appears much more flexible than the wild Andean form.

The geographic distribution of the wild forms corresponds generally to the deciduous and semi-deciduous forests, the transition between very rainy and drier regions. In these regions, the annual rainfall varies between 800 and 1,500 mm, with a marked dry season extending to 3 to 5 months. Temperature is a very important factor and too low values limit the extension of the species in altitudes and latitudes. In particular, in Mexico, the Meso-American form is more frequent in lower elevations

forests are converted into grassland due to shortage of precipitation. This situation might explain a better drought tolerance of the Andean form. This should be, however, checked because average annual rainfall is not the most critical factor in the vegetation distribution. Average annual temperatures are much more important in the altitudinal distribution: the Andean wild form, more adapted to higher elevations, tolerates lower values than the wild Meso-American form.

Some natural populations have been reported in the evergreen forest, on relatively well drained soils (calcareous or sandy) and under sunny conditions. In this environment, a constant humidity facilitates the spread of fungal diseases, such as *Alternaria* and *Phoma* (Maquet and Baudoin, 1997). Only some rustic populations can survive such unfavorable conditions. On the other hand, some populations are reported to be xerophytic. In this case, a nearby river, fog or the presence of a water table in the soil provide the minimum of humidity required for the development of these ecotypes.

In view of *in situ* conservation of wild forms, it is important to point out the instability of the deciduous forests, which are often mismanaged or even destroyed in large parts of the American continent (Schnell, 1987). Nevertheless, some wild *Phaseolus* species and, in particular, the wild forms of *P. lunatus* are well adapted to the secondary anthropic vegetation (Debouck, 1987). Unfortunately, these deciduous forests do not evolve, after deforestation to the canopy restoration and

exposed to annual fire, preventing the establishment of wild forms. Considering the small population size (on average 10 plants per population), this practice has a very detrimental effect on the survival of wild Lima beans.

The extended geographic distribution of *P. lunatus* is mainly due to the adaptation of Meso-American and Andean landraces to extremely diverse ecological conditions, such as coastal deserts, lowland humid and subhumid tropics and highland areas. The cultivated form has also been adapted to warm temperate regions (such as California) and some early cultivars have been bred to thrive well in the southern part of Canada.

In spite of this adaptation to new ecological situations, it is urgent to preserve the local *P. lunatus* landraces before their extinction. Indeed, in the whole of Latin America and in Arizona, drastic changes in traditional agriculture contribute to the replacement of local landraces by modern cultivars being more productive and resistant or tolerant to abiotic and biotic conditions. In particular, *P. lunatus* is being replaced by other food legume species, for which more breeding efforts have been made, such as cowpea and pigeon pea. Varieties of these two species have been improved for higher drought tolerance, better earliness and yield stability.

A case study: Phytogeography in the Central Valley of Costa Rica

The ecology of the Central Valley of Costa Rica: The Central Valley is an intermontane valley located in the geographic center of Costa Rica. It is delimited by two major mountain ranges: The Cordillera Central to the north, and the Cordillera

de Talamanca to the south (Flores, 1991; Bergoing, 1998). The valley encloses a maximum area of 3,246 km², within the altitude range 800-1,800 masl. The maximum length of the area is some 70 km, running from east to west, and the width is about 30 km, running from north to south.

The Central Valley is divided into two smaller valleys: the western valley, or Valle de San José, the larger, including about two-thirds of the total area, and the eastern valley, or Valle de Cartago, located between the Cordillera Central to the north and the Cordillera de Talamanca to the south. Both valleys have access to the coastal lowlands, the former descending slowly along the basin of the Grande de Tárcoles River, and the latter along the basin of the Reventazón River. The eastern valley drains into the Caribbean Sea, while the western valley drains into the Pacific Ocean.

The most striking change in land use in the Central Valley began with the introduction of coffee to Costa Rica in 1808, when the Governor Tomás de Acosta attempted to strengthen the economy of the poverty-stricken colony, and considered that this crop could provide a much-needed commodity to trade with Europe. Although the cultivation of coffee evolved slowly and little progress was made before 1920, these actions resulted in the establishment of most of the Costa Rican population in the Central Valley. The landscape changed dramatically, as the forest was almost totally replaced with agricultural fields. Coffee and sugarcane, with smaller areas of other crops and pastures, dominate the landscape to this day, and the majority of the Costa Ricans still inhabit the Central Valley

(Seligson, 1980; Boucher *et al.*, 1983; Baudoin *et al.*, in press)

In general, the soils of the Central Valley are known for being highly fertile. According to the preliminary soil map of Costa Rica (Pérez *et al.*, 1978), there are eleven soil associations belonging to nine main soil types in the study area. Most soil types have been affected by volcanic activity in the Cordillera Central and typically have developed from volcanic ash deposits or other volcanic tuffs. Most soils can be described as deep, rich in organic matter, and well drained, although some soils are poorly drained. The variation in soil types reflects closely the diversity in topography, climate and geological histories that characterize this area.

As far as climate is concerned, great variation in the micro-geographical distribution of rainfall results from the orientation of the mountain ranges and the location of wind passes. Rainfall is seasonal, with a well-defined dry season during December-April. The dry season in the south western part of the Central Valley is not as severe as in the rest of the valley. This is because these areas do not experience the strong winds that affect the rest of the valley during this time of the year (Fournier *et al.*, 1985). The rainy season also varies in intensity, and there is less rain in the middle of the year (typically in July) than during the months just before and after. This period of less rain is called "veranillo", which means little summer (Coen, 1983). Typically, March and September are the months with the lowest

rainfall is lower in the eastern valley than in the western valley.

Because of its geographical location, temperature is not particularly variable in the Central Valley (Protti *et al.*, 1983; Fournier *et al.*, 1985). The mean annual temperature ranges from 15°C at the top of the Cordillera Central and the Cordillera de Talamanca, to 25°C in the south-eastern part of the study area (near the city of Atenas).

The Central Valley of Costa Rica is very diverse biologically. Two distinct approaches have been used to describe the interactions of its physical environment with biological diversity. These are the ecological maps of Costa Rica, which is based on the Holdridge Life Zone system (Holdridge 1966), and the map of Biotic Units, which is based on the structure and floristic composition of the plant communities (Gómez, 1986).

Distribution and ecogeography of wild P. lunatus in the valley

Wild populations of Lima bean can be found throughout the Central Valley of Costa Rica (Standley, 1937; Rocha *et al.*, 1997; Baudoin *et al.*, 1998; Rocha *et al.*, 2002). The populations are usually found in open and disturbed areas with grasses and scattered trees or bushy thickets; they also colonize the coffee plantations from perennial fence (usually *Erythrina* and euphorbs) bordering the plots (Standley, 1937; Debouck, 1987). They are particularly found where coffee is grown under shade (traditional coffee plantations); as well as in the waste lands around these plantations.

bananas. Typically, agricultural activities are less intense in this agro-ecosystem, and do not rely on heavy use of herbicides for the elimination of weeds (Rocha *et al.*, 1997). However, it has been demonstrated that because of changes in agricultural practices and in land use due to urban development, the populations of Lima bean in the Central Valley are fragmented and undergo local extinction and re-colonization (Rocha *et al.*, 1997).

The geographic location of some 450 populations (defined as groups of Lima bean individuals isolated at least 500 m from any other) was determined during 1992-1994. Further surveys were carried out in subsequent years, which found new populations as well as recorded the disappearance of old ones. In order to analyze the physical and ecological attributes of all locations where *P. lunatus* was found during the course of the project (565 populations in total) a detailed classification of each site was conducted using geographical information system (GIS). This work was carried out in collaboration with Centro de Investigaciones en Desarrollo Sostenible (CIEDES) of the Universidad de Costa Rica. The results may be summarized as follows (Baudoin *et al.*, in press).

P. lunatus is found at sites located between 800 and 1,800 masl, but most populations are found between 1,100 and 1,600 masl. The wild populations are more likely to be found in locations with annual precipitation between 1,900 and 2,400 mm and with mean annual temperature between 19 and 22°C. Lima beans are found in large numbers in all relative humidity categories of the valley, not showing a

clear association with population abundance.

The sites where the plants are present occupy 30 different soil types. However, 46% of the populations (260) are found in deep inceptisol, well drained and permeable with low fertility.

According to Holdridge (1966) classification, Lima beans are found in seven different life zones, being most abundant in the humid premontane (38% of populations) and the very humid premontane forest (54%). Similarly, the species is observed in 15 different biotic units. However, 72% of the populations are found in only three of these, i.e., humid, temperate and subtropical areas with a marked dry season that lasts 3-6 months.

Overall, the project's findings indicate that Lima beans are not randomly distributed in the study area, and that their location may in fact be influenced by environmental factors. This information can be used to make predictions about additional locations where Lima beans could be found, both within and outside of the Central Valley. The relationship between Lima bean distribution and the physical factors examined explain partly the genetic structure of the species revealed by morphological or physiological traits as well as isozyme and molecular markers (Maquet *et al.*, 1996; Rocha *et al.*, 1997; Zoro Bi, 1999; Ouédraogo, 2003).

Genetic diversity of wild Lima beans in the valley

A major component of this project was to evaluate the genetic diversity represented by the wild Lima bean populations found in the Central Valley of Costa Rica. This

diversity was studied at both the intra- and inter-population levels, and results served as a guide to understand and maintain the genetic structure of the species in the area. Once biochemical (protein, isozyme) and molecular (microsatellite) markers had been developed, the genetic diversity was assessed between and within populations, and a study was conducted to determine factors responsible for the genetic organization and micro-geographical patterns of the wild Lima beans.

Despite the relatively small size of the area considered in this study and the small sample size taken from each population (5 seeds from a bulked sample), there was significant phaseolin variation among the wild populations studied, all belonging to the Meso-American gene pool (Vargas *et al.* 2000, 2001).

Using phaseolins, populations could be arranged in groups according to their proximity and phenology. This could be explained, at least in part, by climatic factors, such as dry season. Using isozyme markers on 95 populations well distributed in the valley, results showed a very heterogeneous allelic distribution through all the polymorphic loci (Zoro Bi, 1999). Alleles were present in either very few or numerous populations. Geographical distribution of the alleles was very irregular, with some alleles being only found in specific parts of the Central Valley. This non-random spatial distribution of alleles might result from limited gene flow between populations, and/or very much localized selection pressures caused by biotic or abiotic stresses.

the populations as well as environmental conditions in the Central Valley. For example, wild populations of Lima beans might undergo repeated bottlenecks, as weeding and other agricultural practices only allow a few plants to survive and reproduce. These processes lead to significant reduction in effective population size, and to high levels of inbreeding, favoring the decrease of heterozygotes in the population. The recurrent reduction in population size will also favor genetic differentiation among populations. The discontinuity of the habitats where wild Lima beans are most likely to be found in the valley also promotes genetic differentiation among populations. Such fragmentation is mainly the result of replacement of traditional coffee plantations by modern, intensive plantations and accelerated urban development. Differences in abiotic (climate and soil) and biotic factors will also have affected levels and patterns of genetic variation in the Valley (Rocha *et al.* 1997; Ouédraogo, 2003; Baudoin *et al.*, in press)

Environment of Lima Bean: Ecology and Farming System

According to the natural area of distribution of the wild form, the cultigen *P. luteus* is adapted to the savannah climate of the lowland tropics. During domestication, it has diffused to various ecologies: humid, dry or semi-arid tropics and hot temperate regions. Lima bean tolerates wide-ranging ecological conditions. In its extension zone, it can be found from sea level up to altitudes

It requires a frost-free growing season and a fairly dry period when the pods are ripening. Normal annual rainfall required is 900-1,500 mm, but the crop tolerates as little as 500-600 mm once established. Indeed, like groundnut and pigeon pea, Lima bean is considered to be very drought resistant, thanks in particular to its deep, well-developed root system. *P. lunatus* is much more tolerant of rain during its vegetative development than *P. vulgaris* and is therefore better suited than the latter to the low-altitude humid tropical conditions. The plant will thrive if the average monthly temperature during the growing season lies between 16° and 27°C. Below 13°C, its growth is considerably retarded. The small-seeded forms (cultigroups Sieva and Potato) withstand high temperatures better than the large-seeded ones (cultigroup Big Lima). *P. lunatus* is best cultivated on well-aerated, adequately drained soils with a pH of 6.0-6.8.

The area devoted to Lima beans in the tropics is small compared with that planted to starchy staple and export crops. Farming systems where the species is integrated vary from monocrop systems under intensive agricultural conditions or in drier environment to traditional mixed cropping systems of the rainforest or savannah zones. The bushy determinate varieties are better suited to the intensive cropping system or the drier areas, while the climbing indeterminate types predominate in the traditional (shifting or permanent) system of cultivation or in more humid tropical areas. Climbing plants are very often intercropped with other species, like sorghum, pearl millet, yams, cassava, cotton,

banana; they are also grown in household gardens. In the Yucatan Peninsula of Mexico, Lima beans have always been associated with maize in the ancient system of shifting cultivation known colloquially as 'milpa'. In the desertic coastal region of Peru, where the cv-gr Big Lima predominates, the landraces are characterized by a prostrate indeterminate habit and are usually grown as a sole crop or intercropped principally with maize or cotton. In this environment, plants are irrigated with approximately 3,500 m³ water ha⁻¹ during the initial period of vegetative growth.

Conclusions

In order to promote the potential of *P. lunatus*, an in-depth survey of the wild form and landraces is essential, particularly in some countries of Latin America and underrepresented in the national and international germplasm collections, such as Belize, Salvador, Honduras, Nicaragua, Panama, Colombia, Venezuela, Argentina, Brazil and some Caribbean Islands (Maquet and Baudoin, 1997). It is also recommended to better understand the phytogeography of the wild and cultivated forms, particularly in some countries where collecting missions were carried out on a fragmentary level: this is the case in some regions of Bolivia (Cochabamba, Chuquisaca and Santa Cruz), in the south of Peru, in the Cordillera between Colombia and Venezuela, on the Atlantic Coast of Mexico (particularly in Veracruz and Tamaulipas), in the eastern part of Guatemala and in both the north and the south of Costa Rica.

To preserve the landraces of the Lima bean, it will be useful to identify people

who have developed them and to analyze their cultural traditions. This will apply, for example, to the Hopi of Arizona, the Guaymí of Panama, the Tainos of Cuba and the Ayoreo in the Paraguayan Chaco. In the same context, it is relevant to promote some horticultural cropping systems linked to a subsistence agriculture still present in the New World, such as the milpa or conucos (Maquet and Baudoin, 1987), in the frame of an on-farm *in situ* conservation program.

In each site of collecting missions, maximum ecological data should be gathered. In most Latin American countries, the relief and the influence of oceanic streams have generated numerous micro-climates and some high rainfall gradients on short distances, which also explain the presence of a very much diversified flora and vegetation types. The actual information in these sites is too fragmentary and concern only main climatic zones in Latin America. It is therefore important to complete such data through in-depth ecogeographical studies.

In order to take benefit of the large gene pools of Lima bean, it is also essential to undertake, from the base germplasm collections, a complete screening of the materials so gathered for various agronomical traits (such as earliness, growth habit, resistance to biotic and abiotic constraints, etc.) and, on this basis, identify the most promising parental genotypes to develop improved cultivars in *P. lunatus*.

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