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Genetic diversity of Argentinean common bean and its evolution during domestication

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Summary

Wild populations of common bean pertaining to the Andean gene pool are distributed from southern Peru to northern Argentina. The objectives of this study were to determine the genetic structure of Andean landraces from northern Argentina, as one of the potential domestication sites of the Andean domesticated gene pool, and to establish a correspondence between Andean primitive landraces and wild populations that might have served as the source of domesticated bean. Forty-four landraces and 21 wild populations representing the diversity of common bean in northern Argentina were included in this study. Results indicated that Andean gene pool in Argentina has a large genetic base on the basis of morphological and adaptive variability and biochemical analysis. The existence of introgressed populations with sympatric wild forms was evidenced.

Introduction

Andean domesticated common bean (*Phaseolus vulgaris* L.) germplasm is remarkably diverse in plant and grain morphology and agroecological adaptation, but it has been proved to have a narrow genetic base on the basis of molecular analysis (Beebe et al., 2001) as compared to Mesoamerican germplasm. The genetic structure of common bean landraces reflects the structure of the wild beans; hence a narrow genetic variability for biochemical analysis in Andean domesticated germplasm was also found in wild germplasm (Koenig & Gepts, 1989; Cattan-Toupance et al., 1998). AFLP analysis revealed that wild bean populations in the Andean zone have probably been more isolated from each other as compared to most Mesoamerican wild beans and have been subjected to less genetic mixing, resulting in rather discrete populations in southern Peru, Bolivia, northern Argentina (Tohme et al., 1996) and also in Colombia although it is not entirely clear whether this area constitutes a centre of domestication or an instance of gene flow between wild and domesticated beans (Beebe et al., 1997).

The precise region of domestication for the Andean gene pool as well as the number of domestications has been controversial although previous studies have indicated that Bolivia is the most likely candidate as the primary domestication site of the Andean gene pool of common bean (Beebe et al., 2001). Northern Argentina has furthermore been considered as an unique segment of the Andean gene pool, isolated from other bean germplasm since weedy types have never been described in this area (Tohme et al., 1996; Beebe et al., 2001). Argentinean domesticated germplasm include mostly bean populations of races Nueva Granada and Peru. However, race Chile and Mesoamerica have also been found (Singh et al., 1991) which could be due to a limited germplasm exchange in pre-Columbian times between Mesoamerica and South-America (Kaplan & Kaplan in Gepts, 1988) although more extensive seed movement occurred after the 1500s.

Common bean, mainly from the southern Andes, was brought to Europe since the first visits of Europeans to the Americas which must have taken the nicely coloured, easily transportable seeds with them as a curiosity. Variation in medium- and large-seeded white bean types was greater among germplasm from Europe (Portugal, Spain, Greece, France, Italy and Bulgaria) than from American collections (Singh, 1989). Spain and Portugal, mainly the northern regions, have been considered as a secondary center of genetic diversity for common bean, specially large white seeded common bean cultivars (Santalla et al., 2002). This germplasm have been disseminated to other parts in Europe, and from there to the Middle East and Western Asia and to other parts in the world (Debouck & Smartt, 1995). As a consequence, in more traditional bean-growing areas of Argentina, medium-large white seeded bean types (e.g. 'Alubia, 'Bolita Cristal'. . .) have been grown by Spanish settlers in the northwestern Argentinean provinces of Salta and Tucumán, only to be exported back to Spain and other European countries.

Wild beans of South America are very small-seeded compared with their corresponding domesticated forms. Common bean has evolved during domestication from small- to large-seeded forms, from extreme indeterminate climbing types to determinate bush types, from seed dormancy and water impermeability of the seed coat to lack of dormancy and water permeable seed coat and from highly fibrous pod wall and shattering forms to lack of fibres and nonshattering types (Smartt, 1988; Gepts & Debouck, 1991). There was a loss of seed coat pigmentation and a reduction of the content in toxic materials, which have probably improved the flavour and caused the loss of seed protection against predators (Smartt, 1988). Domesticated beans differ by at least three traits, photoperiod neutrality, heat tolerance, and tolerance to soil abiotic stresses, as compared to their wild progenitors (Gepts et al., 1999). These marked phenotypic differences, named the domestication syndrome (Hammer, 1984; Koinange et al., 1996), result from selection during several thousands of years for adaptation to cultivated environments. Moreover, wild bean populations are believed to represent more genetic variability than domesticated beans (Gepts et al., 1986; Koenig et al., 1990). This evidence suggests a founder effect in the domestication process (Ladizinsky, 1985), which could have excluded valuable genetic variability of wild beans from domesticated populations (Schoonhoven et al., 1983, Debouck & Tohme, 1989; Kipe-Nolt et al., 1992). In addition, the tendency towards increased selfing upon domestication might have limited gene flow between landraces and neighbouring wild common bean populations.

Wild germplasm remains important for the genetic improvement of domesticated types (Cattan-Toupance et al., 1998; Singh, 2001) since hybrids between wild and domesticated beans are fully fertile and have no major barriers (Singh et al., 1995). Their potential as a source of genetic diversity can be illustrated by the arcelin seed protein, which is responsible for resistance to bruchids (*Z. subfasciatus*). This resistance was absent in thousands of populations of domesticated common bean (Schoonhoven & Cardona, 1982), while only a few wild bean populations from Mexico were highly resistant (Acosta-Gallegos et al., 1998). Studies of Andean germplasm including wild and domesticated germplasm will provide sources of diversity to broaden the genetic base of Andean common bean germplasm which are obviously important due to Andean beans have been more difficult to improve for yield potential (White et al., 1992; Kornegay et al., 1992). These studies will offer a composite picture of the possible evidence of gene flow on genetic relationships and would identify populations that show introgression of wild genes. Since growth and development of wild bean populations are affected by many factors in their natural habitat, the phenotypic plasticity of wild and domesticated beans should be compared under controlled conditions (Garcia et al., 1997). The objectives of this study were (i) to evaluate genetic variation in agronomical traits among wild populations and primitive landraces from northwestern Argentina throughout an environmental range comparable to that of wild populations, (ii) to determine the genetic structure of Argentinean bean landraces and wild populations using phaseolin and allozyme markers, and (iii) to establish a correspondence between Argentinean landraces and wild populations that might have served as source of the Andean domesticated bean.

Materials and methods

Plant material

Forty-four primitive landraces and 21 wild populations that had been collected in the northwestern Argentina (Figure 1) were included in this study (Table 1). The accessions span the natural distribution range of wild common bean in northern Argentina (Salta, Jujuy and

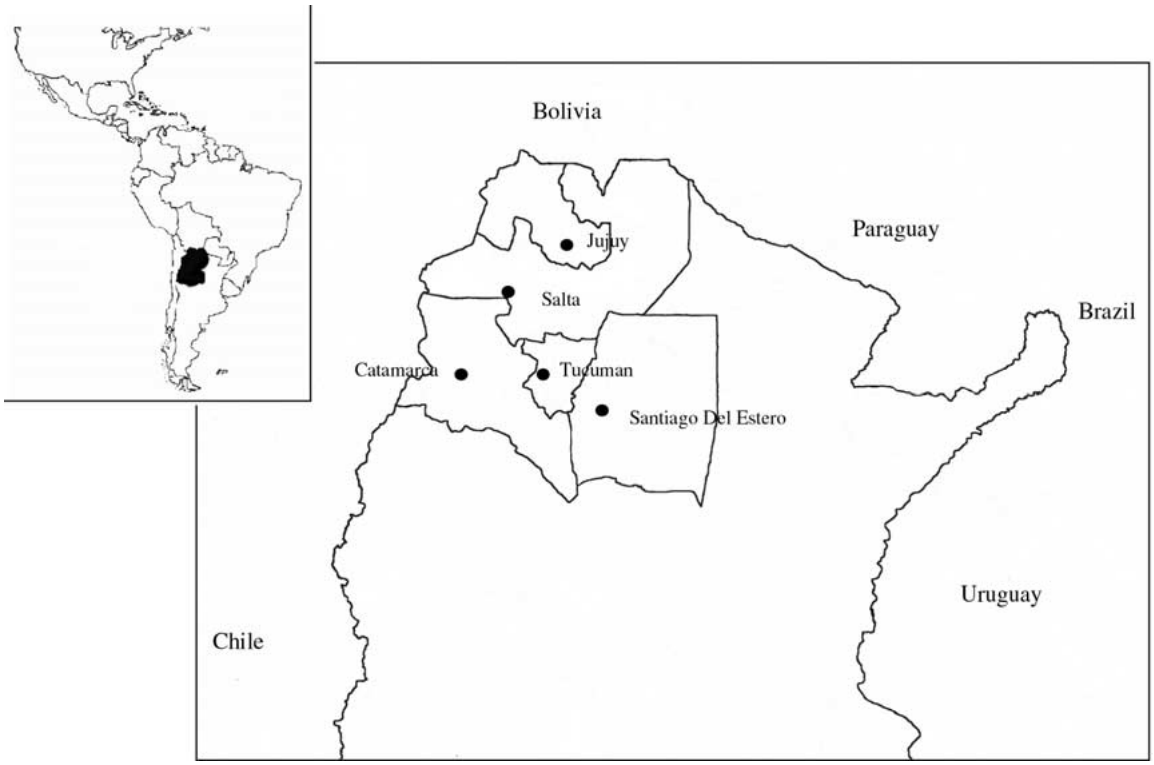

Figure 1. Argentinan regions from which wild populations and primitive landraces of common bean were sampled.

Table 1. Origin and accession number of wild populations and primitive landraces of common bean from northern Argentina

Accession number	Origin
Wild populations	
PHA-0725, PHA-0726, PHA-0727, PHA-0728, PHA-0729, PHA-0730, PHA-0731, PHA-0732, PHA-0733, PHA-0734, PHA-0735, PHA-0739, PHA-0740, PHA-0852, PHA-0856, PHA-0858	Salta
PHA-0736, PHA-0737, PHA-0738	Jujuy
PHA-0741, PHA-0742	Tucumán
Primitive landraces	
PHA-0743, PHA-0744, PHA-0745, PHA-0748, PHA-0749, PHA-0750, PHA-0751, PHA-0752, PHA-0753, PHA-0754, PHA-0755, PHA-0756, PHA-0757, PHA-0758, PHA-0759. PHA-0760, PHA-0761, PHA-0762, PHA-0763, PHA-0764, PHA-0765, PHA-0766, PHA-0767, PHA-0768, PHA-0769, PHA-0770, PHA-0772, PHA-0775, PHA-0777, PHA-0778, PHA-0779, PHA-0780, PHA-0783, PHA-0784, PHA-0785 PHA-0855	Salta
PHA-0746, PHA-0747, PHA-0771, PHA-0773, PHA-0774, PHA-0776, PHA-0781, PHA-0782	Jujuy

Tucumán) that represent the southern limit of the domestication and diversification area. In this area, traditional agriculture based on the cultivation of maize, tubers and beans for food and feed is still practised. Landraces are defined as locally-adapted, domesticated unimproved or primitive genotypes. Four improved cultivars representing Andean dry bean (PHA-0257-canellini and PHA-0838-white kidney) and snap bean (PHA-0135-flatted pod and PHA-0142-rounded pod) market classes were used in the study as controls (Santalla et al., 2001).

Three experiments were conducted in a greenhouse at Misión Biológica de Galicia's research station (42° 24' N, 8° 38' W, 40 m masl elevation). The first experiment was sown on 1995, the second one on 1996, and the third one on 1998. Each experiment consisted of two replications in a randomized complete block design. Plots were grown with adequate irrigation and

natural photoperiod throughout. Mean day and night temperatures were 20 °C and 15 °C, respectively, and during the hotter days windows were opened to reduce the temperature inside the greenhouse. These conditions were chosen to avoid photoperiod sensitivity effects on growth and development. Each plot consisted of a single row, 3.5 m in length, spacing between rows was 0.7 m and within rows was 0.25 m. Seeds from wild populations were scarified mechanically before sowing. Fertilizer was applied at planting and insect and pest controls were implemented as needed.

Data collection

Qualitative traits (determinacy vs. indeterminate; pigmented vs. white flowers and seeds; yellow vs. green pods) were observed in the plots in each experiment. Agronomic data included days to flowering (50% plants had at least one open flower), end of flowering (50% plants had flower abscission) and period of flowering (beginning until 50% plants had flower abscission). The number of seeds per pod, pod weight (weight in grams of 5 fresh pods), pod moisture (%), dry seed weight (determined on 100 dry seeds per plot), pod characteristics (suture string, length, width, thickness and the ratio thickness to width which indicated the shape of the pod according to the values of 1 = rounded pods and 0.5 = flatted pods, were determined on five fresh pods per plot). Seed dimensions (length, width, thickness and the ratios length to width and thickness to width (Puerta Romero, 1961), which are related with the shape of the seed) were measured on 10 random seeds per plot after drying for 72 h at 80 °C. Nutritional seed traits (crude protein, starch and total sugars) were determined on dried material using the Near Infrared Transmittance (NIT) method.

Allozyme and phaseolin data

For each bean population, at least 12 seeds were sown and plant and root tissues collected at the first leaf stage (approximately twenty days after sowing) were analysed. A crude tissue homogenate was produced by grinding the leaf or root apex tissue (depending on the enzymes assayed) in a potassium phosphate grinding buffer 0.1 M pH 7.0 containing 20% sucrose (w/v), 5% PVP-40, 0.5% Triton X-100 and 14 mM 2-mercaptoethanol. The homogenate, absorbed onto paper wicks, was loaded on a 12% starch gel and subjected to electrophoresis in a Lithium borate/tris citrate discontinuous system. Wicks from 24 samples along with one check were inserted into a vertical slice 4 cm from the base of the gel. Electrophoresis was carried out at 25 mA for 20 minutes to load the proteins into the gel. The wicks were then removed and electrophoresis resumed at 30 mA. After the Borate front migrated 8.0 to 9.0 cm, both anodal and cathodal sections of a gel slice 1.5 mm thick were placed in a tray along with the enzyme assayed. The gels were incubated in the dark at 37 °C or at room temperature depending on the enzyme assayed and scored after two hours. Following preliminary assays with 12 enzyme systems to determine the plant tissue with maximum enzyme expression and the polymorphism observed in the bean populations, 6 enzyme systems were assayed: malic enzyme (*Me*; E.C.1.1.1.40), shikimate dehydrogenase (*Skdh*, E.C.1.1.1.25), ribulose bisphosphate carboxylase (*Rbcs*, E.C.4.1.1.39), peroxidase (*Prx*, E.C. 1.11.1.7), malate dehydrogenase (*Mdh*, E.C.1.1.1.37) and diaphorase (*Diap*, E.C.1.6.99). The *Mdh* and *Diap* enzyme systems each had two independent loci. Loci were labelled sequentially with those migrating closest to the anodal end being designated as number 1 (Koenig & Gepts, 1989). The most common allele was designated as 100 and all other alleles were measured in millimetres from the standard. In each gel, the cultivar ICA-Pijao was included as standard. ICA-Pijao has the following genotype at polymorphic enzyme loci: $Rbcs^{100}$, $Skdh^{103}$, Prx^{98}, Me^{100}, $Mdh\text{-}1^{100}$, $Mdh\text{-}2^{100}$, $Diap\text{-}1^{95}$ and $Diap\text{-}2^{105}$.

Five seeds per population were used for polyacrilamide gel electrophoresis analysis of phaseolin-seed storage proteins following the procedures of Brown et al. (1981) and electrophoresed by one-dimensional SDS/PAGE according to the method of Laemmli (1970) as modified by Ma & Bliss (1978). The phaseolin phenotypes of the populations were scored by comparing the patterns to those of reference genotypes (Boyaca 22-'B' type, Sanilac-'S' type, Contender-'C' type, Tendergreen-'T' type and Huevo de Huanchaco-'H' type).

Data analysis

Individual and combined analysis of variance were carried out to determine the variation present in the quantitative traits studied. Analysis of homogeneity of error variance was also conducted. Years and populations were considered as random effects. The sum of squares for populations, populations and year interaction and error were orthogonally divided into components due to the different types of genetic material as primitive landraces, wild populations and

improved cultivars. The least significant difference (LSD) method ($p < 0.05$) was used to compare population means and an harmonic mean was calculated due to the different number of observations within each population group. The analyses were carried out using the SAS package (SAS Institute, 2000). The characters involved in the domestication process (Koinange et al., 1996) were analysed by principal component analysis. In addition, domesticated populations were clustered using the hierarchic agglomerative method by average linkage between populations (UPGMA) of the NTSYS program (Sneath & Sokal, 1973).

Estimation of the allozyme diversity was limited to those populations that showed consistent and scorable polymorphism. Nei's (1973) genetic diversity statistics were used to measure the total genetic diversity (Ht) of the allozyme data as well as the intrapopulation differences (Hs) for each polymorphic locus. Gene diversity due to variation among populations (Dst) was related to the total diversity to determine the proportion residing among populations (Gst). Genotypic frequencies were estimated after grouping bean populations according to their wild and primitive origin. As suggested by Weir (1990) in the case of non-panmictic populations, genotypic frequencies are used rather than allelic frequencies to test for differentiation among groups of populations with contingency tables. In this case, a significance test was performed to evaluate if the genotypic frequencies in the wild and primitive groups were equal. A dendrogram for the primitive landraces, wild populations and improved cultivars based on Nei's (1973) genetic distance was constructed according to the unweighted paired group method or UPGMA (Sneath & Sokal, 1973).

Results

Analyses of variance, mean values and range of variation for agronomic, seed and pod traits are shown in Tables 2, 3, 4 and 5. There were no significant differences in the length of the flowering period between wild populations, primitive landraces and improved cultivars. The pods and dry seeds of wild beans were significatively smaller as compared to primitive landraces and improved cultivars. In terms of seed weight, the largest domesticated beans (primitive landraces and improved cultivars) were approximately 90 g per 100 seeds in weight while the largest wild populations rarely, if ever, exceeded 17 g per 100 seeds in weight (except for PHA-0852 with a seed weight of 26 g/100 seeds) and were commonly much smaller. The variation found in seed shape of primitive landraces was significatively wider that those observed in wild beans. Primitive landraces had often seeds of kidney but also oval and round shapes which vary greatly in colour. Wild populations had flatter seeds than domesticated populations. A significantly greater sugar content was found in domesticated populations (primitive landraces and improved cultivars) than in wild populations. This increase in sugars by domestication was associated with a decrease in the protein and starch level. In addition, primitive landraces and improved cultivars also showed a significantly increased leaf and bracteole size (Table 6). No significant variation was observed in leaf and bracteole shape and lanceolate leaves and bracteoles were found in wild and domesticated germplasm.

A principal component analysis of the quantitative data involved in the domestication process indicated that most studied populations formed three relatively compact groups in two dimensions, wild, domesticated and control groups (Figure 2a). Variation along the first three principal components accounted for 20%, 14% and 11% of the total variation, respectively. Discrimination along the first principal component was accounted by variation in seed, pod and leaflet size and pod moisture and along the second principal component by variation in bracteole size. Two populations (PHA-0750 and PHA-0852) occupied the space between primitive and wild forms. The primitive landrace PHA-0750 displayed a small seed size, while the wild population PHA-0852 had a mottled pattern seed coat, but with a relatively large seed size.

An additional analysis was carried out without wild forms and improved cultivars. The dendrogram created by UPGMA with Euclidean similarity index evidenced several subgroups from the group of Argentinean primitive landraces (Figure 2b). One landrace (PHA-0750) was included in cluster G1 which presented grain and pod types that are relatively close to wild populations with respect to seed and pod size, and range and intensity of grain colours. Landraces in the other groups showed a large seed and pod size and the differences among groups were related to pod and leaflet size. Hence, two landraces were included in cluster G2, which presented the largest pod and leaflet size as compared to the whole of primitive landraces studied considered as primitive based on morphology. Landraces in cluster G3 had smaller pods than those

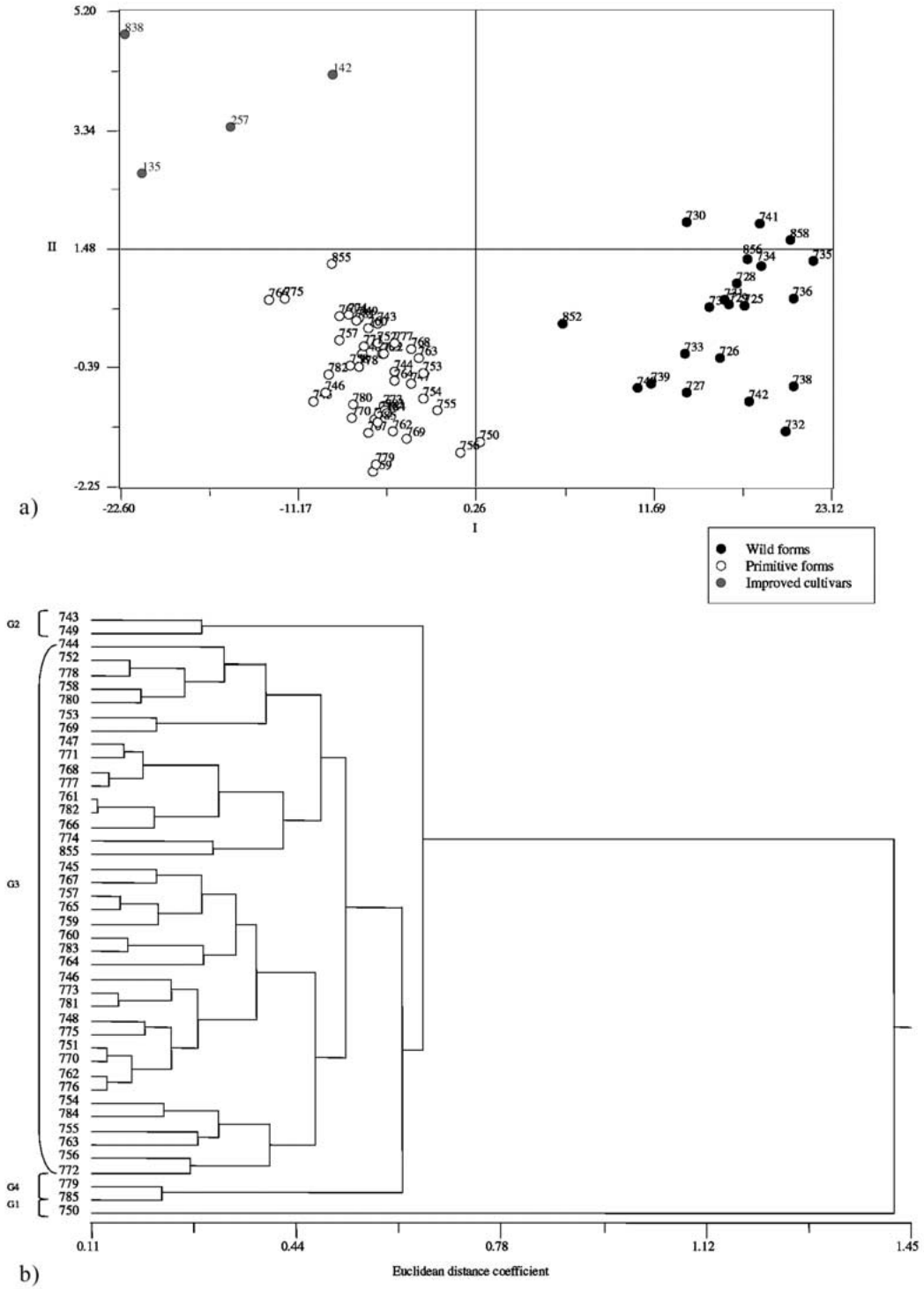

Figure 2. a) Principal component plot derived from principal component analysis of the wild, primitive and improved populations. b) Grouping of primitive landraces by unweighted pair group method with arithmetic mean.

Table 2. Mean squares of the analysis of variance of agronomic traits of wild populations and primitive landraces from northern Argentina and improved cultivars of common bean

Sources of variation	df	Mean squares					
		Days to flowering	End of flowering	Period of flowering	Seeds per pod	Pod weight	Dry seed weight	Pod moisture
		Days				g		%
Year (Y)	2	16637.61 *	22122.02 **	52072.49 **	9.18	8.96	2090.57 **	826.31 *
Replications (Y)	3	1060.30 **	332.10	399.52	1.37	14.84	82.44	31.79
Populations	68	98.91	501.06	390.52	1.14	577.46 **	1547.47 **	48.86 **
Primitive	43	103.39	450.01	371.30	0.91	193.57	253.33 **	9.88 *
Wild	20	95.61	782.30	545.50	0.86	16.84	45.55 **	27.18
Control	3	153.79	203.58	4.75	4.97	888.51	222.60	8.67
Between	2	0.01	0.01	0.01	3.01	13498.62 *	46377.85 **	1133.84 **
Populations*Y	136	104.41	457.46	420.89	0.99 *	173.89 **	71.91 **	5.31
Primitive*Y	86	104.27	406.29	417.30	0.84 **	158.22 **	79.23 **	5.19
Wild*Y	40	111.69	601.20	500.80	0.38	12.68	4.78	3.94
Control*Y	6	67.17	225.75	27.81	4.53	588.61	12.90	1.54
Between*Y	4	77.58	592.84	273.69	2.01	527.13 **	297.56 *	15.43
Error	204	102.70	494.71	357.02	0.56	47.53	32.99	12.19

*, ** null hypothesis rejected at the 0.05 and 0.01 levels respectively.

Table 3. Mean squares of the analysis of variance of seed and pod traits of wild populations and primitive landraces from northern Argentina and improved cultivars of common bean

Sources of variation	df	Mean squares										
		Seed length	Seed width	Seed thickness	Seed length-width	Seed thickness-width	Suture string	Pod length	Pod width	Pod thickness	Protein	Starch	Total sugars
		mm					mm				%		
Year (Y)	2	26.18 **	19.13 **	6.55 **	0.46 **	0.006	2254.2 *	2481.6 *	70.3 **	49.21 **	8.34 *	66.63 **	5.36 **
Replications (Y)	3	1.03	0.28	0.19	0.01	0.001	191.0	194.9	0.4	1.59 *	0.44	0.23	0.02
Populations	68	25.37 **	7.89 **	9.76 **	0.13 **	0.039 **	1576.8 **	1718.9 **	24.3 **	1.60 **	6.34 **	18.86 **	1.31 **
Primitive	43	9.23 **	0.62 **	1.22 **	0.13 **	0.013 **	629.6 **	644.4 **	7.0 **	0.92	2.54	7.55 **	0.36 *
Wild	20	3.51 **	1.19 **	0.54 **	0.01 **	0.004 **	63.0	69.1	1.7 **	0.42	0.97	17.70	0.35
Control	3	9.98	2.22	1.09	0.06	0.004	899.0	2126.8	38.0	3.01	0.73	0.29	0.15
Between	2	614.02 **	239.01 **	288.55 **	1.27 **	0.990 **	38100.7 **	40707.0 **	602.3 **	25.40 *	138.32 **	288.39 *	30.65 **
Populations*Y	136	1.89	0.33	0.34	0.01 **	0.003 **	199.7 **	214.9 **	3.0 **	0.81 **	2.52 **	4.66 **	0.27 **
Primitive*Y	86	0.97	0.30	0.38	0.01 **	0.029 *	127.3 *	132.5	3.10 **	0.67	1.99 *	2.93	0.21
Wild*Y	40	0.71	0.37 *	0.09 *	0.01	0.001	65.5	67.2	0.5	0.47	3.45	13.88	0.95 *
Control*Y	6	2.19	0.36	0.35	0.02	0.006	1822.4 **	1337.9 **	8.7 **	3.01 *	5.34	2.60	0.21
Between*Y	4	3.01	1.30	2.17	0.04	0.010 *	882.7 *	1401.6 *	10.5 *	3.38	7.48	19.20 *	0.41 *
Error	204	1.04	0.37	0.26	0.01	0.002	73.8	78.9	1.6	0.43	0.77	1.66	0.10

*, ** null hypothesis rejected at the 0.05 and 0.01 levels respectively.

in cluster G2, and the last cluster G4 presented smaller pods and leaflet size than the other two groups.

The total genetic diversity (Ht) was 0.248 for the entire array of wild populations and primitive landraces included in this study. There was little within-population diversity (Hs = 0.028) and between population genetic diversity (Dst = 0.220) was moderate. In addition, the coefficient of gene differentiation among populations (Gst) was 0.802. The independence test for the genotype frequencies between primitive landraces and wild populations was significant for most of the loci studied except for *Diap-1* (Table 7). The great majority of wild populations showed the $Me^{98/98}$ and $Rbcs^{100/100}$ genotypes while primitive landraces had the *100/100* and *98/98* genotypes at the *Me* and *Rbcs* loci in a similar frequency. Most Ar-

Table 4. Mean values and range of variation of agronomic traits from wild populations and primitive landraces from northern Argentina and improved cultivars of common bean

Character	Wild	Primitive	Control	LSD[1]	Wild	Primitive	Control
	Mean value				Range of variation		
Days to flowering	62.3	61.4	61.7	n.s.	31.0–112.0	24.0–111.0	37.0–90.0
End of flowering (days)	102	100	101	n.s.	64–174	61–169	75–161
Period of flowering (days)	42.7	40.9	41.4	n.s.	5.0–123.0	3.0–128.0	16.0–91.0
Seeds per pod	3.52	3.80	4.10	n.s.	1.21–5.20	1.00–5.60	2.40–8.80
Pod weight (g)	16.7	41.1	61.7	4.4	8.4–27.2	13.5–68.8	30.2–102.0
Dry seed weight (g)	13.1	53.6	73.9	5.2	8.3–31.9	17.6–90.6	46.0–91.3
Pod moisture (%)	20.0	11.3	10.6	4.2	10.2–36.5	5.1–22.3	6.3–18.4
Pods per plant[2]	11.9	12.6	16.7	n.s.	3.5–27.5	1.6–28.6	1.5–27.0
Seed yield (g/plant)[2]	6	23	54	n.s.	2–15	2–60	18–89

[1] Least significant difference at $p < 0.05$. n.s. = non significant.
[2] Mean values based on one environment.

Table 5. Mean values and range of variation of seed and pod traits from wild populations and primitive landraces from northern Argentina and improved cultivars of common bean

Character	Wild	Primitive	Control	LSD[1]	Wild	Primitive	Control
	Mean value				Range of variation		
Seed length (mm)	8.95	13.13	16.82	0.75	6.83–12.97	9.00–18.41	13.00–20.00
Seed width (mm)	6.18	9.15	8.90	0.47	4.65–8.51	5.80–11.30	6.19–10.20
Seed thickness (mm)	3.24	6.60	6.69	0.38	2.62–5.02	3.55–8.53	5.17–7.51
Seed length/width	1.450	1.443	1.905	0.066	1.150–1.740	0.990–2.070	1.450–2.310
Seed thickness/width	0.526	0.722	0.757	0.033	0.430–0.690	0.530–0.880	0.550–0.840
Pod suture string (mm)	66.9	98.8	130.4	7.6	44.3–88.4	55.0–140.6	58.7–170.0
Pod length (mm)	68.4	99.3	141.7	7.9	45.3–89.8	55.6–140.6	59.3–179.6
Pod width (mm)	9.12	13.73	12.03	0.01	6.33–11.03	7.33–18.60	6.73–19.80
Pod thickness (mm)	4.49	5.24	5.43	0.54	2.80–6.05	2.24–8.31	2.59–8.34
Protein (%)	30.51	27.35	26.95	0.61	26.50–35.20	22.70–31.50	25.30–29.60
Starch (%)	51.1	45.7	44.3	0.9	42.5–61.4	41.0–53.1	42.9–45.6
Total sugars (%)	2.88	4.49	4.55	0.12	1.30–4.50	2.80–6.20	4.00–5.50

[1] Least significant difference at $p < 0.05$.

Table 6. Mean values and range of variation of leaflet and bracteole traits from wild populations and primitive landraces from northern Argentina and improved cultivars of common bean

Character	Wild	Primitive	Control	LSD[1]	Wild	Primitive	Control
	Mean value				Range of variation		
Leaflet length (cm)[2]	11.5	16.0	17.1	1.5	8.0–14.0	12.0–18.0	16.0–19.0
Leaflet width (cm)[2]	7.3	10.6	12.5	1.0	5.1–9.3	8.1–13.6	11.5–13.8
Leaflet length/width[2]	1.58	1.51	1.38	n.s.	1.40–1.85	1.20–1.85	1.26–1.46
Bracteole length (mm)[2]	4.08	4.77	6.38	0.20	2.50–5.10	3.00–6.00	6.00–7.00
Bracteole width (mm)[2]	2.44	2.98	3.92	0.23	1.00–4.00	2.00–4.00	3.00–5.00
Bracteole length/width[2]	1.83	1.87	1.88	n.s.	1.00–2.00	1.00–2.00	1.00–2.00

[1] Least significant difference at $p < 0.05$. n.s. = non significant.
[2] Mean values based on one environment.

Table 7. Distribution of absolute genotypic frequencies in wild populations and primitive landraces of common bean from northern Argentina

Origin	n[1]	*Skdh*		*Me*			*Rbcs*		
		103/103	*100/100*	*102/102*	*100/100*	*98/98*	*102/102*	*100/100*	*98/98*
Wild	21	24	221	0	53	192	38	183	24
Primitive	44	12	474	0	214	272	18	198	270
Test[2]		18.67 **		35.25 **			150.47 **		

Origin	n[1]	*Diap-1*			*Mdh-1*			*Mdh-2*	
		100/100	*100/95*	*95/95*	*103/103*	*100/100*	*98/98*	*102/102*	*100/100*
Wild	21	238	3	4	12	210	23	10	235
Primitive	44	479	3	4	93	382	11	3	483
Test[2]		n.s.[3]			41.78 **			11.19 **	

[1] Total number of accessions analyzed.
[2] Independent chi-square test for all loci.
[3] n.s.=non significant. *, ** null hypothesis rejected at the 0.05 and 0.01 levels respectively.

gentinean populations showed the *100/100* genotype at the *Skdh*, but the genotype *103/103* was observed in a significantly higher frequency in wild populations than primitive landraces. In addition, primitive populations had a significantly higher frequency of the genotype *103/103* at the *Mdh-1* as compared to wild populations.

The cluster analysis based on Nei's (1973) genetic distance (Figure 3) reveals the separation of two major groups of populations within the Argentinean germplasm. The first group consisted of cluster Ga, occupied a position separate from the other major population group, and included wild populations PHA-0852 and PHA-0858 and landrace PHA-0750 from Salta. These populations had a similar plant and seed morphology and shared the Mesoamerican allele 103 at the *Skdh* locus. Cluster Ga is also characterized by 'T', 'C', and 'H' phaseolin types. The second group consisted of two branches sharing the Andean allele 100 at the *Skdh* locus. Most of the populations in the lowest branch (clusters Gb, Gc and Gd) were wild forms (but this branch included the improved snap bean cultivars PHA-0142 and PHA-0135), while the populations in the upper branch (clusters Ge and Gf) were primitive forms, and improved dry bean cultivars. Cluster Gb, comprising two wild populations and one landrace, was characterized by the *Rbcs*$^{102/102}$ genotype. Clusters Gc and Gd were characterized by the genotype*100/100* at the *Rbcs* locus, but cluster Gc presented the *Me*$^{100/100}$ genotype and consisted of two wild populations and eleven landraces while cluster Gd showed the *Me*$^{98/98}$ genotype and included 12 wild populations and seven landraces. Clusters Ge and Gf are characterized by allele *98/98* at the *Rbcs* locus, but cluster Ge showed the *Mdh-1*$^{103/103}$ genotype and cluster Gf was characterized by the *Mdh-1*$^{100/100}$ genotype and three wild populations were included in this last cluster.

Discussion

All the Argentinean wild populations and primitive landraces showed an indeterminate growth habit (type IV), while the improved cultivars had determinate (type I) or indeterminate growth habits (types II and IV). This reflects the fact that modern common bean breeding select dwarf forms. However, the climbing growth habit of the primitive landraces is still favoured by agricultural practices of the Andean natives, who grow beans together with maize or use ripening maize stalks as a natural support for their climbing habit (Brücher, 1988). The lack of significant differences between wild and domesticated populations for flowering traits could be due to the strong susceptibility of these traits to the conditions under which bean materials were evaluated (Voysest & Dessert, 1991). In addition, it has been suggested (Smartt, 1988) that one of the first traits observed by the ancient seed gatherers of wild beans could have been earliness in flowering which, along with the uniformity of the flowering period, could have been used as criteria for cultivar selection.

The heavy load of inmaturing pods and dry seeds in Argentinean domesticated populations was also associated with a stronger vegetative structure than

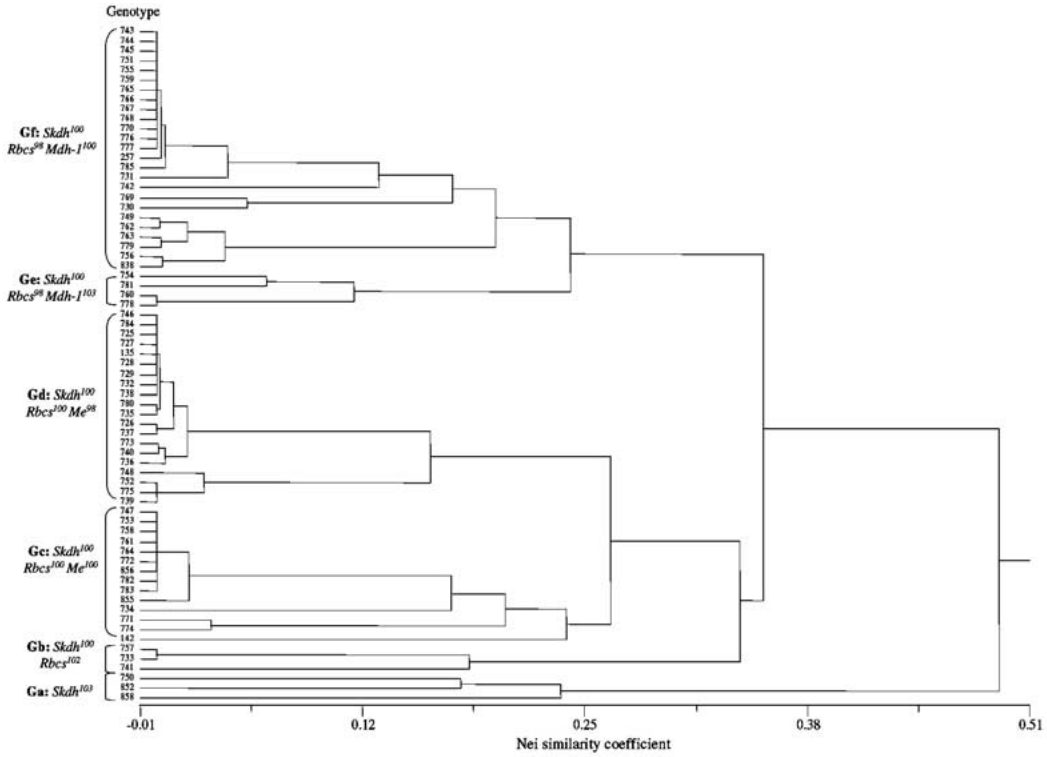

Figure 3. Dendrogram of allozyme diversity in wild populations and primitive landraces from northern Argentina and improved cultivars of common bean. To the left of the dendrogram is the number of the population. Letters a to d indicate clusters of accessions sharing a common allozyme (as indicated to the right of the cluster letter) profile.

wild populations, although some landraces scarcely surpassing that of wild populations. In general in domesticated crops, the parts exploited by humans show gigantism and a less profuse system of branching with a reduced number of leaves although with more robust stems necessary to support large and heavy leaves (Smartt, 1988). These reductions may have come about by response to selections pressure for a more manageable growth habit better suited to cropping systems. The loss in leaf area could be compensated in part at least by increased leaf and flower size as it was found in Argentinean domesticated populations. Populations with large leaves would have a relative advantage in habitats with diffuse light, such as the high-altitude regions of the Andes and Oceanic climate areas of the temperate regions.

Domestication and subsequent evolution in Northern Argentina led to populations with a wide variety of flower and seed colours and colours patterns, some of which are not observed among wild beans such as the lilac and white flower colours. Argentinean wild populations showed pink (38.1%) and purple (61.9%) flowers, and primitive landraces had white (56.8%), pink (19.6%), purple (16.8%) and lilac (6.8%) flowers. Miranda (1967) and Garcia et al. (1997) observed purple and pink flowers in many wild populations from Mexico, while Brücher & Brücher (1976) did not observe white flowers in a large sample of wild beans in northern Argentina. Smartt (1988) observed that the production of pigmented flowers is characteristic of wild forms while reduced pigmentation or its total loss is more common in domesticated forms. Populations with intensely pigmented seed coat also tend to produce pigmented flowers, although the range of colours expressed in seed coats is considerably greater than that in other parts of the plant (Smartt, 1988). Purple and pink wild-type flowers confer black and mineral-brown seed coats (Basset et al., 1995). Hence, the mottled seed coat pattern was frequent in Argentinean wild bean seeds and it was considered (Brücher, 1988) as a phylogenetically ancient feature with a marked selection advantage because mottled seeds are nearly invisible to animals such as birds and rats when they fall onto a debris-covered soil. However, with the harvesting of bean seed under cultivation the adaptive value of mottled seeds was lost and a greater range

of colours became established, although mottled seeds persist but in a modified form as in the cranberry Argentinean primitive landraces.

Common bean is generally considered to be an autogamous species but outcrossing rates as high as 60–70% have been reported (Wells et al., 1988). In addition, it seems that even the lowest rates of outcrossing reported are sufficient to generate broad variability over hundred or thousands of years (Beebe et al., 1997). Some evidences of gene flow between wild and domesticated populations were observed in the Argentinean germplasm studied. The primitive landrace PHA-0750 from Salta displayed characteristics, especially small seed size, indicative of introgresion from wild types. The wild population PHA-0852 is considered by local farmers to be totally wild, having a mottled pattern seed coat, but its relatively large seed size is suggestive of gene flow from domesticated forms. These populations shared the Mesoamerican allele *103* at the *Skdh* locus. Working with a smaller group of wild populations from Argentina, Koenig & Gepts (1989) found that all populations had the allele *100* of *Skdh*, while allele *103* was only observed in populations from Mexico, southern Peru, Colombia and Central America although Debouck et al. (1993) also found the allele *103* of the *Skdh* in wild populations from Ecuador and northern Peru. These populations could be considered as weedy types (weedy 'crop-like' and weedy 'wild-like' types) arose from crosses between primitive landraces and sympatric wild forms as it was evidenced by Beebe et al. (1997) in other Andean zones. The weedy 'crop-like' type (landrace PHA-0750) presented phaseolin 'T', and 'H' types while among populations classified as landraces based on morphology, the great majority were classified as having 'T' type (92%). This weedy type could have resulted from an outcross between a wild bean with a 'H' phaseolin and a domesticated type with a 'T' phaseolin, or conversely. Additionally, the weedy 'wild-like' type (population PHA-0852) showed phaseolin 'T', 'H' and 'C' types while populations considered as wild based on morphology, had 'T' type (82%) followed by 'C' (11%) and 'H' (7%) types. Gepts et al. (1986) also found a predominance of 'T' type phaseolin pattern in Andean wild beans, although they only studied four accessions from Argentina.

The use of allozymes and phaseolin complements the information provided by the study of plant and seed phenotypic diversity. Considerable morphological variation exist with respect to seed type in spite of the uniformity at the allozyme level. Most of the primitive landraces had seeds with spots around the hilum and a kidney shape, typical of cultivars of race Nueva Granada, although some populations had round and oval seeds similar to seeds of cultivars of race Peru and Chile (Singh et al., 1991). The existence of the allele *100* at the*Mdh-1* combined with the 'T' phaseolin type, that was the predominant allozyme and phaseolin pattern found in the domesticated populations studied, are characteristics of race Nueva Granada (Singh et al., 1991). However, 19% of the primitive populations showed the *Mdh-1*^{103} allele and morphologically they belonged to the 'ñuña' and 'bolon' types from the race Peru (Singh et al., 1991). The *Mdh-1*^{103} allele was furthermore observed at a very low frequency in wild germplasm which could indicate germplasm introgression from other Andean zones. The total genetic diversity for the entire array of wild populations and primitive landraces was higher than that found in wild bean (Koenig & Gepts, 1989) but it was lower than in domesticated common bean populations from Spain (Santalla et al., 2002). There was little within-population diversity and between population genetic diversity was moderate. These results suggest that introgression appears to have modestly broadened the genetic base of Argentinean populations. However, the magnitude of gene flow appear to be insufficiently low to maintain population differentiation. In addition, geographical isolation among some of these populations could also be one major cause of genetic differentiation between Argentinean populations in common bean.

These results help to understand bean variability in northern Argentina, with respect to both domestication and gene introgression. Gigantism was observed in Argentinean domesticated populations. Argentinean primitive beans present grain, pod and leaf types that are relatively distant from wild beans with respect to size and range and intensity of grain colours. This suggests that domestication pressures were originally more diverse and not oriented to a single use, which affected mainly harvested parts and traits (seeds, pods and leaves) and concomitantly seed nutritional quality. In addition, the fact that most of the domesticated bean populations belonged to a same group and were not therefore interspersed among the wild or weedy populations suggested that gene flow between wild and domesticated beans appears to have been limited and has not appreciably modified the organisation of the domesticated gene pool in northern Argentina. The exception concerns the clustering of wild or weedy and domesticated populations from Salta (PHA-0750,

PHA-0852 and PHA-0858), which suggested some level of outcrossing on a local level.

The Andean gene pool in Argentina has a large genetic base on the basis of morphological and adaptative variability. Farmers of the Andean zone may have been avid plant selectors to create such variability. Argentinean landraces of common bean present also several phaseolin types and allozyme variation. This large genetic base could suggest that domestication of Argentinean beans occurred within a diverse genetic wild structure. The existence of introgressed accessions is furthermore surprising and is in contrast with previous earlier results (Tohme et al., 1996; Beebe et al., 2001), which is probably due to differences in the samples of wild accessions studied. The present study employed a larger sample of accessions from northern Argentina than in other studies of Argentinean wild germplasm. These introgressed types displayed an allozyme profile similar to that of wild beans from Ecuador and northern Peru, with the presence of Mesoamerican genes. Wild populations from that region of the Andes have been considered as a third gene pool, which was not involved in the domestication of the Andean cultivated gene pool (Debouck et al., 1993). A model was proposed by Kami et al. (1995) suggesting that an evolutionary lineage derived from this ancestral material in Ecuador and northern Peru produced Mesoamerican wild beans and another lineage resulted in Andean wild beans. This evidence could explain the allozyme profiles found in some wild and primitive populations from northern Argentina. Previous studies (Beebe et al., 2001) identified Bolivia as the primary domestication site for the Andean cultivated gene pool. Wild bean accessions from Bolivia clustered with wild germplasm from southern Peru and Argentina (Tohme et al., 1996; Beebe et al., 2001) and they were close to wild germplasm from Ecuador and northern Peru (Freyre et al., 1996). In addition, common bean is no longer as important in Bolivia as it is in Argentina. Hence, it is not clear at this stage if a single domestication or multiple domestications could have taken place, followed by divergence in the Andean domesticated gene pool.

These findings on the large genetic base of Argentinean landraces are significant for genetic improvement of Andean beans. Previous studies (White et al., 1992) reveal that Andean cultivars have been more difficult to improve. Hence, it is important to broaden the genetic base of the Andean cultivars. The introgression of additional genetic diversity into the Andean domesticated gene pool may acquires added importance in light of genetic bottlenecks induced by domestication in common bean (Sonnante et al., 1994). One option could be populations that already display introgression with wild beans such as the weedy types observed in this study. These forms could serve to transfer genetic diversity from wild to domesticated gene pools which may be difficult in modern breeding programs due to linkage with undesirable dominant non-domesticated traits in wild beans (Gepts & Debouck, 1991). The lack of adaptation to temperate zones of wild cultivars could also be overcome using this germplasm.

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