PLANT GENETIC RESOURCES

AFLP Fingerprinting of *Phaseolus lunatus* L. and Related Wild Species from South America

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ABSTRACT

The taxonomic classification of the wild Lima bean complex needs to be assessed to select species for use in breeding programs and to identify genetic resources for conservation. The objectives of this study were to determine the genetic relationships among, and the phylogeny of, wild Lima bean (*Phaseolus lunatus* L.) and related species (*P. augusti* Harms, *P. bolivianus* Piper, *P. pachyrrhizoides* Harms, and *P. rosei* Piper) from South America and to identify specific genetic reserves for conservation. These relationships were investigated by means of amplified restriction fragment length polymorphism (AFLPs) on total genomic DNA. The 122 accessions formed a cluster that was distant from common bean (*P. vulgaris* L.), confirming earlier morphology and hybridology data. Two gene pools of wild Lima beans were confirmed. One was widely distributed in neotropical lowlands, while the other was restricted to the western Andes, in Ecuador and northern Peru. The study also revealed the existence of a third group of wild Lima bean distributed in the Departments of Boyacá and Cundinamarca, Colombia. The three species *P. augusti*, *P. bolivianus*, and *P. pachyrrhizoides* differed very little, certainly not sufficiently to merit a separate taxonomic ranking at the species level. The accessions could be grouped instead according to four geographic origins: Ecuador and northern Peru; Department of Junín, Peru; Departments of Cuzco and Apurímac, Peru; and Bolivia and northwestern Argentina. Results from this study should result in a better selection of parental materials in breeding programs and point to areas where germplasm collections and conservation are needed.

THE GENUS *Phaseolus* includes five grain legumes of worldwide or regional economic importance, and approximately 50 species, all of neotropical origin (Delgado Salinas, 1985; Lackey, 1983; Maréchal et al., 1978). Although floristic surveys of the tropical Western Hemisphere are largely incomplete, evidence suggests that more biological species exist in North America than in South America (Delgado Salinas, 1985). Approximately 45 species of wild beans are distributed from Panama to southern Canada (Debouck, 1991; Delgado Salinas, 1985), whereas only four to six wild species are native to South America.

Most South American *Phaseolus* sensu stricto species have their range of distribution apparently limited to the Andean orographic system from the Venezuelan Andes to the Sierra de Córdoba in Argentina. Exceptions are *P. mollis* Hooker, which is restricted to the Galapagos archipelago (Wiggins and Porter, 1971), and *P. lunatus*, which is present in the eastern tropics of the South American lowlands (Lewis, 1987). No wild *Phaseolus* species have been reported in Chile (Lackey, 1983).

The range of wild *P. vulgaris*, the ancestor of common bean, in South America, extends in the Andes from western Venezuela to San Luis in Argentina (Toro Ch. et al., 1990). *Phaseolus polyanthus* Greenman (synonyms: *P. flavescens* Piper, *P. harmsianus* Diels) is cultivated in the northern Andes (Berglund-Brücher and Brücher, 1974) and exists as a feral species in secondary forests in the Andes from western Venezuela, south to the Department of Apurímac, Peru (Schmit and Debouck, 1991). Wild *P. lunatus*, the Lima bean, exists as two major groups of morphologically distinct populations. One is distributed in the lowlands of eastern South America, stretching from the Caribbean coast, through Brazil and eastern Peru, to Salta, Argentina. The other group is distributed in the western Andes, in Ecuador and northern Peru (Debouck et al., 1987; Gutiérrez Salgado et al., 1995). *Phaseolus vulgaris* and the two wild forms of *P. lunatus* appear to be genuine floristic components of natural dry and subhumid South American forests. Molecular markers studies have shown intrinsic differences within the wild forms of common bean (Khairallah et al., 1992; Tohme et al., 1996) and Lima bean present in Mesoamerica (Gutiérrez Salgado et al., 1995; Maquet et al., 1994). These studies have also shown that wild forms are more polymorphic than the cultivated ones.

Two species, *P. augusti* Harms and *P. pachyrrhizoides* Harms, from Huancavelica and Junín, Peru, respectively, were described by Harms (1921), the latter having larger peduncles and bracts. Macbride (1943), however, observed that few differences exist between these two taxa, although *P. pachyrrhizoides* sometimes displays asymmetrical, lobed, lateral leaflets. *Phaseolus bolivianus* Piper was described as a new species from Cochabamba, Bolivia (Piper, 1926). This species and *P. pachyrrhizoides* were mentioned in this review, but not *P. augusti*, and were not cross-referenced to either of them (Piper, 1926). *Phaseolus bolivianus* was not sufficiently distinct from *P. augusti* and later was considered synonymous (Macbride, 1943). In a numerical taxonomy analysis, Maréchal et al. (1978) placed *P. augusti* close to *P. coccineus* and endorsed the synonymous classification. Lackey (1983) recognized *P. pachyrrhizoides* and *P. bolivianus*, but not *P. augusti*. A species from Ecuador, *P. rosei* Piper, was considered as probably annual (Piper, 1926), whereas Toro Ch. et al. (1993) considered

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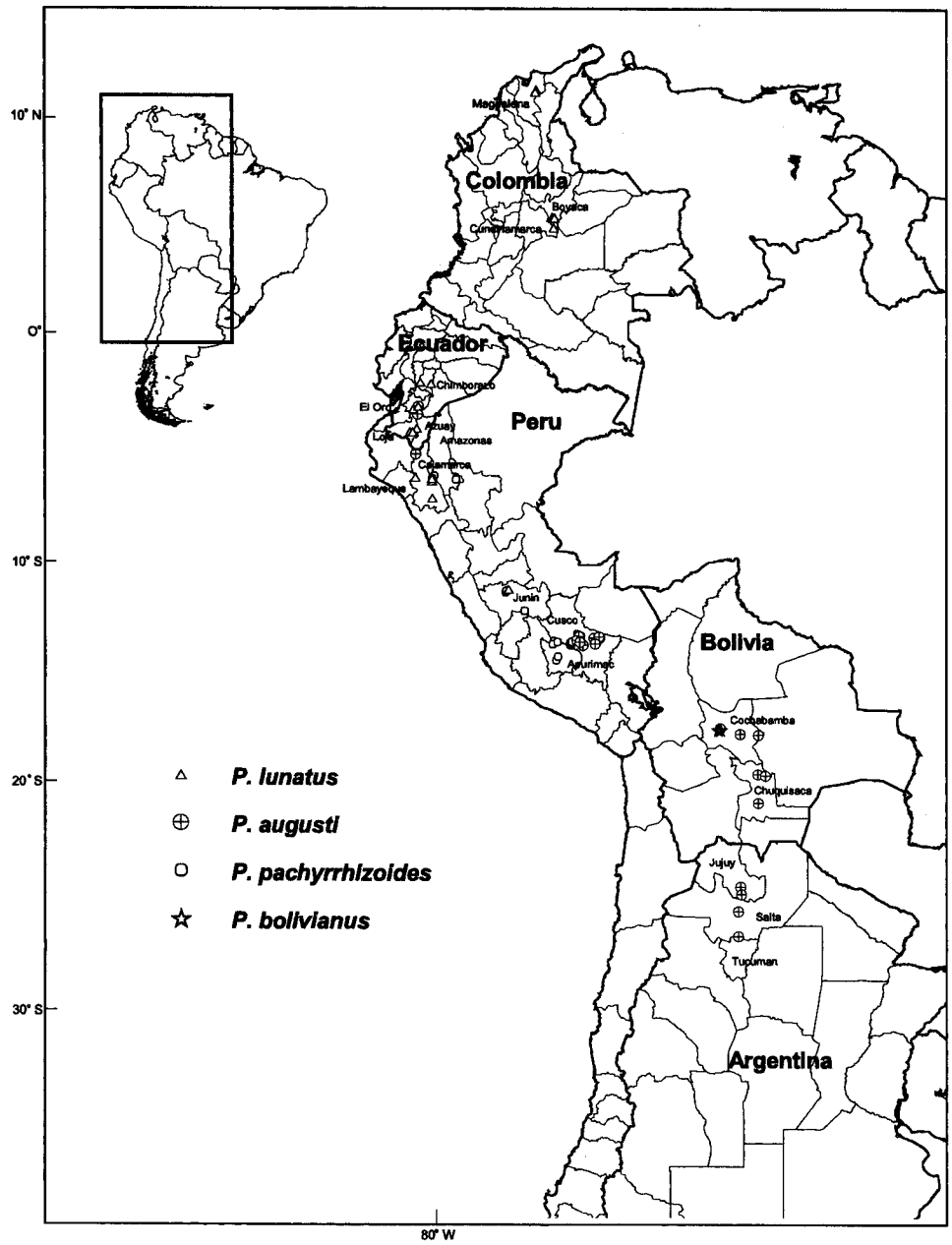

Fig. 1. Distribution map of genotypes of *Phaseolus* species used in this study.

this species to be not different from the Andean wild form of Lima bean. In a catalogue of Peruvian flowering plants, Brako and Zarucchi (1993) recognized only *P. augusti*, *P. lunatus*, *P. pachyrrhizoides*, *P. polyanthus*, and *P. vulgaris*. *Phaseolus augusti* extends from Ecuador (Debouck et al., 1989), through Peru (Brako and Zarucchi, 1993) and Bolivia (Foster, 1958), to Argentina (Palacios and Vilela, 1993). In contrast, the distribution of *P. pachyrrhizoides* is restricted to the Peruvian Andes (Brako and Zarucchi, 1993; Macbride, 1943).

Several questions need to be answered: (i) are *P. augusti* and *P. pachyrrhizoides* related to *P. lunatus*, (ii) are they distinct species (iii) can *P. bolivianus* be merged taxonomically with *P. augusti*, and (iv) is *P. rosei* different from the Andean wild Lima bean. The AFLP fingerprinting technique (Vos et al., 1995) has been successfully applied to the study of genetic diversity of a core collection of wild common bean held at CIAT (Tohme et al., 1996). The objectives of this study were (i) to determine the genetic relationships among species from South America thought to be related to the Lima bean by means of AFLP fingerprinting and (ii) to identify specific genetic resources for conservation.

MATERIALS AND METHODS

A diverse collection of 71 populations of South American species (with one or two individuals per population and totaling 122 genotypes) was obtained from the world collection of *Phaseolus* maintained at CIAT (Fig. 1 and Table 1). Populations collected at the type locality for the species *P. bolivianus* (BBLCO123), *P. pachyrrhizoides* (PPEJU102), and *P. rosei* (LECCH112/113) were included. Five wild accessions and one cultivated line of *P. vulgaris* (ICA-Pijao) were also included to determine genetic distances.

Table 1. List of *Phaseolus* genotypes used in AFLP analysis.

Genotype number	CIAT introduction number	CIAT G number	Collector's ID number	Species	Country of origin	Department	Germination habit†	Altitude
								m
AARJU97	S29355		DGD-1710	*P. augusti*	Argentina	Jujuy	H	1630
AARJU98	S29355		DGD-1710	*P. augusti*	Argentina	Jujuy	H	1630
AARSA48	S32754		VAV 6386	*P. augusti*	Argentina	Salta	H	1400
AARSA49	S32754		VAV 6386	*P. augusti*	Argentina	Salta	H	1400
AARJU99	S29420		DGD-1774	*P. augusti*	Argentina	Salta	H	1550
AARTU95	S29354		DGD-638	*P. augusti*	Argentina	Tucuman	H	1520
AARTU96	S29354		DGD-638	*P. augusti*	Argentina	Tucuman	H	1520
ABLCH24	S31167		DGD-2490	*P. augusti*	Bolivia	Chuquisaca	H	2650
ABLCH25	S31167		DGD-2490	*P. augusti*	Bolivia	Chuquisaca	H	2650
ABLCH26	S31174		DGD-2496	*P. augusti*	Bolivia	Chuquisaca	H	2040
ALBCH27	S31174		DGD-2496	*P. augusti*	Bolivia	Chuquisaca	H	2040
ABLCH20	S31183		DGD-2504	*P. augusti*	Bolivia	Chuquisaca	H	2600
ABLCO21	S31159		DGD-2482	*P. augusti*	Bolivia	Cochabamba	H	2530
ABLCO21	S31159		DGD-2482	*P. augusti*	Bolivia	Cochabamba	H	2530
ABLCO22	S31160		DGD-2483	*P. augusti*	Bolivia	Cochabamba	I	1870
ABLCO23	S31160		DGD-2483	*P. augusti*	Bolivia	Cochabamba	I	1870
ABLCO29	S31185		DGD-2506	*P. augusti*	Bolivia	Cochabamba	H	2920
AECLO43	S32383		DGD-2869	*P. augusti*	Ecuador	Loja	I	2080
AECLO44	S32383		DGD-2869	*P. augusti*	Ecuador	Loja	I	2080
APECU16	S30438		DGD-2312	*P. augusti*	Peru	Cuzco	I	2970
APECU17	S30439		DGD-2313	*P. augusti*	Peru	Cuzco	H	2850
APECU18	S30439		DGD-2313	*P. augusti*	Peru	Cuzco	H	2850
APECU19	S30441		DGD-2317	*P. augusti*	Peru	Cuzco	H	2780
APECU30	S31250		DGD-2571	*P. augusti*	Peru	Cuzco	H	2980
APECU31	S31250		DGD-2571	*P. augusti*	Peru	Cuzco	H	2980
APECU32	S31254		DGD-2575	*P. augusti*	peru	Cuzco	H	2750
APECU33	S31263		DGD-2584	*P. augusti*	Peru	Cuzco	H	2740
APECU34	S31266		DGD-2587	*P. augusti*	Peru	Cuzco	H	2320
APECU35	S31268		DGD-2589	*P. augusti*	Peru	Cuzco	H	2760
APECU36	S31270		DGD-2591	*P. augusti*	Peru	Cuzco	H	2870
APECU37	S31270		DGD-2591	*P. augusti*	Peru	Cuzco	H	2870
APECU38	S31277		DGD-2598	*P. augusti*	Peru	Cuzco	H	2620
APECU39	S31277		DGD-2598	*P. augusti*	Peru	Cuzco	H	2620
APECU85	S30441		DGD-2317	*P. augusti*	Peru	Cuzco	H	2780
APECU86	S31253		DGD-2574	*P. augusti*	Peru	Cuzco	H	2930
BPECU45	S5257		PI260412	*P. augusti*?	Peru	Cuzco	I	
APEPI41	S31818		DGD-2816	*P. augusti*	Peru	Piura	I	1980
APEPI42	S31818		DGD-2816	*P. augusti*	Peru	Piura	I	1980
AECAZ87	S32401		DGD-2887	*P. augusti*?	Ecuador	Azuay	E	1570
AECAZ88	S23401		DGD-2887	*P. augusti*?	Ecuador	Azuay	E	1570
BBLCO80	S33681		DGD-3001	*P. bolivianus*	Bolivia	Cochabamba	H	2830
BBLCO92	S33681		DGD-3001	*P. bolivianus*	Bolivia	Cochabamba	H	2830
BBLCO123	S33680		DGD-3000	*P. bolivianus*	Bolivia	Cochabamba		2650
LAR 65	S20685	G26404	MEF 3917	*P. lunatus*	Argentina		E	
LCOBO70	S32875	G26530	OT-141	*P. lunatus*	Colombia	Boyaca	E	1600
LCOBO74	S33207		OT-284	*P. lunatus*	Colombia	Boyaca	E	1640
LCOBO75	S33347		OT-435	*P. lunatus*	Colombia	Boyaca	E	1440
LCOBO76	S33347		OT-435	*P. lunatus*	Colombia	Boyaca	E	1440
LCOBO77	S33347		OT-435	*P. lunatus*	Colombia	Boyaca	E	1440
LCOBO78	S33347		OT-435	*P. lunatus*	Colombia	Boyaca	E	1440
LCOBO79	S33384		OT-473	*P. lunatus*	Colombia	Boyaca	E	1240
LCOBO81	S33211		OT-288	*P. lunatus*	Colombia	Boyaca	E	1320
LCOBO82	S33211		OT-288	*P. lunatus*	Colombia	Boyaca	E	1320
LCOBO93	S33211		OT-288	*P. lunatus*	Colombia	Boyaca	E	1320
LCOBO122	S32875	G26530	OT-141	*P. lunatus*	Colombia	Boyaca	E	1600
LECCH67	S32402	G26467	DGD-2888	*P. lunatus*	Colombia	Chimborazo	E	890
LECCH69	S32402	G26467	DGD-2888	*P. lunatus*	Colombia	Chimborazo	E	890
LCOCU73	S33188		OT-264	*P. lunatus*	Colombia	Cundinamarca	E	1680
LECLO66	S32394	G26465	DGD-2880	*P. lunatus*	Colombia	Loja	E	1630
LECLO71	S32393	G26545	DGD-2879	*P. lunatus*	Colombia	Loja	E	1800
LCOMA50	S26144	G25819	P&F 4392	*P. lunatus*	Colombia	Magdalena	E	580
LCOMA51	S26144	G25819	P&F 4392	*P. lunatus*	Colombia	Magdalena	E	580
LCOMA62	S26145	G26309	P&F 4393	*P. lunatus*	Colombia	Magdalena	E	50
LCOMA63	S26145	G26309	P&F 4393	*P. lunatus*	Colombia	Magdalena	E	50
LCOMA115	S26144	G25819	P&F 4392	*P. lunatus*	Colombia	Magdalena	E	580
LCOMA116	S26144	G25819	P&F 4392	*P. lunatus*	Colombia	Magdalena	E	580
LCOSA125	S29217	G25968	DGD-2124	*P. lunatus*	Costa Rica	San Jose	E	970
LECCH46	S32378		DGD-2863	*P. lunatus*	Ecuador	Chimborazo	E	1550
LECCH112	S32378		DGD-2863	*P. lunatus*	Ecuador	Chimborazo	E	1550
LECCH113	S32378		DGD-2863	*P. lunatus*	Ecuador	Chimborazo	E	1550
LECEL47	S32399		DGD-2885	*P. lunatus*	Ecuador	El oro	E	800
LECEL114	S32399		DGD-2885	*P. lunatus*	Ecuador	El oro	E	800
LECLO89	S32389	G26463	DGD-2875	*P. lunatus*	Ecuador	Loja	E	1530
LECLO90	S32394	G26465	DGD-2880	*P. lunatus*	Ecuador	Loja	E	1630
LECLO119	S32394	G26465	DGD-2880	*P. lunatus*	Ecuador	Loja	E	1630
LECLO120	S32394	G26465	DGD-2880	*P. lunatus*	Ecuador	Loja	E	1630

Continued next page.

Table 1. Continued.

Genotype number	CIAT introduction number	CIAT G number	Collector's ID number	Species	Country of origin	Department	Germination habit†	Altitude
								m
LGUSA124	S28874	G25912	DGD-1700	*P. lunatus*	Guatemala	Sacatepequez	E	1400
LPECA52	S29849	G25913	DGD-1944	*P. lunatus*	Peru	Cajamarca	E	1810
LPECA53	S29849	G25913	DGD-1944	*P. lunatus*	Peru	Cajamarca	E	1810
LPECA54	S29850	G25914	DGD-1945	*P. lunatus*	Peru	Cajamarca	E	1750
LPECA55	S29850	G25914	DGD-1945	*P. lunatus*	Peru	Cajamarca	E	1750
LPECA56	S29850	G25914	DGD-1945	*P. lunatus*	Peru	Cajamarca	E	1750
LPECA57	S29850	G25914	DGD-1945	*P. lunatus*	Peru	Cajamarca	E	1750
LPECA58	S29861	G25915	DGD-1957	*P. lunatus*	Peru	Cajamarca	E	2020
LPECA59	S29861	G25915	DGD-1957	*P. lunatus*	Peru	Cajamarca	E	2020
LPECA60	S29881	G25916	DGD-1981	*P. lunatus*	Peru	Cajamarca	E	1720
LPECA61	S29881	G25916	DGD-1981	*P. lunatus*	Peru	Cajamarca	E	1720
LPECA64	S31859	G26348	DGD-2857	*P. lunatus*	Peru	Cajamarca	E	1100
LPECA117	S29861	G25915	DGD-1957	*P. lunatus*	Peru	Cajamarca	E	2020
LPECA118	S29861	G25915	DGD-1957	*P. lunatus*	Peru	Cajamarca	E	2020
LPEJU72	S30322	G26547	DGD-2148	*P. lunatus*	Peru	Junin	E	980
LPEJU91	S30322	G26547	DGD-2148	*P. lunatus*	Peru	Junin	E	980
PPEAM104	S27161		DGD-1266	*P. pachyrrizoides*	Peru	Amazonas	E	2110
PPEAP121	S30417		DGD-2260	*P. pachyrrizoides*	Peru	Apurimac	H	2760
PPEAP2	S30328		DGD-2154	*P. pachyrrizoides*	Peru	Apurimac	H	2800
PPEAP3	S30328		DGD-2154	*P. pachyrrizoides*	Peru	Apurimac	H	2800
PPEAP4	S30332		DGD-2158	*P. pachyrrizoides*	Peru	Apurimac	H	2050
PPEAP5	S30333		DGD-2159	*P. pachyrrizoides*	Peru	Apurimac	H	2770
PPEAP6	S30333		DGD-2159	*P. pachyrrizoides*	Peru	Apurimac	H	2770
PPEAP7	S30334		DGD-2160	*P. pachyrrizoides*	Peru	Apurimac	H	2470
PPEAP8	S30336		DGD-2162	*P. pachyrrizoides*	Peru	Apurimac	H	2540
PPEAP9	S30417		DGD-2260	*P. pachyrrizoides*	Peru	Apurimac	H	2760
PPEAP10	S30425		DGD-2293	*P. pachyrrizoides*	Peru	Apurimac	H	2900
PPEAP11	S30426		DGD-2294	*P. pachyrrizoides*	Peru	Apurimac	H	2750
PPEAP12	S30428		DGD-2296	*P. pachyrrizoides*	Peru	Apurimac	I	2440
PPEAP13	S30432		DGD-2306	*P. pachyrrizoides*	Peru	Apurimac	H	1950
PPEAP14	S30434		DGD-2308	*P. pachyrrizoides*	Peru	Apurimac	H	2600
PPEAP15	S30434		DGD-2308	*P. pachyrrizoides*	Peru	Apurimac	H	2600
PPEAP83	S30329		DGD-2155	*P. pachyrrizoides*	Peru	Apurimac	H	2560
PPEAP84	S30329		DGD-2155	*P. pachyrrizoides*	Peru	Apurimac	H	2560
PPEAP108	S30355		DGD-2185	*P. pachyrrizoides*	Peru	Apurimac	H	2830
PPEAP109	S30355		DGD-2185	*P. pachyrrizoides*	Peru	Apurimac	H	2830
PPEAP110	S30355		DGD-2185	*P. pachyrrizoides*	Peru	Apurimac	H	2830
PPECA105	S29884		DGD-1984	*P. pachyrrizoides*	Peru	Cajamarca	I	2210
PPECA106	S29884		DGD-1984	*P. pachyrrizoides*	Peru	Cajamarca	I	2210
PPECA107	S29910		DGD-2011	*P. pachyrrizoides*	Peru	Cajamarca	E	2360
PPECU111	S30440		DGD-2314	*P. pachyrrizoides*	Peru	Cuzco	E	2620
PPEJU1	S30325		DGD-2151	*P. pachyrrizoides*	Peru	Junin	H	2730
PPEJU100	S30318		DGD-2143	*P. pachyrrizoides*	Peru	Junin	H	2800
PPEJU101	S30318		DGD-2143	*P. pachyrrizoides*	Peru	Junin	H	2800
PPEJU102	S30323		DGD-2149	*P. pachyrrizoides*	Peru	Junin	H	2390
PPEJU103	S30324		DGD-2150	*P. pachyrrizoides*	Peru	Junin	H	3080
VCOO		Ica Pijao		*P. vulgaris*	Bred Line		E	
VECAZ127	S31770C	G23580C		*P. vulgaris*	Ecuador	Azuay	E	1930
VPEHU128	S4910	G12856		*P. vulgaris*	Peru	Huanuco	E	
VPECU129	S31313	G23569		*P. vulgaris*	Peru	Cuzco	E	2940
VBLTA130	S31180B	G23445B		*P. vulgaris*	Bolivia	Tarija	E	2100
VAR131	S29356	G21197		*P. vulgaris*	Argentina		E	1850

† Germination habit: E, epigeal; H, hypogeal; I, intermediate.

Total genomic DNA was extracted from young leaves (González et al., 1995). AFLP fingerprinting was carried out as per Tohme et al., 1996. Five hundred nanograms of total DNA were placed in a digestion cocktail (5 U *Eco*RI, 5 U *Mse*I, 1× One-Phor-All buffer, 5 m*M* dithiothreitol (DTT), completed to volume of 50 μL with DNA) and incubated for 1 h at 37°C. Ten microliters of ligation cocktail were added in the same tube (5 pmoles double stranded adapter complementary to the *Eco*RI site, 50 pmoles double stranded adapter complementary to the *Mse*I site, 1.2 m*M* ATP, 1× One-Phor-All buffer, 2.5 m *M* DTT, 1 u T4 DNA ligase), and incubated for 3 h at 37°C.

Primers complementary to adapter sequences, having one additional nucleotide on their 3′ end, were used to carry out a selective pre-amplification of the DNA template. The primer sequence complementary to the *Eco*RI end was: 5′-GACTGCGTACCAATTCA-3′(E + A). The *Mse*I primer sequence was: 5′-GATGAGTCCTGAGTAAG-3′ (M + G). Underlined letters correspond to the first selective nucleotide. Five microliters of digested and ligated DNA were separated and mixed with 25 μL of primer cocktail (75 ng E + A, 75 ng M + G, 200 μ*M* dNTPs) and 20 μL of Taq polymerase cocktail (1 unit Taq polymerase, 1× PCR buffer). Samples were amplified with a PTC-100 programmable thermal controller (MJ Research, Inc, Watertown, MA) with the following PCR profile: 35 cycles of 30 s at 94°C, 30 s at 55°C, and 60 s at 72°C. To verify adequate amplification, 20 μL of the amplification reaction were observed as smears on an ethidium bromide stained agarose gel. The rest of the amplification product was diluted 20 fold in TE buffer. Two primer pairs were used to carry out the final selective amplification of the DNA restriction fragments. The primer complementary to the *Eco*RI end of the DNA template, E + AAC (5′-GACTGCGTACCAATTCAAC-3′), was used in combination with the *Mse*I

primer M + GTA (5′-GATGAGTCCTGAGTAAGTA-3′). Another *Eco*RI primer, E + AGT (5′-GACTGCGTACCAATTCAGT-3′) was used in combination with the *Mse*I primer, M + GAC (5′-GATGAGTCCTGAGTAAGAC-3′). The primer combinations will be referred to as PE1A/PM1A, and PE1C/PM1C respectively. *Eco*RI primers (5 ng) were labeled radioactively with γ-^{32}P (0.2 μL [γ-^{32}P]ATP 3000 Ci/mmol, 1× One-Phor-All buffer, 0.1 U T4PNK, adjusted to a volume of 0.5 μL with water) in a water bath at 37°C for 30 min. The phosphonucleokinase was then inactivated by placing in a water bath at 70°C for 10 min.

The PCR reaction mixture consisted of 5 μL of the diluted PCR +1/+1 product, along with 5 μL of primer mix (5 ng labeled *Eco*RI primer, 30 ng *Mse*I primer, 200 μ*M* dNTPs), and 10 μL of the amplification mixture (0.4 U Taq polymerase, 1× PCR buffer). The PCR reaction was carried out in a PTC-100 with the following profile: 12 cycles of 30 s at 94°C, 30 s at 65°C (−0.7°C/cycle), and 30 s at 72°C, 25 cycles of 30 s at 94°C, 30 s at 56°C, and 60 s at 72°C. Amplification products were mixed with an equal volume of stop solution (Amersham), denatured at 95°C, and 4 μL were loaded into a 6% (w/v) polyacrylamide gel in 1× TBE electrophoresis buffer. Gels were dried out on Whatman 3MM paper (Whatman Lab, Hillsboro, OR) and exposed at least fourteen hours to Kodak, X-OMAT LS 35.3 × 43.2 cm films (Sigma-Aldrich Co., Saint Louis, MO) at room temperature. In each gel, a common bean accession, ICA-Pijao, was included three times to allow comparisons of bands among gels. Data were computed as 1/0, corresponding to presence or absence, respectively, of heavy bands. Minor or slightly marked bands were ignored.

Genetic similarities among all accessions were calculated with the Nei-Li coefficient (Nei and Li, 1979), that is, $S = 2a/(2a + b + c)$, where a = bands shared by both individuals, b = bands presented by individual (1) but not by (2), and c = bands presented by individual (2) but not by (1). Dendrograms were constructed with SAHN clustering of NTSYS-PC, version 1.80 (Rohlf, 1993), by the UPGMA method (unweighted paired grouped mean arithmetic average). Confidence intervals at 95% of the Nei-Li similarity indexes for selected nodes of the dendrogram were calculated by bootstrap analysis, by SAS (SAS Institute, 1989). Multiple correspondence analysis was performed with "CORRESP" of SAS; three dimensions were sufficient to explain most of the observed variation.

Heterogeneity or gene diversity indices for each observed gene pool were calculated according to formulas proposed by Nei (1987). Because alleles belonging to a single locus could not be identified in the AFLP fingerprinting, each band was considered as a character with two possible states (alleles): presence and absence. Heterogeneity was calculated for each band and then averaged out for the total measure. Heterogeneity values measured in this manner result in the overestimation of the number of real loci, whereas the number of alleles per locus is underestimated, and heterozygotes are not detected. Thus, heterogeneity measures have only relative value, and cannot be compared with values obtained by other molecular markers or even in other AFLP studies.

RESULTS AND DISCUSSION

The whole set of accessions was evaluated with two combinations of primers, PE1A/PM1A and PE1C/PM1C, that were selected for the high number of bands and polymorphism found in previous studies of *P. vulgaris* (Tohme et al., 1996). Indeed, a sufficient level of polymorphism was found (Table 2). For each primer, a subset of accessions was re-run through the whole process (DNA extraction, digestion-ligation, amplification, and band scoring). In all cases, the original band pattern was obtained, indicating high reproducibility of data.

Two phenetic dendrograms were calculated by Nei's similarity indexes for each primer combination used (data not shown), and another was calculated with both primer combinations (Fig. 2). Correlation coefficient between similarity matrices for each primer was 0.86, indicating that each primer combination provided similar information about this group of accessions.

Phaseolus vulgaris (Cluster 1) was clearly separated from the other accessions at similarity values ranging from 0.13 to 0.26 (Fig. 2), thus indicating considerable genetic distance from *P. lunatus* (Clusters 2–4) and related species (Clusters 5–10). *Phaseolus vulgaris* is as distant from *P. lunatus* as it is from the group of *P. augusti*, *P. bolivianus*, and *P. pachyrrhizoides* (Table 3). *Phaseolus lunatus* is the next species to separate, at a range of 0.56 to 0.69. Wild Lima beans were separated into three subclusters. (i) Accessions from northern Peru and southern Ecuador (including four accessions from Boyacá, Colombia) (Cluster 4). This subcluster separates from the following two at a range of 0.58 to 0.71. (ii) Accessions from Central America, Colombia (Caribbean departments), and tropical South America (Junín, Peru; and Salta, Argentina) (Cluster 3). (iii) Accessions from the Department of Boyacá, Colombia (Cluster 2). One population, DGD-2887 (AECAZ87, AECAZ88), handled originally as wild *P. augusti*, but later reclassified as *P. lunatus* falls within the Andean group of wild Lima bean, as expected.

The species *P. augusti*, *P. pachyrrhizoides*, and *P. bolivianus* were separated at higher levels of similarity (range 0.65–0.78), and split according to geographic origin (Junín; Cuzco; Ecuador and northern Peru; Argentina and Bolivia; Cuzco and Apurímac), rather than on morphological differences. Means of similarity coefficients between these species varied between 0.70 and 0.81, thus indicating a high level of similarity (Table 3). Such values are comparable with, or lower than, the similarities separating the different geographic groups of wild Lima bean (Table 3). In multiple correspondence analysis (Fig. 3 and 4), once *P. vulgaris*, and *P. lunatus* are excluded, Dimension 1 shows differences between the Ecuador-northern Peru group and the southern Peru-Argentina-Bolivia group. Dimension 2 helps separate accessions from Junín, while Dimension 3 separates accessions from Bolivia-Argentina from those of Cuzco-Apurímac.

Total genetic diversity or heterogeneity was calculated for the *P. lunatus* and the *P. augusti–P. pachyrrhizoides–P. bolivianus* groups (Table 4). Heterogeneity

Table 2. Level of polymorphism found in a group of wild *Phaseolus* accessions and genotypes from South America by means of AFLPs as indicated by primer combination.

Primer combination	Number of bands		
	Analyzed	Polymorphic	Per accession
PE1A/PM1A	106	102	27 to 41
PE1C/PM1C	73	68	12 to 33
Both	179	170	42 to 63

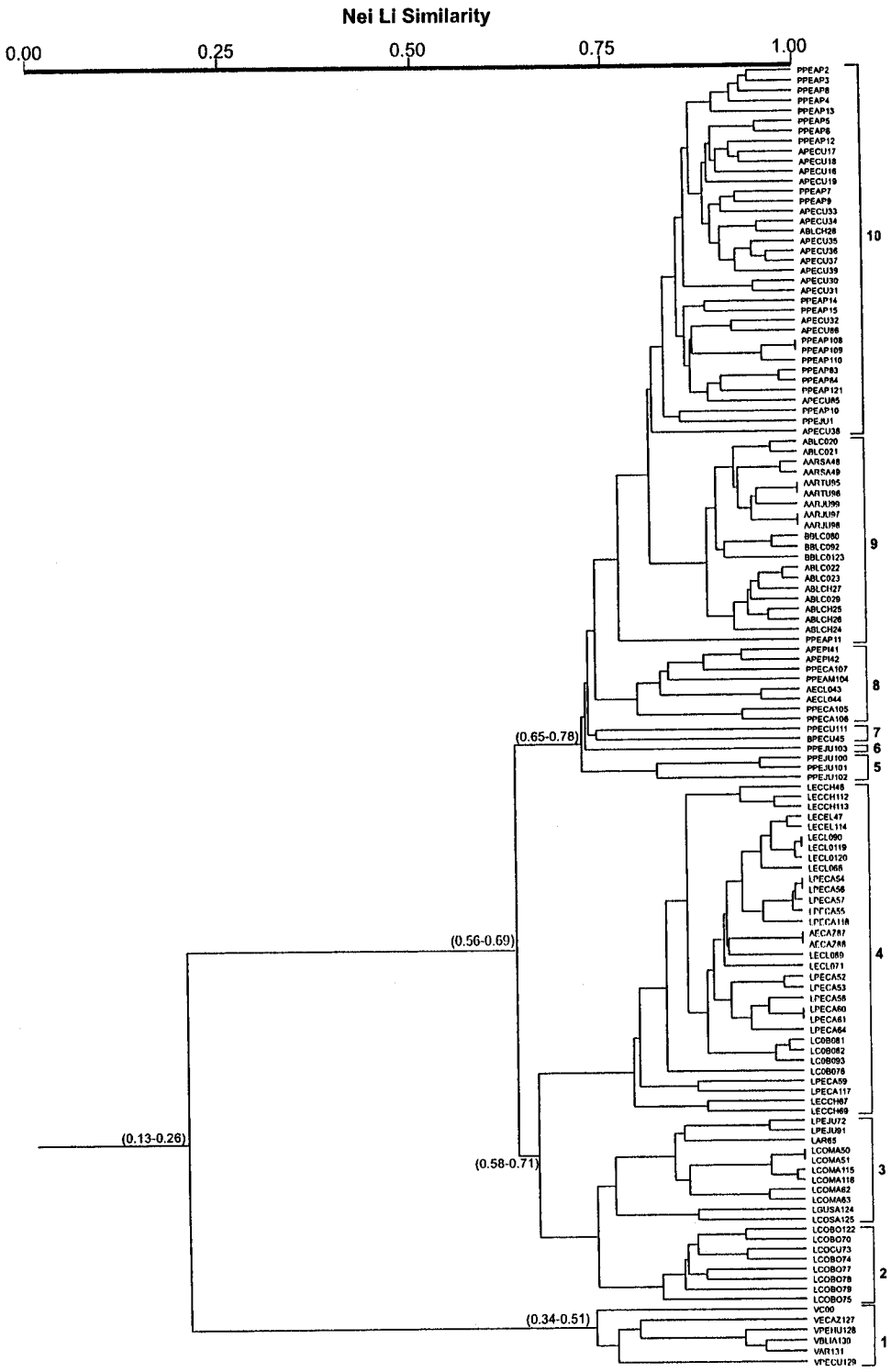

Fig. 2. Dendrogram developed from the UPGMA method of Nei-Li similarity values for both two primer combinations. Confidence intervals, using bootstrap analysis, are reported for key nodes. Figures at right refer to clusters discussed in the text. Cluster 1 refers to *P. vulgaris* accessions; Cluster 2 refers to *P. lunatus* genotypes from Colombia; Cluster 3 to small seeded wild Lima beans; Cluster 4 to slightly larger seeded wild Lima beans from Ecuador, Peru and Colombia; Cluster 5 includes *P. pachyrrhizoides* genotypes from Junin, Peru; Cluster 6 the same; Cluster 7 genotypes from Cuzco, Peru; Cluster 8 includes genotypes of *P. augusti* and *P. pachyrrhizoides* from Ecuador and northern Peru; Cluster 9 genotypes of *P. augusti*, *P. bolivianus* and *P. pachyrrhizoides* from Argentina, Bolivia and southern Peru; and Cluster 10 genotypes of *P. augusti* and *P. pachyrrhizoides* from Apurimac, Cuzco and Junin in Peru.

Table 3. Similarity indexes and genetic distances between gene pools for both primer combinations, above and below the diagonal, respectively. Similarity indexes are mean values for each accession included in the comparisons; distances are calculated as 1 − *S*.

	Gene pools					
	Phaseolus augusti, P. pachyrrhizoides, and *P. bolivianus*			*Phaseolus lunatus*		
	Peru (Junin)	Southern Peru	Bolivia-Argentina	Colombia	Ecuador-Peru	*Phaseolus vulgaris*
Phaseolus augusti, P. pachyrrhizoides,* and *P. bolivianus						
Ecuador-northern Peru	0.695 ± 0.03	0.730 ± 0.03	0.745 ± 0.03	0.61 ± 0.05	0.661 ± 0.05	0.191 ± 0.03
Junin		0.725 ± 0.03	0.715 ± 0.03	0.598 ± 0.03	0.605 ± 0.03	0.194 ± 0.03
Southern Peru			0.807 ± 0.03	0.595 ± 0.03	0.634 ± 0.04	0.194 ± 0.03
Bolivia-Argentina				0.612 ± 0.03	0.658 ± 0.04	0.206 ± 0.02
P. lunatus						
Colombia					0.694 ± 0.09	0.208 ± 0.02
Ecuador-Peru						0.19 ± 0.02
P. vulgaris	0.809	0.807	0.806	0.794	0.810	

was greater in the *P. vulgaris* group (H_t = 0.15) than in either the *P. lunatus* group (H_t = 0.11) or the highly cohesive *P. augusti–P. pachyrrhizoides–P. bolivianus* group (H_t = 0.08). More heterogeneity was observed within the *P. lunatus* group, even though composed of only one species, than within the *P. augusti–P. pachyrrhizoides–P. bolivianus* cluster, which, presumably, is made up of three distinct species. These two groups were taken to be discrete populations, and divided into various subpopulations or gene pools, as defined by the clustering analysis. Heterogeneity (H_{si}) was calculated for each subpopulation (i). These measures were used to determine the amount of heterogeneity within each subpopulation of the whole population (H_s) and the genetic differentiation between subpopulations with respect to the total heterogeneity present in the population (G_{st}) (Table 4). Diversity for the Colombian *P. lunatus* subpopulation was of the same magnitude as total diversity for the whole *P. lunatus* population (H_t). The presence of extensive diversity in the Colombian *P. lunatus* may support the hypothesis that a third gene pool exists in the center of the country. Heterogeneity was found to be greater within gene pools (subpopulations) than between them, suggesting the absence of discrete separation between these gene pools and the possible presence of gene flow between sympatric populations. These results are especially significant in the *P. augusti–P. pachyrrhizoides–P. bolivianus* group, as gene flow between such populations would imply that they are in reality a single species, perhaps with ecological variants.

These results suggest four points for discussion. First, compared with species such as *P. vulgaris* (Cluster 1), *P. lunatus* and its related taxa are relatively distant. Although our analysis includes only a few *Phaseolus* species, these results are consistent with those obtained by Maréchal et al. (1978), who organized this genus into gene pools, with the common bean and the Lima bean at the extremes of an orthogenetic sequence. Our results are also consistent with cpDNA analysis (Schmit et al., 1993), which showed significant distances between *P. lunatus* and the group of species related to *P. vulgaris*. In addition, our results are consistent with experimental hybrid data (Leonard et al., 1987; Mok et al., 1978), which showed severe incompatibility between the two taxa. Despite our having analyzed too few species, *P. au-*

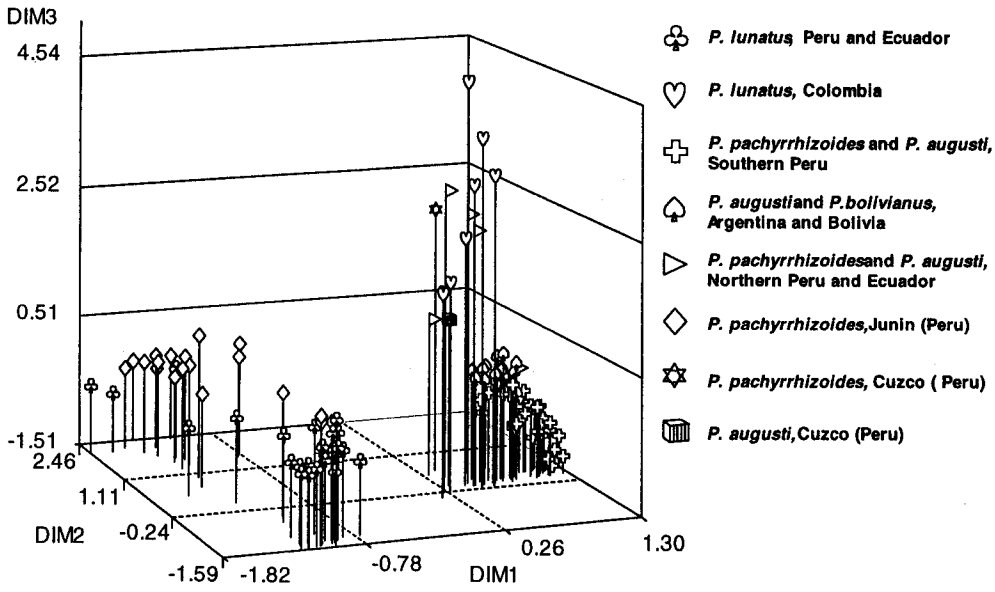

Fig. 3. Multiple correspondence analysis (excluding *P. vulgaris*) showing separation of accessions of *P. lunatus, P. augusti, P. pachyrrhizoides,* and *P. bolivianus* according to geographic origin.

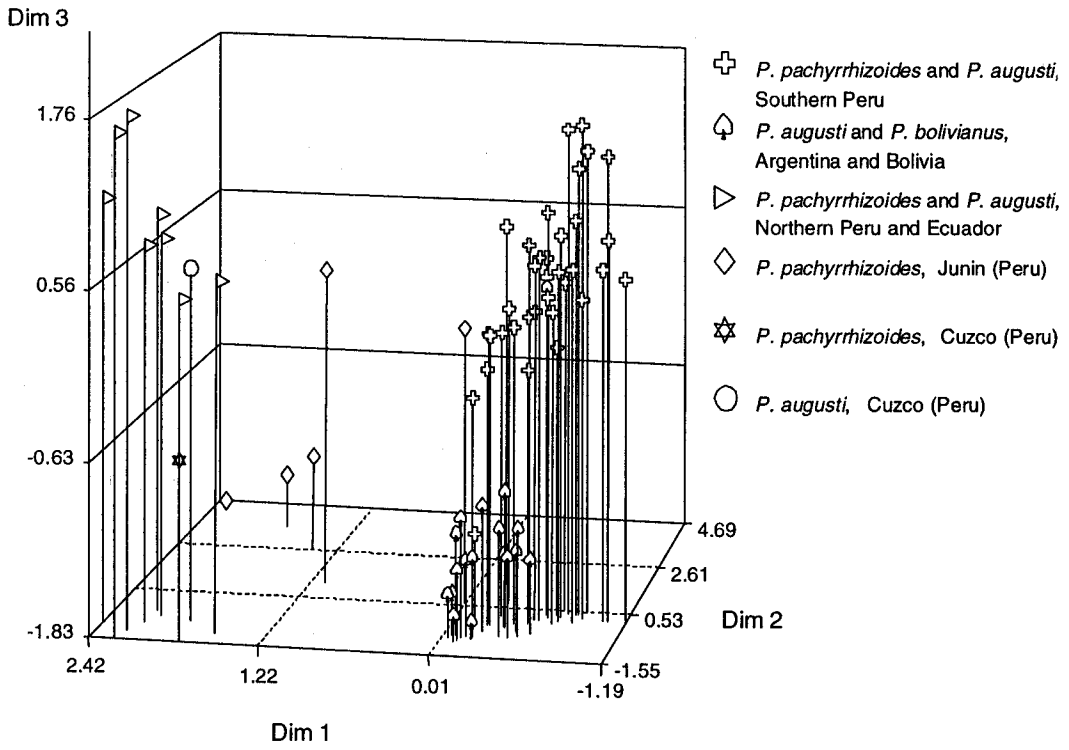

Fig. 4. Multiple correspondence analysis (excluding *P. vulgaris* and *P. lunatus*) showing separation of accessions of *P. augusti*, *P. pachyrrhizoides*, and *P. bolivianus* according to geographic origin.

gusti and *P. pachyrrhizoides* are related to *P. lunatus*, confirming earlier observations (Debouck, 1991).

Second, our results confirmed, with Clusters 2, 3, and 4, the existence of two major gene pools in wild Lima bean—also evidenced by polymorphism in seed storage proteins (Gutiérrez Salgado et al., 1995; Lioi, 1996), allozymes (Maquet et al., 1994), RAPDs of genomic DNA (Nienhuis et al., 1995; Fofana et al., 1997), and RFLPs of rRNA genes (Jacob et al., 1995). The wild form (Cluster 4), with slightly larger seeds and present in the western Andean range of Ecuador and northern Peru, clearly separates (0.58–0.71, $P > 0.05$, Fig. 2) from the form distributed in Central America and the eastern lowland South American tropics. Interestingly, in Cluster 3, the small-seeded wild Lima beans from Central America separate from those of South America, but the sample is too small to make a definitive conclusion. However, some Colombian genotypes from the Andean Departments of Boyacá and Cundinamarca, although close to the lowland neotropical wild Lima bean (Cluster 3), form Cluster 2. Some genotypes found in this region of Colombia, although having an Andean morphotype, had seed storage protein profiles close to that of wild "Mesoamerican" Lima bean (Toro Ch. et al., 1993), and fell within Cluster 4.

This finding raised the question of the origin of Cluster 2. Either it had resulted from crosses between the two gene pools of Lima bean, which are sympatric, or, because it does not fall exactly in between as would a hybrid group, it is related to something else. A comparison can perhaps be made with wild *P. vulgaris* from Ecuador and northern Peru with type "I" phaseolin: initially thought to result from crosses between the two major gene pools (Koenig et al., 1990). These beans were eventually shown to be a separate group with unique diversity (Kami et al., 1995; Khairallah et al., 1992; Tohme et al., 1996). Additional wild material from southwestern Colombia, particularly in the transition zone to Ecuador, is needed to answer this question.

The population of *P. rosei* collected at the type locality (LECCH112/113 in Cluster 4 in Fig. 2) falls within the Andean group of wild Lima beans (Cluster 4) as

Table 4. Values of total heterogeneity (H_t), heterogeneity due to variation within the defined subpopulations at individual (H_{si}) and total level (H_s), and the relative magnitude of gene differentiation among subpopulations (G_{st}) in gene pools defined by the dendrogram obtained with two primer combinations.

Gene pools	Heterogeneity			
	H_t	H_{si}	H_s	G_{st}
Phaseolus augusti/P. pachyrrhizoides/P. bolivianus **(total)**	**0.083**		**0.055**	**0.34**
Ecuador and northern Peru		**0.072**		
Junin (Peru)		**0.096**		
Southern Peru		**0.062**		
Bolivia-Argentina		**0.042**		
Phaseolus lunatus **(total)**	**0.111**		**0.088**	**0.26**
Colombia		**0.113**		
Ecuador and Peru		**0.079**		
Phaseolus vulgaris	**0.151**			

just another population among them. *Phaseolus rosei* would thus be an Andean wild form of Lima bean (and the formal name for it), as claimed elsewhere on the basis of morphological and biochemical evidence (Toro Ch. et al., 1993). This raises the question of whether one should separate, by a formal taxonomic nomenclature, the Andean gene pool of Lima bean from the one distributed in the lowland neotropics, and if so, at what level. For the time being, we favor maintaining the Lima bean as a single biological entity, with the name *P. lunatus*, and with two major gene pools (with a possibility of a third, minor, pool in Colombia), and passing "*Phaseolus rosei*" into synonymy.

A third implication of our results is that *P. augusti*, *P. bolivianus*, and *P. pachyrrhizoides* seem to form a continuum (Clusters 5–10) rather than three different clear-cut entities. Although we could not include the type for *P. augusti*, we found populations that matched the type description, particularly those from the southern range. These populations, the type locality population of *P. bolivianus* (BBLCO123 in Fig. 2), and *P. pachyrrhizoides* (PPEJU102 in Fig. 2) separate at a lower level than do the gene pools of Lima bean (Table 3). Their level of separation is comparable with the separation of gene pools in *P. vulgaris* (Cluster 1). Such a low level of separation makes us question the maintenance of these three taxa as separate species, instead of one polymorphic species. Germination habit is indeed polymorphic. While most populations of Clusters 5 to 10 had hypogeal germination and a tuberous root system, three populations (PPEAM104, PPECA107, and PPECU111) had epigeal germination and fibrous roots. Such varying modes of germination, while uncommon in the genus, has also been reported in *P. leptostachyus* Bentham (Delgado Salinas, 1985). Polymorphism also exists in leaflet shape. While all populations of Clusters 5 to 10 have ovate leaflets with variable pubescence, seven populations (APEPI41, AECLO43, AARJU99, PPEJU103, PPEAM104, PPECA107, and PPECU111) have lanceolate leaflets. One (APECU16) displays lobulate lateral leaflets (as noted previously by Macbride, 1943). In both cases, they did not separate clearly from the bulk of other populations. AFLPs also revealed polymorphism within populations. In five cases (ABLCH24-25, ABLCH26-27, APECU19-85, APECU38-39, and PPEAP9-121), individuals from the same population fell into other Clusters, indicating higher levels of polymorphism within populations than between them. Such levels of high polymorphism within populations, revealed by biochemical (Schmit et al., 1992) and molecular (Llaca et al., 1994; Schmit et al., 1993) markers, have also been found in wild *P. coccineus*, a polymorphic species from Mexico and Guatemala.

In contrast to results from isozyme analysis reported by Maquet and Baudoin (1996), we could not observe a clear-cut separation between the taxa *P. augusti*, *P. pachyrrhizoides*, and *P. bolivianus*. However we did see some groupings according to major geographic regions. These were (i) southern Peru (Apurímac, Cuzco) (Cluster 10), (ii) northwestern Argentina and central-southern Bolivia (Cluster 9), and (iii) northern Peru (Cajamarca, Piura) and southern Ecuador (Loja) (Cluster 8). The populations from Junín, Peru, however, were very different from each other, as was recognized earlier (Debouck, 1987), and did not link easily with other clusters. Clusters 5 and 6 display a relatively high number of polymorphisms over short geographic distances (PPEJU101 and PPEJU102, separated by roughly 15 km). Cluster 7 includes two populations with epigeal germination (BPECU45 and PPECU111). BPECU45 was initially collected as *P. lunatus* and temporarily classified as *P. augusti*. Cluster 8 includes populations from northern Peru and Ecuador. Cluster 9 includes populations from Bolivia and Argentina, and, although it has a long range, from Cochabamba (Bolivia) to Tucumán (Argentina), this cluster is somewhat less variable. Cluster 10 includes mostly populations from the Departments of Apurímac and Cuzco in southern Peru, and is relatively variable. Although we have not seen the type of *P. augusti*, we suspect it falls within the natural morphological variation of the taxa *P. bolivianus* and *P. pachyrrhizoides*. In our view, only one name should be kept and used for this somewhat polymorphic species, distributed from southern Ecuador to northern Argentina. Populations from Junín, and two others from Cuzco (BPECU45 and PPECU111) differ from each other and from the other groups, but not at a level that is high enough to justify a nomenclature treatment.

A fourth implication of our results is that we can make inferences about the phylogeny and evolution of this group of *Phaseolus* beans. According to Lackey (1983) and Polhill et al. (1981), woody stems, associated with tuberous roots and a perennial habit, may be regarded as a primitive character in the Phaseolinae subtribe. To a somewhat constant degree throughout the genus, these traits are also associated with hypogeal germination. Accordingly, the complex *augusti–pachyrrhizoides* with hypogeal germination probably constitute an ancestral stock. Two scenarios can be envisioned for the early formation of this stock. One is that it may have been formed in South America only. However, cpDNA evidence (Bruneau et al., 1995; Delgado Salinas et al., 1993) suggests that the genus does not have a polyphyletic origin, but instead forms a natural group, with a large number of species currently distributed in Central America. This leads us to a second scenario. Central America may have been where most speciation took (and is still taking) place. A group of species including the ancestral branch of Lima bean would have been formed there, giving millenia afterwards species such as *P. maculatus* Scheele and *P. ritensis* Jones (Debouck, 1991). Geographic isolation would explain why this group of Mesoamerican (Mexican) species is the tertiary gene pool, and why genetic distances from the group of species related to *P. vulgaris* are so noticeable. A few mutations in the ancestral stock would have led to the forms with epigeal germination, particularly *P. lunatus*. If this second scenario is correct, then the species *P. lunatus* would have an Andean origin. The tropical small-seeded pool of wild Lima bean would have separated from the northern Andes to diffuse towards Mexico (Sinaloa, Tamaulipas) and Argentina (Salta), where it is known today (Gutiérrez Salgado et al., 1995). The

lower diversity observed in Argentina, compared with that in the northern and central Andes, would be compatible with migration from a nuclear area in northern South America. Some of the above statements need further support from additional analyses of cpDNA and mtDNA, and/or sequencing ITS (internal transcribed spacer) on larger and more geographically diverse samples. These analyses would help towards a better understanding of the *Phaseolus* group's evolution and the Lima bean's phylogenetic affinities within this group, thus aiding future plant breeding programs.

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REFERENCES

Berglund-Brücher, O., and H. Brücher. 1974. Murutungo, eine semidomestizierte wildbohne (*Phaseolus flavescens* Piper) aus den tropischen gebirgen Südamerikas. Angew. Bot. 48:209–220.

Brako, L., and J.L. Zarucchi. 1993. Catalogue of the flowering plants and gymnosperms of Peru. Missouri Botanical Garden, St. Louis.

Bruneau, A., J.J. Doyle, and J.L. Doyle. 1995. Phylogenetic relationships in Phaseoleae: evidence from chloroplast DNA restriction site characters. p. 309–330. *In* M.D. Crisp and J.J. Doyle (ed.) Advances in legume systematics. Part 7 Phylogeny. Royal Botanic Gardens, Kew, England.

Debouck, D.G. 1987. Recolección de germoplasma de *Phaseolus* en el centro y centro-sur del Perú. International Board for Plant Genetic Resources, Rome.

Debouck, D.G. 1991. Systematics and morphology. p. 55–118. *In* A. van Schoonhoven, and O. Voysest (ed.) Common beans, research for crop imporvement. Commonwealth Agricultural Bureaux International, Wallingford, England.

Debouck, D.G., R. Castillo, and J. Tohme. 1989. Observations on little-known *Phaseolus* germplasm of Ecuador. FAO/IBPGR (Int. Board Plant Genet. Resour.) Plant Genet. Resour. Newsl. 80:15–21.

Debouck, D.G., J.H. Liñan Jara, A. Campana Sierra, and J.H. De la Cruz Rojas. 1987. Observations on the domestication of *Phaseolus lunatus* L. FAO/IBPGR (Int. Board Plant Genet. Resour.) Plant Genet. Resour. Newsl. 70:26–32.

Delgado Salinas, A. 1985. Systematics of the genus *Phaseolus* (Leguminosae) in North and Central America. Ph.D. dissertation. University of Texas, Austin, TX.

Delgado Salinas, A., A. Bruneau, and J.J. Doyle. 1993. Chloroplast DNA phylogenetic studies in New World Phaseolinae (Leguminosae: Papilionoideae: Phaseoleae). Syst. Bot. 18:6–17.

Fofana, B., X. Vekemans, P. du Jardin, and J.P. Baudoin. 1997. Genetic diversity in Lima bean (*Phaseolus lunatus* L.) as revealed by RAPD markers. Euphytica 95:157–165.

Foster, R.C. 1958. A catalogue of the ferns and flowering plants of Bolivia. Contr. Gray Herb. Harv. Univ. 184:1–223.

González, D.O., N. Palacios, G. Gallego, and J. Tohme. 1995. Protocolos para marcadores moleculares. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia.

Gutiérrez Salgado, A., P. Gepts, and D.G. Debouck. 1995. Evidence for two gene pools of the Lima bean, *Phaseolus lunatus*, in the Americas. Genet. Resour. Crop Evol. 42:15–28.

Harms, H. 1921. Einige neue *Phaseolus*-Arten. Notizbl. Bot. Gart. Mus. Berlin-Dahlem 7:503–508.

Jacob, M., D. Zink, and W. Nagl. 1995. RFLPs of the rRNA genes in the genus *Phaseolus*. Genet. Resour. Crop Evol. 42:97–106.

Kami, J., V. Becerra Velásquez, D.G. Debouck, and P. Gepts. 1995. Identification of presumed ancestral DNA sequences of phaseolin in *Phaseolus vulgaris*. Proc. Natl. Acad. Sci. (USA) 92:1101–1104.

Khairallah, M.M., B.B. Sears, and M.W. Adams. 1992. Mitochondrial restriction fragment length polymorphisms in wild *Phaseolus vulgaris* L.: insights on the domestication of the common bean. Theor. Appl. Genet. 84:915–922.

Koenig, R.L., S.P. Singh, and P. Gepts. 1990. Novel phaseolin types in wild and cultivated common bean (*Phaseolus vulgaris*, Fabaceae). Econ. Bot. 44:50–60.

Lackey, J.A. 1983. A review of generic concepts in American Phaseolinae (Fabaceae, Faboideae). Iselya 2:21–64.

Leonard, M.F., L.C. Stephens, and W.L. Summers. 1987. Effect of maternal genotype on development of *Phaseolus vulgaris* L. × *P. lunatus* L. interspecific hybrid embryos. Euphytica 36:327–332.

Lewis, G.P. 1987. Legumes of Bahia. Royal Botanic Gardens, Kew, England.

Lioi, L. 1996. Phaseolin diversity in wild Lima bean (*Phaseolus lunatus* L.) in American centres of origin. Genet. Resour. & Crop Evol. 43:575–580.

Llaca, V., A., Delgado Salinas, and P. Gepts. 1994. Chloroplast DNA as an evolutionary marker in the *Phaseolus vulgaris* complex. Theor. Appl. Genet. 88:646–652.

Macbride, J.F. 1943. Flora of Peru—Family Leguminosae. Field Mus. Nat. Hist. Publ. Bot. Ser. 13:1–507.

Maquet, A., and J.P. Baudoin. 1996. New lights on phyletic relations in the genus *Phaseolus*. Annu. Rep. Bean Improv. Coop. (USA) 39:203–204.

Maquet, A., B. Wathelet, and J. Baudoin. 1994. Etude du réservoir génétique de la légumineuse alimentaire *Phaseolus lunatus* L. par l'analyse électrophorétique d'isozymes. Bull. Rech. Agron. Gembloux 29:369–381.

Maréchal, R., J.-M. Mascherpa, and F. Stainier. 1978. Etude taxonomique d'un groupe complexe d'espèces des genres *Phaseolus* et *Vigna* (Papilionaceae) sur la base de données morphologiques et polliniques, traitées par l'analyse informatique. Boissiera 28:1–273.

Mok, D.W.S., M.C. Mok, and A. Rabakoarihanta. 1978. Interspecific hybridization of *Phaseolus vulgaris* with *P. lunatus* and *P. acutifolius* Theor. Appl. Genet. 52:209–215.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

Nei, M., and W. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl Acad. Sci. (USA) 76:5269–5273.

Nienhuis, J., J. Tivang, P. Skroch, and J.B. dos Santos. 1995. Genetic relationships among cultivars and landraces of lima bean (*Phaseolus lunatus* L.) as measured by RAPD markers. J . Am. Soc. Hortic. Sci. 120:300–306.

Palacios, R.A., and A.E. Vilela. 1993. Las especies argentinas de *Phaseolus*, un recurso genético que necesita protección. p. 123–139. *In* A.M. Clausen et al. (ed.) Recursos genéticos hortícolas. Instituto Nacional de Tecnología Agropecuaria, Mar del Plata, Argentina.

Piper, C.V. 1926. Studies in American Phaseolinae. Contr. US Nat. Herb. 22:663–701.

Polhill, R.M., P.H. Raven, and C.H. Stirton. 1981. Evolution and systematics of the Leguminosae. p. 1–26. *In* R.M. Polhill, and P.H. Raven (ed.) Advances in legume systematics – Part 1. Royal Botanic Gardens, Kew, England.

Rohlf, F.J. 1993. NTSYS-PC numerical taxonomy and multivariate analysis system. Version 1.8. Exeter Publ., Setauket, NY.

SAS Institute. 1989. SAS/STAT users guide, 6th ed. SAS Institute, Cary, NC.

Schmit, V., Baudoin, J.P., and Wathelet, B. 1992. Contribution à l'étude des relations phylétiques au sein du complexe *Phaseolus vulgaris* L.–*Phaseolus coccineus* L.–*Phaseolus polyanthus* Greenman. Bull. Rech. Agron. Gembloux 27:199–207.

Schmit, V., and D.G. Debouck. 1991. Observations on the origin of *Phaseolus polyanthus* Greenman. Econ. Bot. 45:345–364.

Schmit, V., P. du Jardin, J.P. Baudoin, and D.G. Debouck. 1993. Use of chloroplast DNA polymorphisms for the phylogenetic study of seven *Phaseolus* taxa, including *P. vulgaris* and *P. coccineus*. Theor. Appl. Genet. 87:506–516.

Tohme, J., D.O. González, S. Beebe, and M.C. Duque. 1996. AFLP analysis of gene pools of a wild bean core collection. Crop Sci. 36:1375–1384.

Toro Ch., O., L. Lareo, and D.G. Debouck. 1993. Observations on a noteworthy wild lima bean, *Phaseolus lunatus* L., from Colombia. Annu. Rep. Bean Improv. Coop. (USA) 36:53–54.

Toro Ch., O., J. Tohme, and D.G. Debouck. 1990. Wild bean (*Phaseolus vulgaris* L.): description and distribution. Centro Internacional de Agricultura Tropical, Cali, Colombia.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23:4407–4414.

Wiggins, I.L., and D.M. Porter. 1971. Flora of the Galapagos Islands. Stanford University Press, Stanford, CA.

Occurrence of Fungal Endophytes in Species of Wild *Triticum*

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ABSTRACT

Seedborne, nonpathogenic, fungal endophytes are commonly found in symbiotic relationships with many members of the cool-season grass subfamily *Pooideae*. The beneficial effects on plants possessing fungal endophytes, and the detrimental effects on consumers of fungal endophyte-infected plants are widely known. The objective of our research was to determine if fungal endophytes exist in indigenous, wild *Triticum* (wheat) species from Turkey. From the *Triticum* species collected, we found two different fungal endophytes. Fungi identified morphologically as members of the genus *Neotyphodium* were found in the diploid *Triticum* species *T. dichasians* (Zhuk.) Bowden and *T. tripsacoides* (Jaub. & Spach) Bowden. The second endophyte, an *Acremonium* species, was found in *T. columnare* (Zhuk.) Morris & Sears, *T. cylindricum* Ces., *T. monococcum* L., *T. neglecta* Morris & Sears, *T. recta* Morris & Sears, *T. triunciale* (L.) Raspail, *T. turgidum* L., and *T. umbellulatum* (Zhuk.) Bowden. No fungal endophytes were found in *T. kotschyi* (Boiss.) Bowden, *T. ovatum* (L.) Raspail, *T. peregrinum* Morris & Sears, *T. speltoides* (Tausch) Gren. ex Richter, and *T. tauschii* (Coss.) Schmal., although the number of samples tested was small for some of these species. Both *Acremonium* endophyte-infected and *Acremonium* endophyte-free plants of *T. triunciale* were found to occur at different frequencies at four collection sites on the Anatolian Plateau. Through two selfed generations of the plants, it was found that the *Neotyphodium* endophyte was transmitted to 100% of the progeny of *T. dichasians* and *T. tripsacoides*. However, the *Acremonium* endophytes were not transmitted in all plants that originally possessed them. We concluded that fungal endophytes of the genera *Neotyphodium* and *Acremonium* inhabit some wild wheat species grown indigenously in Turkey. These endophytes may influence the ecology and distribution of *Triticum* species, and may also serve as a source of biological control agents of pests or abiotic stress factors in wheat.

To economically feed an increasing world population, it is important that food production be increased while the cost of producing the food be decreased. The strategic use of naturally occurring organisms to control pest populations and increase production of major crops represents a viable option to host-plant resistance and pesticide-based pest control. One group of biological control agents that provide a source for novel pest control are the mutualistic fungal symbionts belonging to the genus *Epichloë* (Clay, 1989). These fungi, in association with their host grass plant, produce a range of deterrence to various insects and some plant diseases (Latch, 1993). In addition, improved growth and drought tolerance are characteristic of some plants possessing fungal endophytes (West, 1994). The basis of much of the pest deterrence in grasses possessing endophytes is the production of alkaloids by the endophytes (Siegel et al., 1991). Endophyte-infected grasses have caused toxicity-related problems in livestock, such as cattle, sheep, and horses, that graze on the infected pastures (Hoveland, 1993). Humans ingesting food products derived from endophyte-infected grasses probably would also suffer similar toxicity, because the fungal endophytes involved are relatives of the sclerotial (ergot) forming *Clavicipiteae*, whose toxicity effects are widely known (Groger, 1972). Thus, if fungal endophytes are to be safe and effective biological control agents of food crops, the means must be found to either eliminate any potentially harmful toxic compounds or to select toxic-specific fungal strains.

The systemic, seedborne, nonpathogenic, fungal endophytes of most interest as biological control agents belong to the genus *Neotyphodium* Glenn, Bacon, Price, and Hanlin (formerly *Acremonium* section *Albolanosa* Morgan-Jones and Gams) (Glenn et al., 1996). These fungi are conidial anamorphs of *Epichloë* spp. (Persoon:Fries) Tulasne (Schardl and Phillips, 1997). Another group of fungal endophytes of grasses that have been identified are the p-endophytes, which as a group are closely related to each other, and have been found to sometimes coexist in plants with *Neotyphodium* endophytes (An et al., 1993). However, the biology and ecology of the p-endophytes are relatively unknown. Fungal

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