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Phylogenetic study on wild allies of Lima bean, *Phaseolus lunatus (Fabaceae)*, and implications on its origin

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Abstract: An investigation was made of the phylogenetic relationships among wild accessions of Lima bean (*Phaseolus lunatus*) and wild allies of Mesoamerican and Andean origins, using electrophoresis of seed storage proteins and isozymes. Mesoamerican wild species are phylogenetically more distant from *P. lunatus* than Andean species, and apparently belong to the tertiary gene pool of Lima bean. The Andean wild species, which are investigated for the first time, reveal a high similarity to the Lima bean, and particularly with its Mesoamerican gene pool. These Andean species probably constitute a secondary gene pool of Lima bean, and are thus of considerable interest in the context of genetic improvement of the crop. Based on these observations, an Andean origin is suggested for the Andean wild species and for *P. lunatus*. These results point out the importance of collecting and conserving Andean *Phaseolus* germplasm.

Key words: *Fabaceae*, *Phaseolus*, *Phaseolus lunatus*, Taxonomy, germplasm, evolution, origin, allozyme, Latin America.

Phylogenetic studies in genera of cultivated plants are of considerable interest in the context of the conservation of genetic resources. Phylogenetic relationships among *Phaseolus* species are far from being fully understood, partly due to the paucity of available living germplasm in many species, and to the limitation in interspecific hybridisation programmes. Delgado S. (1985) reported about 40 species of American distribution in the genus *Phaseolus*, while Debouck (1991) tentatively recognised 55 species. Much more information is thus needed before adopting any decisive taxonomic treatment.

Based on limited material, the numerical taxonomy study of *Phaseolus* by Maréchal et al. (1978) identified two main opposite poles: the *P. vulgaris* L. – *P. coccineus* L. complex and *P. lunatus* L. The Lima bean, *P. lunatus*, is a tropical food legume characterised by a high genetic diversity and yield potential (Baudoin 1991). Among the cultivated species of the genus, it ranks second after the common bean, *P. vulgaris*. In the study of Maréchal et al. (1978), wild species of Mesoamerican distribution such as *P. ritensis* Jones, *P. polystachyus* B. S. P., and *P. pedicellatus* Benth. showed some similarity with *P. lunatus* which remained, however, isolated. For all the species morphologically similar to *P. lunatus*, the term 'ally', as used by Piper (1926), is choosen instead of 'related' as no inference is made on phyletic relations between these species. Interspecific hybridisation studies (Baudoin et al. 1985, Hucl and Scoles 1985, Federici and Waines 1988, Cabral and Crocomo 1989, Katanga 1989, Baudoin and Katanga 1990, Smartt 1990) were performed to investigate relationships between Lima bean and its wild allies from different geographical origins in the context of the gene pool concept of Harlan and De Wet (1971) modified by Smartt (1990). According to the results, the primary gene pool of *P. lunatus* comprises the wild accessions and the landraces of Lima bean. These accessions can be grouped into two main races: Andean and Mesoamerican races. Each race is characterised by specific morphological characters (Debouck et al. 1987, Maquet 1995), ecological adaptation (Maquet and Baudoin 1997), seed storage proteins (Debouck

et al. 1989, Lioi et al. 1991, Gutiérrez S. et al. 1995, Maquet 1995), patterns of allozyme (Lioi and Lotti 1996, Maquet et al. 1997) and RAPD marker (Nienhuis et al. 1995, Fofana et al. 1997) polymorphism. Escaped form and weedy form (natural hybrids between the wild form and a landrace) are observed throughout Latin America (Maquet and Baudoin 1997). Currently, no natural interspecific hybrids involving *P. lunatus* have been reported. Thus, none of the investigated relatives of Lima bean appear to belong to the secondary gene pool, that is defined as species between which viable or partially fertile hybrids are easily obtained with the crop species. The tertiary gene pool includes the species between which hybridisations are possible, but the resulting hybrids are sterile, lethal or abnormal. In accordance with the study made by Katanga and Baudoin (1990) and Baudoin (1991), the tertiary gene pool of Lima bean comprises tentatively seven wild species, all belonging to the Mesoamerican region: *P. polystachyus*, which is mainly distributed in the USA; *P. maculatus* Scheele and *P. ritensis*, which grow both in USA and Mexico; *P. jaliscanus* Piper, *P. marechalii* Delgado, *P. salicifolius* Piper, and *P.* sp. NI702 (not yet botanically identified and conserved in the collection of the Belgium National Botanical Garden), which are only found in Mexico. A detailed description of synonyms and of botanical and agronomic characters of these species is given in Delgado S. (1985) and Katanga (1989). Results from hybridisation studies show higher crossability between *P. lunatus* and these seven wild species than between *P. lunatus* and the *P. vulgaris* – *P. coccineus* complex (Honma and Heeckt 1958, Al-Yasiri and Coyne 1966, Leonard et al. 1987, Kuboyama et al. 1991).

Only wild species of Mesoamerican origin were involved in the former studies as well as in more recent phylogenetic investigations using molecular (Hamann et al. 1995, Jacob et al. 1995) or biochemical markers (Jaaska 1996). On the basis of phytogeographic, morphological and ecological arguments, Burkart (1952), Macbride (1943), Maréchal et al. (1978), and Debouck (1991) suggested however that Lima bean might be closer to the Andean wild species of the genus: *P. augusti* Harms, which has the largest geographical distribution (from Ecuador up to Argentina); *P. bolivianus* Piper, a wild species reported by Piper (1926) distributed in Peru and Bolivia, but for which recent collecting missions failed to gather specimens (Debouck 1986a, b, 1988, 1989, 1990; Debouck and Tohme 1988; Freyre et al. 1996); a Peruvian wild accession, S05257, of the *Phaseolus* world collection which was originally collected from Cuzco and is identified as *P.* aff. *bolivianus*; *P. pachyrrhizoides* Harms distributed toward the Peruvian 'Ceja de Selva' (mid-elevation on the western side of the Andes), and *P. rosei* Piper, which has been rediscovered recently by Debouck (1990) in Ecuador.

The question of the origin of *P. lunatus* has long been debated. Linnaeus (1753) suggested an origin located in Bengal (India). To determine the native areas of cultivated plants, De Candolle (1883) relied mainly upon the location of the primitive wild populations. He is the first botanist to propose an American origin (Centre of Brazil) for the Lima bean, taken into account knowledge in taxonomy, phytogeography, archaeology and linguistics. In 1935, Vavilov considered two centres of origin for cultivated Lima bean: one Mesoamerican centre for the small-seeded landraces, and one Andean centre for the large-seeded landraces (in Vavilov 1992). Mackie (1943) recognised a unique centre in Guatemala. Currently, by integrating evidence such as the presence of older archaeological sites in the Andes, the lack of large-seeded remains in Mesoamerican burials (Kaplan 1971, Kaplan and Kaplan 1988), the observation of two major clusters in phylogenetic studies which comprise each wild and cultivated accessions (Nienhuis et al. 1995, Fofana et al. 1997), and the use of different vernacular names in the Andes and in Mesoamerica (Maquet 1995) associated with differences in seed size (Debouck 1986a, Debouck et al. 1989), two independent domestication events are hypothesised in the Lima bean. The Andean domestication centre is probably located in the western valleys of medium elevation in Ecuador and in northern Peru (Gutiérrez S. et al. 1995, Maquet 1995). The Mesoamerican centre could not be located precisely because wild and cultivated forms belonging to this gene pool are distributed from Mexico up to Argentina.

In this study, we investigate phylogenetic relationships among wild allies of *P. lunatus*, including three species of Andean origin, using seed protein and allozyme polymorphisms. Wild accessions of Lima bean, belonging to the Mesoamerican and Andean races, as well as two wild accessions of *P. vulgaris* are included in the analysis for comparison. The objectives are (1) to clarify the relationships between *P. lunatus* and its Andean and Mesoamerican wild allies, and (2) to identify a putative centre of origin for *P. lunatus*.

Materials and methods

Plant material. Forty-nine accessions were included in this study using seed protein (SP) or allozyme (A) markers (Table 1). These accessions were obtained from the Lima

Table 1. Wild accessions of *Phaseolus* spp. included in the analysis, seed protein and/or allozyme markers used identification number (Id. No.) origin (country and department), altitude, latitude and longitude ([1]*SP* seed protein, *A* allozyme; [2]*BOL* Bolivia, *COL* Colombia, *CRI* Costa Rica, *ECU* Ecuador, *GTM* Guatemala, *MEX* Mexico, *PER* Peru, *USA* United States of America)

Taxon	Marker[1]	Id. No.	Origin[2]	Altitude (m a.s.l.)	Latitude	Longitude
P. augusti	SP	S29355				
P. augusti	SP, A	S31159	BOL, Cochabamba	2530	17.38S	65.28W
P. augusti	SP	S31183				
P. augusti	SP, A	S31250	PER, Cuzco	2980	13.36S	71.45W
P. augusti	SP	S31818				
P. augusti	SP	S32383				
P. aff. *bolivianus*	SP, A	S05257	PER, Cuzco			
P. jaliscanus	A	NI1090	MEX, Jalisco	2200	20.46N	103.52W
P. lunatus	A	G25221	MEX, Veracruz	20		
P. lunatus	A	G25230	MEX, Colima	3	19.03N	104.14W
P. lunatus	SP	G25704	MEX, Jalisco	1390	20.48N	103.24W
P. lunatus	A	G25713	MEX, Campeche	100	19.21N	89.40W
P. lunatus	A	G25738	MEX, Campeche	80	20.13N	89.55W
P. lunatus	A	G25759	MEX, Campeche	40	19.34N	90.15W
P. lunatus	A	G25819	COL, Magdalena	580	10.53N	74.03W
P. lunatus	A	G25844	GTM, Sacatepequez	1740	14.29N	90.43W
P. lunatus	A	G25915	PER, Cajamarca	2020	7.11S	78.50W
P. lunatus	A	S29700	CRI, Alajuela	1170	10.07N	84.23W
P. lunatus	SP	S32386	ECU, Loja	1780	4.11S	79.12W
P. lunatus	A	S32389	ECU, Loja	1530	4.09S	79.17W
P. lunatus	A	S32394	ECU, Loja	1630	4.21S	79.47W
P. lunatus	A	S32401	ECU, Azuay	1570	3.13S	79.12W
P. lunatus	A	S32402	ECU, Chimborazo	890	2.08S	79.02W
P. lunatus	SP	S32418	ECU, Loja	1700	4.11S	79.10W
P. lunatus	SP	S32419	ECU, Loja	1600	3.50S	79.22W
P. maculatus	A	NI0696	MEX, Zacatecas	2250	23.29N	103.36W
P. maculatus	A	NI1237	MEX, Nuevo Leon	970	24.43N	99.50W
P. maculatus	SP	S13169				
P. marechalii	A	NI1252	MEX, Morelos	1900		
P. marechalii	SP	S30610	MEX			
P. marechalii	SP	S31075	MEX			
P. pachyrrhizoides	SP	S17033	PER, Apurimac			
P. pachyrrhizoides	SP	S27161	PER			
P. pachyrrhizoides	SP	S29710	PER			
P. pachyrrhizoides	SP	S29884	PER			
P. pachyrrhizoides	SP	S30318	PER			
P. pachyrrhizoides	A	S30325	PER, Junin	2730	12.01S	74.53W
P. pachyrrhizoides	SP	S30333	PER			
P. pachyrrhizoides	A	S30355	PER, Apurimac	2830	13.36S	73.28W
P. pachyrrhizoides	A	S30428	PER, Apurimac	2440	13.37S	73.12W
P. pachyrrhizoides	SP	S30440	PER			
P. polystachyus	A	NI0430	USA, Florida			
P. polystachyus	A	NI0563	USA, Florida			
P. ritensis	A	NI0796	USA, Arizona	2050		
P. ritensis	A	NI0798	USA, Arizona	1850		
P. rosei	SP	S32378	ECU, Chimborazo	1550	2.15S	78.55W
P. salicifolius	A	NI1132	MEX, Sinaloa	2000		
P. vulgaris	A	NI0407	MEX, Guerrero			
P. vulgaris	A	NI1422	BOL			

bean world collection held at C.I.A.T. in Cali, Colombia (G and S accession numbers) and from the Belgium National Botanical Garden in Meise (NI accession numbers).

For the seed protein electrophoresis, four accessions from *P. lunatus* were chosen on the basis of the results obtained by Maquet (1995): accessions S32419 and G25704, which showed the most frequent seed storage protein pattern ('M1') in the Mesoamerican gene pool; accessions S32418 and S32386, which had the most common Andean patterns ('A1' and 'A3', respectively).

For isozyme electrophoresis, 13 accessions from wild Lima bean belonging either to the Andean gene pool (G25915, S32389, S32394, S32401, S32402) or to the Mesoamerican gene pool (G25221, G25230, G25713, G25738, G25759, G25819, G25844, S29700), according to their seed protein pattern, were chosen on the basis of the classification depicting the genetic relationships among *P. lunatus* (Maquet et al. 1997). In order to represent a large variability, these accessions were sampled in different cluster groups based on allozyme frequencies. In addition, two accessions from *P. vulgaris* were used as a taxonomic outgroup: a wild Mexican accession (NI0407) and a wild Bolivian accession (NI1422).

Seed protein analysis. Three to five seeds per accession were analysed by one-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Seed protein extracts were prepared by removing the seedcoat and grinding at room temperature ca 30 mg of cotyledon in a 0.5 M NaCl adjusted at pH 2.4. The suspension was centrifuged at 14000 rpm for 10 min and an aliquot of the supernatant was mixed with an equal volume of cracking buffer (0.625 M Tris-HCl pH 6.8, 2 mM EDTA; 2% w/v SDS; 40% w/v sucrose; 1% w/v 2-mercaptoethanol and 0.01% w/v bromophenol blue). The resulting mixture was boiled during 5 min (Brown et al. 1981).

Polyacrylamide gels were prepared according to Laemmli (1970). The loading gel had a 4% w/v acrylamide concentration in a 0.5 M Tris-HCl pH 6.8 buffer with 10% w/v SDS, 1‰ w/v ammonium persulfate and 0.26‰ TEMED. The separator gel was prepared with 15% w/v acrylamide in a 1.5 M Tris-HCl pH 8.8 buffer with 10% w/v SDS, 1‰ w/v ammonium persulfate and 0.45‰ TEMED. The electrode buffer was a 0.025 M Tris, 0.192 M glycine buffer at pH 8.3 with 1% w/v SDS.

Samples were run for 10.5 h at 80 mA and 250V in a BioRad Protean II apparatus. Polyacrylamide gel were stained with 0.25% w/v Coomassie Blue for 12 h and then washed with a bleaching solution (14 H_2O: 6 methanol: 1 acetic acid) (Hussain et al. 1988).

Each polyacrylamide gel contained two standards: a wild Mexican accession (G25704) and a wild Peruvian accession (G25916) of *P. lunatus*. A protein standard (LMW Calibration Protein Standards of Pharmacia) and an accession of *P. vulgaris* ('Tendergreen' cultivar, G07476) were also used. According to this sample size, no variation was observed within the accessions but Maquet (1995), studying more *P. lunatus* accessions noted a variation within some genotypes. On the other hand, for the species with more than three accessions analysed (i.e. *P. augusti*, *P. lunatus*, *P. pachyrrhizoides*), variation within the species has also been noted.

Isozyme analysis. For each accession, three to five seeds were analysed. Eight enzyme systems, obtained from the cotyledon tissues, were assessed: alcohol dehydrogenase (ADH, E.C. 1.1.1.1), cytosol amino-peptidase (CAP, E.C. 3.4.11.1), endopeptidase (END, E.C. 3.4.23.6), glucose-6-phosphate isomerase (GPI, E.C. 5.3.1.9), isocitrate dehydrogenase (IDH, E.C. 1.1.1.42), malate dehydrogenase (MDH, E.C. 1.1.1.37), phosphogluconate dehydrogenase (PGDH, E.C. 1.1.1.44), and phosphoglucomutase (PGM, E.C. 5.4.2.2).

Electrophoretic analyses were conducted on horizontal 10% starch (Sigma # S-4501) gels containing 3% sucrose (Sigma # S-8501), according to the method of Maquet et al. (1994). Five-day old imbibed cotyledons were ground in a potassium phosphate 0.1 M pH 7.0 buffer as described by Hussain et al. (1988). The crude homogenate absorbed onto paperwicks was used for starch gel electrophoresis following the method described by Murphy et al. (1990) and using the histidine-citrate pH 5.7 buffer system. The histochemical staining procedures used were those of Hussain et al. (1988) for CAP, END, GPI, and MDH and those of Murphy et al. (1990) for ADH, IDH, PGDH, and PGM.

Loci were labelled sequentially with those migrating closest to the anodal end designated as number 1 (Koenig and Gepts 1989). The first and the last stacks correspond to the accession G25221, a Mexican wild accession, considered as a standard for our analysis. The allele from this genotype is designated as 100 and all other allozymes are measured by their relative distance from the standard. The genetics of the tested enzyme systems has been investigated in previous works (Maquet 1995, Zoro Bi et al. 1997).

For all those species for which more than one accession have been evaluated, variations within accession and within species have been observed. Allelic frequencies for each locus and accession were used to compute Nei's genetic distances, according to Nei (1978), between each pair of accession. Four study groups were considered in the analysis: Andean wild species (six accessions); Mesoamerican wild species (nine accessions); Andean gene pool of *P. lunatus* (five wild accessions); and Mesoamerican gene pool of *P. lunatus* (eight wild accessions). Average Nei's genetic distances between pairs of accessions within or among study groups were computed. Genetic differentiation between pairs of study groups was tested using a permutation test described by Van Rossum et al. (1997), that consists in computing the observed difference between the average genetic distance within and between the two groups, and testing it against an ad hoc distribution of 1000 values of this statistic generated by randomly assigning accessions to each of the two groups and computing the corresponding average distances. These data analyses were performed using GEN-SURVEY, a program written by one of the authors (X. Vekemans).

For phylogenetic analysis, accessions of each species (except *P. lunatus* and *P. vulgaris*, that were divided each

into an Andean and a Mesoamerican gene pool) were pooled and a matrix of presence and absence of each allele at each locus was computed. This matrix was used as input for a Wagner parsimony analysis using procedure MIX from the PHYLIP package (Felsenstein 1993). One thousand bootstraps were performed and a consensus tree was built using procedure CONSENSE. The phylogenetic tree obtained was rooted using the two accessions from *P. vulgaris* as outgroups. The tree was drawn using the program TreeView (Page 1996).

Results

Seed proteins. In Lima bean, the major difference at the seed protein level between Mesoamerican and Andean accessions is revealed by protein of molecular weights ranging from 23 kDa to 26.5 kDa (Fig. 1). Wild and cultivated accessions of *P. lunatus* from the Andes show a first band around 23 kDa and a second one around 25.7 kDa, while Mesoamerican accessions

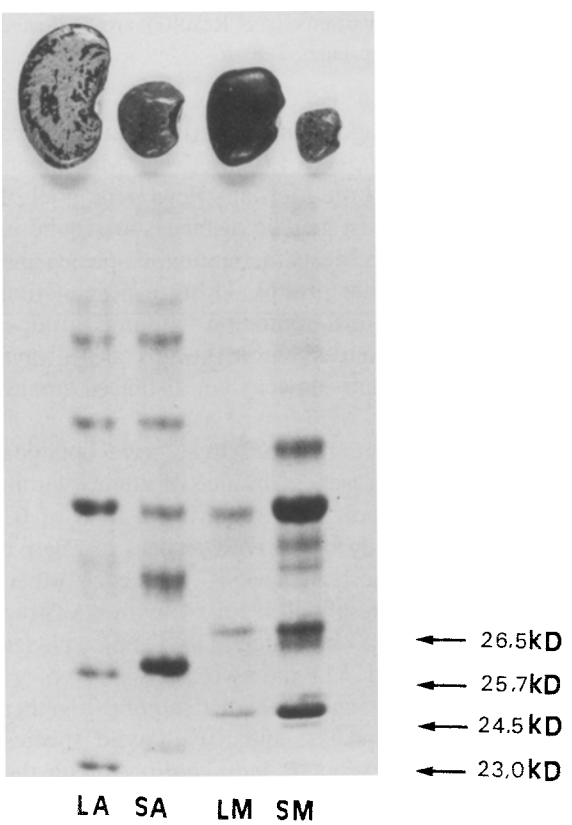

Fig. 1. SDS-PAGE of seed storage protein of *P. lunatus* characterising both Andean and Mesoamerican gene pools. LA Landrace from Ecuador (seed protein pattern 'A3'), SA wild accession from Ecuador (seed protein pattern 'A12'), LM landrace from Guatemala (seed protein pattern 'M1'), SM wild accession from Guatemala (seed protein pattern 'M2')

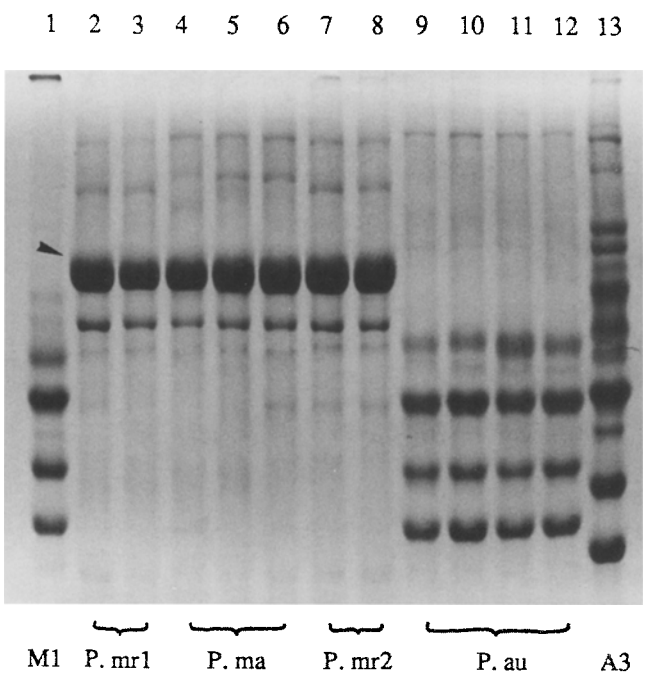

Fig. 2. Seed protein patterns (SDS-PAGE) of some wild related species of *P. lunatus*. *M1, A3 P. lunatus*; *P. mr1, P. mr2 P. marechalii* (S31075 and S30610, respectively); *P. ma P. maculatus* (S13169); *P. au P. augusti* (S31253). *P. vulgaris* like phaseolin protein is indicated by the arrowhead

are characterised by two bands around 24.5 kDa and 26.5 kDa. The former seed protein pattern is labelled by 'A', while the latter is designed by 'M', with a suffix number distinguishing the variants inside these two families (Maquet 1995).

The analysed wild species are characterised by different seed protein patterns in relation to their origin, as illustrated in Fig. 2. Wild species from Mesoamerica (i.e. *P. marechalii* and *P. maculatus*) have seed protein patterns which are different from those observed in Lima bean, but which are similar to that of *P. vulgaris*, represented in Fig. 3, in showing a phaseolin protein around 45 kDa. In contrast, wild Andean species (i.e. *P. augusti*, *P. pachyrrhizoides* and *P. rosei*) show seed protein patterns similar to those observed in Lima bean (Figs. 2, 3), which lack the *P. vulgaris* like phaseolin protein. In addition, it appears that the seed protein patterns of *P. augusti* and *P. pachyrrhizoides* are much closer to the Mesoamerican than to the Andean patterns of *P. lunatus*. *Phaseolus* aff. *bolivianus* also shows a pattern similar to a Mesoamerican *P. lunatus* (data not shown). The seed protein pattern of the Andean wild species *P. rosei*, however, is closer to an Andean *P. lunatus*, and is very similar to the profile 'A1' which is the most common among the Andean wild accessions of *P. lunatus*.

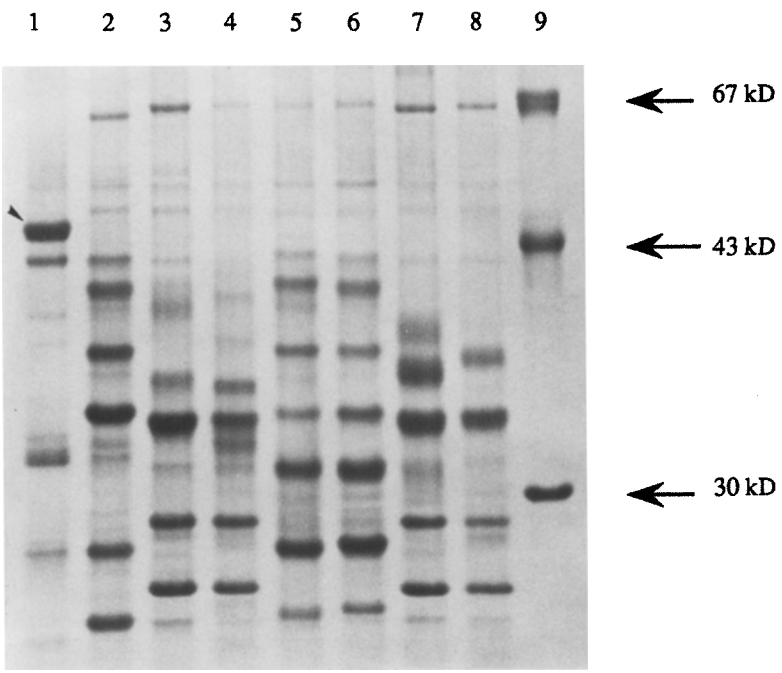

Fig. 3. Seed protein patterns (SDS-PAGE) of different Andean wild related species of *Phaseolus lunatus*, V *P. vulgaris* characterised by its phaseolin (arrow-head): P. lu *P. lunatus*; P. ro *P. rosei*; P. au *P. augusti*, P. pa *P. pachyrrhizoides*, MM protein standard with molecular weights indicated at the right side. Pattern variants (see Results) are indicated below the lanes 2–8

Allozymes. Table 2 shows the allelic distribution according to the wild species and the geographic regions. Twelve alleles are only found within one species and in one gene pool: *Adh-1*^{70}, *Adh-2*^{61}, *Adh-2*^{128}, *Cap*^{94}, *Cap*^{145}, *End*^{77}, *Gpi-1*^{105}, *Idh*^{90}, *Idh*^{120}, *Mdh-2*^{200}, *Mdh-3*^{70}, and *Pgdh-2*^{135}. Some alleles are common in *Phaseolus*: *Adh-2*^{100}, *Idh*^{100}, *Mdh-2*^{140}, and *Mdh-3*^{100}. Only three alleles are observed exclusively among three or more species originating from Mesoamerica: *Adh-1*^{65}, *Cap*^{136} and *Gpi-1*^{86}. The allele *Pgdh-2*^{119}, observed among all Mesoamerican wild species, is also present but at low frequency in landraces of Lima bean (Maquet et al. 1997). The Andean wild species do not share a unique allele.

We also checked the presence of the alleles discriminating the two *P. lunatus* gene pools, in wild relatives of the species. The alleles characterising the Mesoamerican wild accessions of Lima bean (Maquet et al. 1997) are either absent in wild related species (i.e. *Adh-2*^{61}, *Cap*^{94}, and *Mdh-2*^{100}), or observed among the Andean wild species only (i.e. *Gpi-1*^{100} and *Pgm-1*^{90}). The alleles identifying the Andean wild *P. lunatus* accessions do not exhibit any clear-cut pattern.

Average Nei's genetic distances between pairs of accessions for comparisons within and among study groups are given in Table 3. Within groups, average genetic distances range from 0.20 (within each of the two gene pools of *P. lunatus*) to 0.34 (within the group of Andean wild species). Between groups, average genetic distances range from 0.43 (between the two gene pools of *P. lunatus*) to 1.01 (between Mesoamerican wild species and the Mesoamerican gene pool of *P. lunatus*). The highest genetic distances are found in comparisons between Mesoamerican wild species and each of the three other groups. Using a permutation procedure to test differentiation among groups, average genetic distances within groups are always found to be significantly lower than distances among groups.

Three equally parsimonious trees were obtained from the matrix of presence/absence of alleles within each species/gene pool, each requiring a total of 63 steps. These trees only differ with respect to relative position of Mesoamerican species with each other. One such tree is represented in Fig. 4, with bootstrap values higher than 500 indicated. Three major clades can be distinguished: (1) the two accessions of *P. vulgaris*; (2) the two gene pools of *P. lunatus* together with Andean wild species; and (3) all wild species from Mesoamerica except *P. marechalii*. Among the different most parsimonious trees, the Mesoamerican species *P. marechalii* occurs as a sister group either to clade (2) or to clade (3). Within clade (2), the Andean species *P. augusti* and *P. pachyrrhizoides* are closely related to each other, but the relative positions of the Andean and Mesoamerican gene pools of *P. lunatus* are unresolved. Within clade (3), all phylogenetic relationships are unresolved.

Table 2. Allozyme distribution among the wild form and the wild allies of *Phaseolus lunatus*. The observed alleles are resumed in this table without regarding their frequences

Species	*ADH1*			*ADH2*			*CAP*			*END*			*GPI*			*IDH*			*MDH2*			*MDH3*		*PGDH1*		*PGDH2*			*PGM1*		
	65	70	91	100	61	100	128	94	100	108	118	136	145	77	88	100	119	139	86	96	100	105	80	90	100	120	100	140	200	70	100	86	92	100	100	109	119	135	167	90	100	107	125
P. lunatus																																										
Andean wild				x		x			x	x						x	x			x	x				x								x	x						
Mesoamerican wild				x	x	x		x	x	x					x	x	x	x				x	x			x									x					x	x
Andes																																										
P. augusti				x		x					x	x			x							x															x				
P. bolivianus				x		x					x											x																			
P. pachyrhizoides				x		x					x						x					x																			
Mesoamerica																																										
P. jaliscanus												x	x		x	x				x																				x	
P. maculatus				x								x	x		x	x				x																				x	
P. marechalii				x		x						x			x	x				x																				x	
P. polystachyus		x				x		x				x	x			x				x																				x	
P. ritensis													x			x				x																				x	
P. salicifolius		x				x							x			x				x																				x	
Outgroup *P. vulgaris*														x		x				x																					
Andean Form			x			x						x				x				x																				x	
Mesoamerican Form			x			x						x				x				x																				x	

Table 3. Average Nei's genetic distances between pairs of accessions within and among study groups of *Phaseolus* and permutation tests of genetic differentiation among groups

Group	Andean wild species	Mesoamerican wild species	*P. lunatus*, Andean gene pool	*P. lunatus*, Mesoamerican gene pool
Andean wild species	0.343			
Mesoamerican wild species	1.003 $P<0.001$	0.276		
P. lunatus, Andean gene pool	0.579 $P<0.05$	0.689 $P<0.01$	0.199	
P. lunatus, Mesoamerican gene pool	0.547 $P<0.001$	1.008 $P<0.001$	0.427 $P<0.01$	0.196

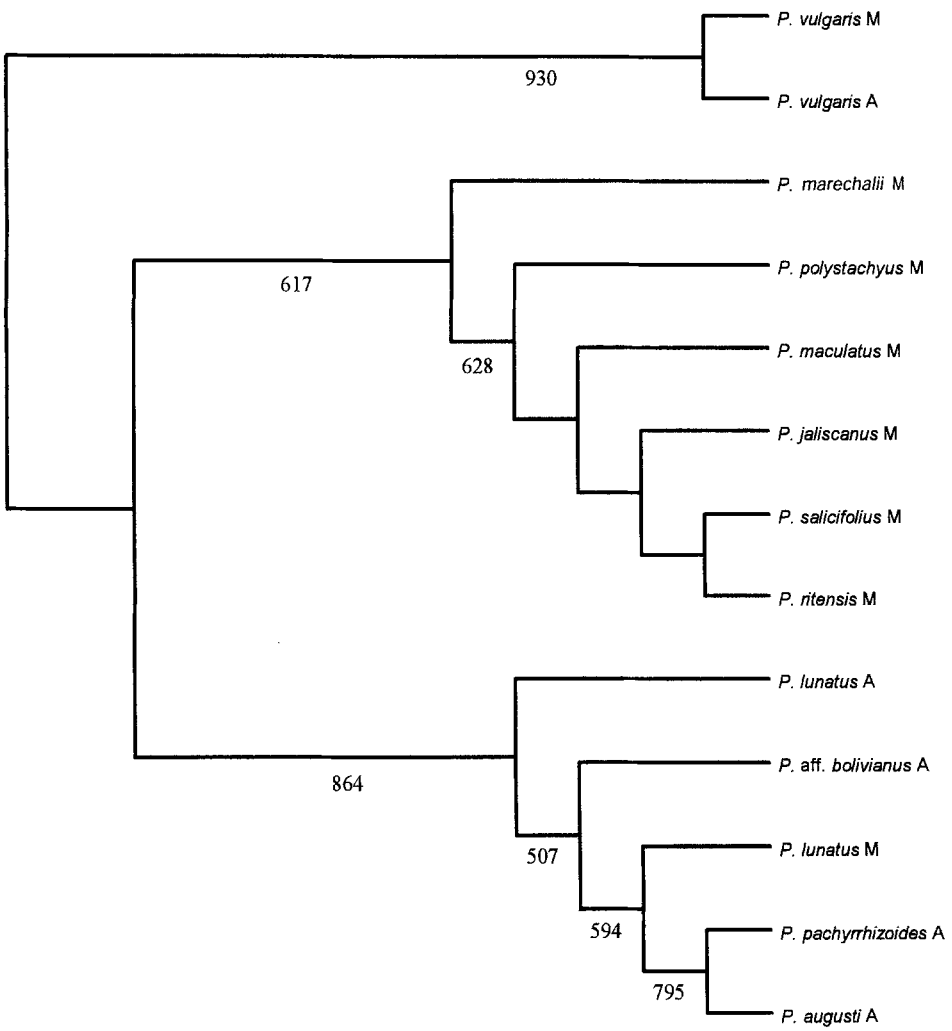

Fig. 4. One of three most parsimonious cladograms of *Phaseolus lunatus* and its wild allied species. A Andean origin, M Mesoamerican origin. Only bootstrap values above 500 are indicated

the Andean wild species, made here for the first time, shows the existence of a Lima bean species complex including its two gene pools (Mesoamerican and Andean) and diverse wild species like *P. augusti*, *P.* aff. *bolivianus*, and *P. pachyrrhizoides*. These three species might be included in the secondary gene pool of *P. lunatus*, and in this context, interspecific hybridisation experiments with the Lima bean would

be of great value. The characterisation of this species complex opens new prospects for the genetic improvement of *P. lunatus*.

The existence of the Lima bean species complex raises also the question of the origin of *P. lunatus*. Mexico is considered as the centre of diversity of the genus *Phaseolus* (Sousa S. and Delgado S. 1993). Wild species are particularly numerous along the Occidental Sierra Madre and the volcanic transversal axis. These two mountain ranges have a recent origin; the first dating from the Oligocene-Miocene while the second dating from the end of the Tertiary era or the Pliocene. The current diversity in *Phaseolus* would date from the Oligocene ($\cong$ 25 millions years ago), or from a more recent time during the upthrust of these mountains (Delgado S. 1985). In the case of *P. lunatus*, the co-occurrence of its two gene pools in South America, as well as its close phylogenetic relationship with the Andean wild species, can be viewed as arguments for an Andean origin of the species, as suggested by Carter (1945) on the basis of archaeological data. Recently, Kami et al. (1995) and Tohme et al. (1996) suggested also an Andean origin for *P. vulgaris*, on the basis of molecular markers. Hereafter, we propose a hypothetical evolutionary model for the Andean species complex involving *P. lunatus*. After the contact between Central America and South America (4 to 2 million years ago) a Mesoamerican ancestor, probably characterised by tuber roots and adaptation to high altitudes, migrated to South America. Indeed, in Mexico most *Phaseolus* species are adapted to higher altitudes, and have a tuberous root system, a hypogeal germination except for the wild form of *P. vulgaris* and *P. polyanthus* Greenm., and are currently characterised by a narrow geographic distribution (Delgado S. 1985). This putative ancestor could explain the distribution of the wild species at high elevation in the Andean area (Debouck 1988, 1989, 1990; Maquet and Baudoin 1997), and the presence of taxa with tuber roots, as *P. augusti* and *P. pachyrrhizoides* (Harms 1921). The migration of a Mesoamerican species adapted to high altitudes is possible despite the low altitudes (0–500 m. a.s.l.) in Nicaragua. Indeed, *P. coccineus*, a species adapted mainly to altitudes higher than 2000 m. a.s.l., is distributed from Mexico up to Panama (Schmit 1988, 1989). The uprising of the Andes started in the Pliocene (5 to 2 million years ago) and stopped at the beginning of the Pleistocene (Forero 1988). This rising of the Andes would favour a diversification of the putative ancestor by the appearance of various microclimates and the relative isolation of the valleys. Climatic changes characterising the Pleistocene (glaciation era) influenced also greatly the development of the flora and the vegetation (Dejoux 1994). During this period, a new variant could have emerged in the Andes, and subsequently colonise lower altitudes with higher temperatures. In this new environment, many genotypes have acquired small seeds (Maquet and Baudoin 1997) and could became photoperiod-insensitive, a physiological modification observed in similar climates by Masaya and White (1991) in *P. vulgaris*. The day-length insensitivity would have favoured the wide distribution of this new variant up to higher latitudes, as Central America and Mexico. According to this scenario, the small-seeded accessions could have an Andean origin.

Debouck (1988, 1989, 1990), Freyre et al. (1996), and Maquet and Baudoin (1997) report that the species included in the Andean complex are currently threatened. Consequently, to exploit the genetic potential of this species complex in *Phaseolus* improvement, it is urgent to activly conserve it by collecting and by establishing genetic reserves as these species are poorly represented in the world collection held at C.I.A.T. (Hidalgo 1991). In addition, increasing the genetic sampling of these species will improve the knowledge of the relationships among the wild species allied to *P. lunatus* and a phylogeny based on sequences which evolved according to the molecular clock model would help to propose a deeper-rooted model on species divergence.

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