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Molecular characterisation of wild populations and landraces of common bean from northwestern Argentina

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Abstract
Information on the variability of wild bean populations and landraces is essential to set conservation strategies and design breeding programmes aimed at enlarging the genetic base of commercial beans. Nineteen Argentinean common bean landraces and wild populations were characterised and their diversity was analysed by means of inter-simple sequence repeat (ISSR) markers and seed proteins. Populations were successfully identified as belonging to the Andean gene pool of origin by phaseolin electrophoresis, whereas ISSR markers revealed high levels of inter- and intra-population variability. Four of 10 primers produced polymorphic and reproducible DNA profiles, which were used to generate UPGMA (unweighted pair group method with arithmetical averages) and NJ (neighbour-joining) trees. ISSR markers revealed a high level of variability both within wild bean populations and landraces. Genetic variability of wild samples was associated with their geographic distribution. By contrast, landraces were clustered, at least to some degree, based on their seed colour and shape, showing no clear discrimination among sites. The results presented here suggest that, to a certain extent, hybridisation between wild beans and landraces occurs in the wild, a hypothesis that needs to be tested through further analyses.

Keywords: *Genetic variability, introgression, ISSR, phaseolin,* Phaseolus vulgaris

Introduction

Phaseolus vulgaris L., common bean, is one of the most important crops worldwide, both due to its role as a major source of protein for humans and its ability to adapt to different environments. The primary gene pool of *P. vulgaris* includes cultivars and wild populations, the latter being the immediate ancestors of common bean cultivars (Burkart & Brücher 1953). Although wild populations are distributed throughout the Americas from Mexico (Chihuahua) to Argentina (Córdoba) (Gepts et al. 1986; Koenig et al. 1990; Toro et al. 1990; Menéndez Sevillano 2002), the nucleus of diversity is in Ecuador and northern Peru (Kami et al. 1995; Tohme et al. 1996), which was probably the area from where wild beans dispersed both northwards and southwards. From these two places, prior to domestication, two major gene pools arose, one in Mesoamerica and the other in the Andes of South America (Gepts 1998).

Bolivia and northwestern Argentina represent the southernmost limit of the Andean-domesticated gene pool (Gepts et al. 1986; Beebe et al. 2001; Islam et al. 2002). In isolated valleys of this area, farmers still grow bean landraces for home consumption. De Ron et al. (2004) described the phenotypic variation of a group of landraces collected in Argentina and suggested that the high diversity observed in some areas could be due to their proximity to wild bean populations. Furthermore, they described the existence of some weedy types in this region (De Ron et al. 2004). During the last decade, considerable changes have been made in bean production systems. Many traditional landraces have been replaced by more profitable crops or by genetically improved varieties, thereby

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threatening the *in situ* conservation of the common bean germplasm (Debouck & Tohme 1988). Landraces evolved through long periods of cultivation are now in danger of extinction due to the destruction of their original habitats. For this reason, the collection and analysis of bean landraces and their neighbouring wild populations is important to obtain a better understanding of landrace genetic variability. Traditional landraces represent reservoirs of genetic variation of relevance to improving the tolerance of the species to pathogens, diseases and/or environmental stresses.

Molecular markers such as restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) have been used to analyse the variability of cultivated and wild common bean populations not only to estimate bean diversity, but also to cluster accessions based on their gene pool of origin (Khairallah et al. 1992; Tohme et al. 1996; Galván et al. 2001, 2006; Maciel et al. 2001). The inter-simple sequence repeat (ISSR) is a PCR technique based on the amplification of DNA sequences between two inverted microsatellites (Zietkiewicz et al. 1994). This technique is superior to RAPD for the analysis of population diversity because of its higher reproducibility (Zietkiewicz et al. 1994; Kojima et al. 1998; Bornet & Branchard 2001) and its greater information content, even among closely related bean populations (Galván et al. 2003; González et al. 2005; Zizumbo-Villarreal et al. 2005; Marotti et al. 2007). Our purpose was to characterise the genetic variability and relationships of wild populations and landraces of *P. vulgaris* from northwestern Argentina by means of electrophoretic phaseolin profiles and ISSR markers.

Materials and methods

Plant material

Nine wild populations and 10 landraces of common bean were collected in the provinces of Jujuy, Salta and Tucumán (northwestern Argentina), between 22° and 28° S latitude, 64°–66° W longitude and from 1300 to 2600 m a.s.l. The accessions were stored in the Germplasm Bank of the "Laboratorio de Recursos Fitogenéticos N. I. Vavilov" of the Facultad de Agronomía, Universidad de Buenos Aires. Accession numbers and geographical information are provided in Table I and Figure 1. Aspects of seed morphology are listed in Table II.

Genotyping

The phaseolin profiles of five seeds per population were analysed by SDS polyacrylamide gel electrophoresis, following Brown et al. (1981), and were compared with those of reference genotypes (Gepts

Table I. Wild populations and landraces of common bean included in this study. Accession numbers and geographical information of the collection sites.

	ID number[b]	Province	Department	Altitude (m a.s.l.)	Latitude S	Longitude W
Wild[a]						
S1	VAV-6364	Salta	Santa Victoria	2300	22°15′	64°58′
S2	VAV-6365	Salta	Santa Victoria	2600	22°15′	64°58′
J1	VAV-6362	Jujuy	Tumbaya	1900	23°50′	65°28′
J2	VAV-6363	Jujuy	Tiraxi	1600	24°02′	65°23′
J3	VAV-6366	Jujuy	S. S. de Jujuy	1500	24°11′	65°18′
T1	VAV-6368	Tucumán	Trancas	1300	26°13′	65°27′
T2	VAV-6369	Tucumán	Trancas	1500	26°13′	65°27′
T3	VAV-6370	Tucumán	Trancas	1500	26°13′	65°27′
T4	MCM-146	Tucumán	Chicligasta	1600	27°28′	65°55′
Landraces						
s1	VAV-5867	Salta	Santa Victoria	2500	22°15′	64°58′
s2	MCM-18	Salta	Santa Victoria	2400	22°15′	64°58′
s3	MCM-79-A	Salta	Santa Victoria	2400	22°15′	64°58′
i1	VAV-3716-G	Salta	Iruya	2900	22°50′	65°14′
i2	VAV-3716-T	Salta	Iruya	2900	22°50′	65°14′
i3	VAV-3716-U	Salta	Iruya	2900	22°50′	65°14′
i4	MCM-69-A	Salta	Iruya	2900	22°50′	65°14′
i5	MCM-109	Salta	Iruya	2900	22°50′	65°14′
i6	MCM-110	Salta	Iruya	2900	22°50′	65°14′
j1	VAV-5674-D	Jujuy	Tilcara	2400	23°34′	65°22′

[a]Nomenclature used for the accessions: Capital letters were used for wild beans and lower case letters for cultivated beans. S: Salta; J: Jujuy; T: Tucumán; s: Santa Victoria; i: Iruya; j: Tilcara. [b]VAV: Plant Genetic Resources Laboratory "N.I.Vavilov", University of Buenos Aires; MCM: name given to accessions collected by Maria del Carmen Menéndez.

Table II. Colour, pattern and seed shape of the landraces analysed.

Landraces	ID number	Shape	Seed colour/pattern
s1	VAV-5867	Oblong	White
s2	MCM-18	Oblong	Brown, purple/mottled
s3	MCM-79-A	Elliptic	White
i1	VAV-3716-G	Oblong	Brown, purple/mottled
i2	VAV-3716-T	Oblong	Brown, purple/striped
i3	VAV-3716-U	Oblong	Brown, purple, black/striped
i4	MCM-69-A	Oblong	Black
i5	MCM-109	Elliptic	Brown
i6	MCM-110	Round	White, purple/mottled
j1	VAV-5674-D	Oblong	White, brown, red/tri-colour

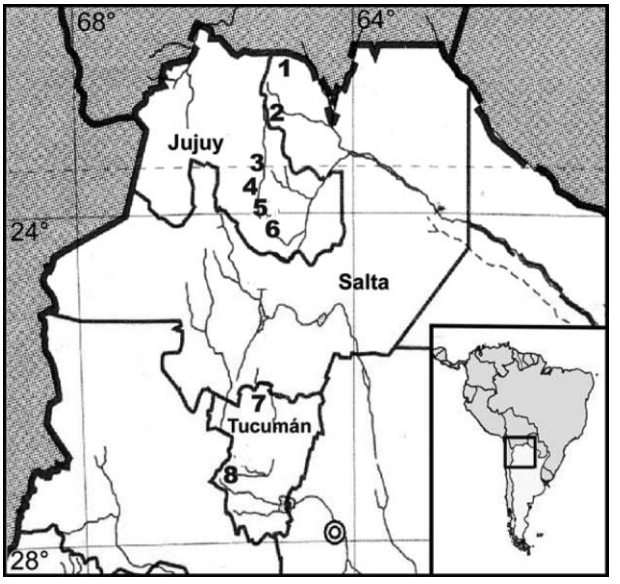

Figure 1. Geographic distribution of the sites of collection of the wild populations and landraces from northwestern Argentina. Numbers indicate the site of collection reported in Table I. Salta province: (1) Santa Victoria, (2) Iruya; Jujuy province: (3) Tilcara, (4) Tumbaya, (5) Tiraxi, (6) San Salvador de Jujuy; Tucumán province: (7) Trancas, (8) Chicligasta.

et al. 1986; Koenig et al. 1990). For ISSR analysis, genomic DNA was extracted from 200 mg of fresh leaf tissue by a CTAB protocol (Bornet & Branchard 2001) and was amplified using five anchored and five non-anchored ISSR primers in 25-µl reactions containing 12 ng template DNA, 100 pM primer, 200 µM dNTP and 1.25 U *Taq* DNA polymerase in reaction buffer (2 mM Tris–HCl, pH 8.0; 10 mM KCl; 0.01 mM EDTA; 0.1 mM DTT). The amplification programme began with an incubation at 94°C for 1 min, followed by 30 cycles at 94°C for 1 min, annealing for 1 min (Table III) and 72°C for 4 min, ending with an incubation at 72°C for 7 min. The products of the reaction were electrophoresed through 2% agarose gels containing 0.2 µg/µl ethidium bromide. Each reaction was repeated at least twice and three plants were considered as replicates of the accessions tested.

Table III. Primer sequences, annealing temperatures and amplified bands of the primers used to generate ISSR markers that characterise landraces and wild populations of common bean from northwestern Argentina.

Name	Sequence (5′–3′)	Annealing temp (°C)	Total number of amplified bands	Number of polymorphic bands
A	$(AC)_8$	57	8	2
C	$(AG)_8CG$	54	14	12
D	$(AG)_8$	49	12	3
G	$GAG(CAA)_5$	52	10	7

Data analysis

DNA fragment size was estimated by comparison with a 1-kbp DNA ladder (Promega Biotech Corp., Madison, WI). The bands were recorded as present (1) or absent (0) and were assembled in a data matrix. Pairwise comparisons were calculated using Jaccard's similarity coefficient (Sneath & Sokal 1973). A dendrogram was generated from a similarity matrix using the unweighted pair group method with arithmetical averages (UPGMA) (Sneath & Sokal 1973) using NTSYSpc version 2.0 (Rohlf 1998). The cophenetic correlation coefficient (CCC) was calculated as suggested by Sneath and Sokal (1973). The similarity matrices were also used to obtain a neighbour-joining (NJ) tree (Felsenstein 1988) using PHYLIP version 3.6 (Felsenstein 2005). The robustness of the groups was evaluated by bootstrapping (Felsenstein 1985), with 1000 replications.

An analysis of the molecular variance (AMOVA; Excoffier et al. 1992) was applied to measure the genetic structure based on the correlation between haplotypes. Permutation tests were used to estimate the significance of the variance components. Correlation between collection altitude, location and genetic distance was estimated with Mantel's (1967) test.

Results

The gene pool of origin of wild beans and landraces were successfully identified via their phaseolin profiles. Types "T" and "J", previously described as typical for the Andean gene pool (Gepts et al. 1986), were identified among the materials analysed. While type "T" was observed in 86% of the entries, "J" was observed in only 13%, in agreement with analysis of other wild populations from the same region (Cattan-Toupance et al. 1998). Four ISSR primers generated 44 distinct fragments, of which 24 were variable (Table III). The fragments ranged in size from 390 to 2300 bp (Figure 2).

The AMOVA based on the ISSR data showed that the wild beans significantly differed from the landraces, with 93% of the variation remaining

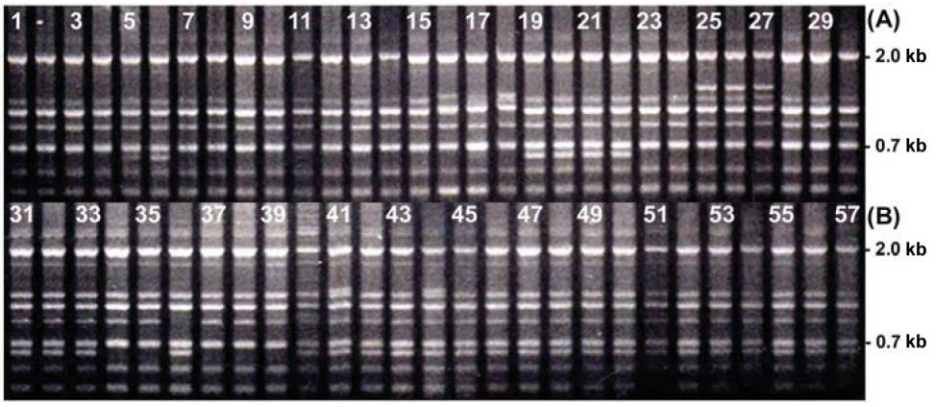

Figure 2. Representative reaction of ISSR molecular markers of landraces and wild bean populations from northwestern Argentina. The amplification reaction was performed with primer G. (A) Landraces populations: 1–3: i1; 4–6: i2; 7–9: i3; 10–12: j1; 13–15: s1; 16–18: s2; 19–21: i4; 22–24: i5; 25–27: i6; 28–30: j1. (B) Wild populations: 31–33: J1; 34–36: S1; 37–39: S2; 40–42: J2; 43–45: J3; 46–48: T1; 49–51: T2; 52–54: T3; 55–57: T4.

Table IV. Results of the AMOVA for ISSR phenotypes of wild populations and landraces of common bean from northwestern Argentina considering four sites (Santa Victoria, Iruya, Jujuy and Tucumán).

Source of variation	% of total variance	*p*-value*	φ-statistics
Among sites	21.85%	<0.001	φst = 0.2185
Within sites	78.15%		

**p*-value: probability of obtaining a larger variance component by chance under the null hypothesis that the variance component is zero (estimated from 1000 permutations).

within groups (φst = 0.0186; $p < 0.001$). There was also a significant effect of altitude and a high level of variability within each site (78% of the total variation; Table IV). An intra-population analysis could not be generated due to the small number of individuals in some of the populations.

In both the UPGMA and the NJ trees, the wild bean populations and landraces grouped into two distinct clusters, with Cluster 1 containing most of the landraces and Cluster 2 containing most of the wild materials (Figures 3 and 4). UPGMA Cluster 1 included most of the landraces from Salta (Iruya and Santa Victoria) and Jujuy (Tilcara), which were collected from sites between 2400 and 2900 m a.s.l. and from 22° to 24° S latitude. Subcluster 1a contained some wild materials (S1c, T1a, T1b and J3b). Cluster 2 contained exclusively wild bean samples, from sites between 1300 and 1600 m a.s.l. and 23°–28° S latitude. The collection sites of materials within Subclusters 2a and 2b were Jujuy and Tucumán, respectively. The latter also included two individuals from Jujuy (J3a and J3c) associated at low similarity levels. Clusters 3 and 4 of the UPGMA were distantly associated with Clusters 1 and 2 (similarity levels < 0.63) and included landraces (Cluster 3) and wild beans (Cluster 4) from Santa Victoria (2300–2600 m a.s.l. and 22°15′ S latitude). The fit of the UPGMA to the similarity matrix was significant (CCC = 0.77). The UPGMA grouping was confirmed by the NJ tree (Figure 4). Wild beans from Jujuy and Tucumán clustered together, except for T1c, T1a, J3b and J1a, which clustered with landraces i2c and i2b from Iruya. T1b was associated with i4 and S1c, but the latter seemed to be genetically more distant.

The correlation between the genetic variation measured by the ISSR distance matrix and the geographic distance between sites, considering all the accessions analysed, was significant but small ($r = 0.195$, $p < 0.01$). This was more noticeable with wild bean genotypes, where the pattern of genetic variability was associated with their geographic distribution. A significant correlation was found between the similarity matrix, the differences in altitudes of sample sites ($r = 0.59$, $p < 0.001$) and the geographic distances between sites ($r = 0.50$, $p < 0.001$).

Discussion

The morphological and biochemical analysis of landraces and wild beans from the Andean gene pool in Argentina revealed a large genetic base (Menéndez Sevillano 2002; De Ron et al. 2004; Santalla et al. 2004). Galván et al. (2006) confirmed these findings within wild beans by means of RAPD markers. The diversity within the landrace clusters was as high as that within wild clusters, as has been previously described in Mexican bean populations (González et al. 2005). This high genetic variability may result from a combination of efficient selection by the farmers and seed exchange and/or hybridisation with sympatric wild populations. The ISSR profiles analysis clustered wild materials according to their collection site. Wild beans from Jujuy and

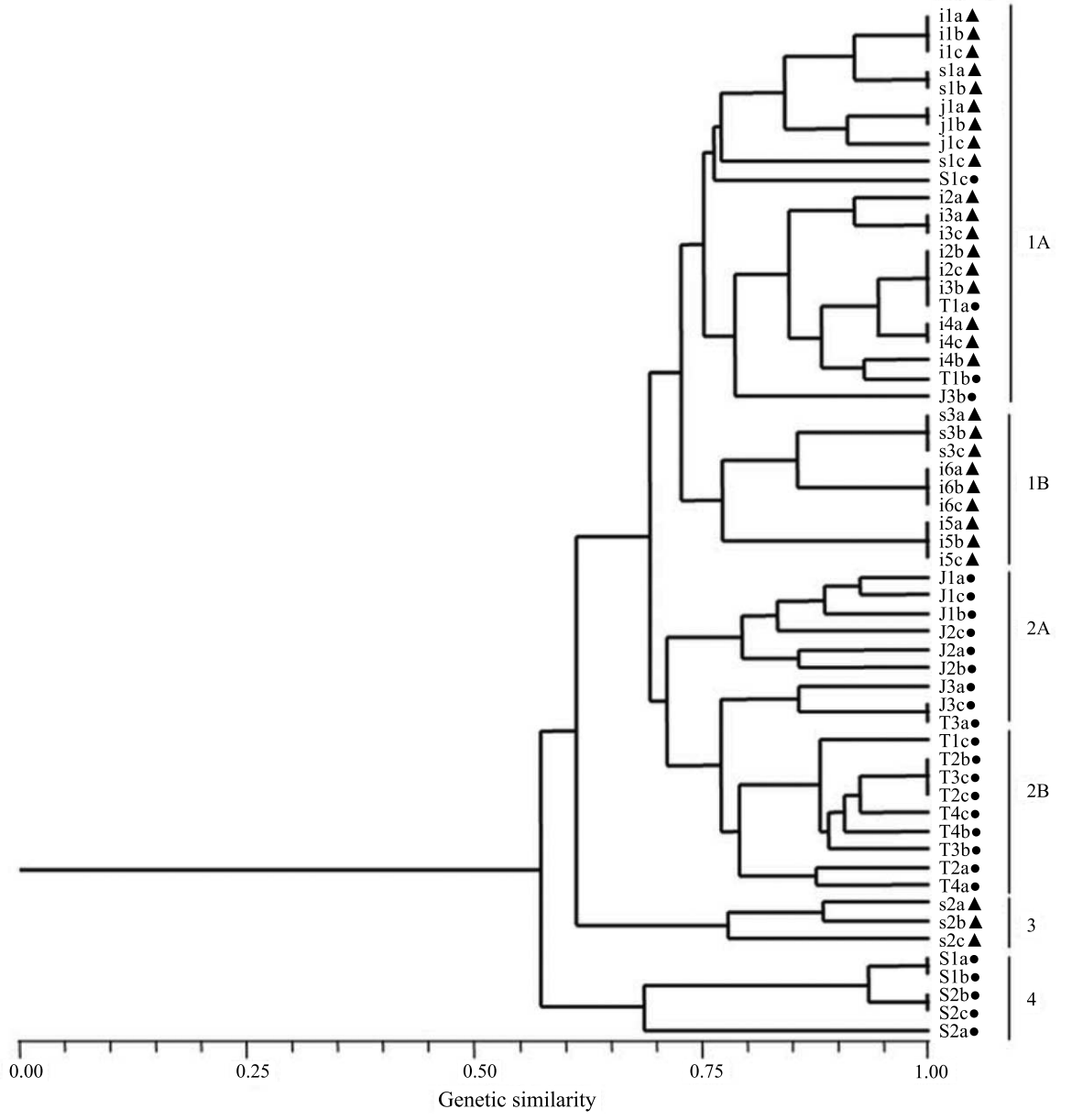

Figure 3. UPGMA of 57 individuals corresponding to wild populations and landraces of common bean from northwestern Argentina, based on 24 ISSR polymorphic bands. Scale of the UPGMA indicates Jaccard's similarity values. The major clusters and subclusters are indicated in the right margin. For nomenclature, see Table I (▲ = Landraces; • = Wild accessions).

Tucumán were genetically more similar to one another than those from Salta, in agreement with conclusions based on RAPD and morpho-agronomic data (Galván et al. 2006). The topography of the northwest of Argentina favours the geographic isolation of wild populations, leading to the maintenance of distinct ecogenotypes. A certain degree of outcrossing and gene flow among neighbouring populations has probably helped to maintain high levels of variability.

Unlike wild beans, the pattern of genetic variability of landraces was unrelated to their geographic distribution. Although high levels of genetic variability were found within sites, differences between sites were not so clear. De Ron et al. (2004), when analysing diversity based on morpho-agronomic characters of Argentinean landraces, made similar observations. The low degree of differentiation among sites in the sampled area could be explained by a homogeneous selection exerted by farmers and by seed exchange in local markets. Similar observations have been made in Mexico (Papa & Gepts 2003). González et al. (2005) reported a strong association between ISSR haplotypes and seed colour and suggested that multilocus associations could occur, given that ISSR loci are widely distributed in the bean genome as are the genes controlling seed colour (McClean et al. 2002).

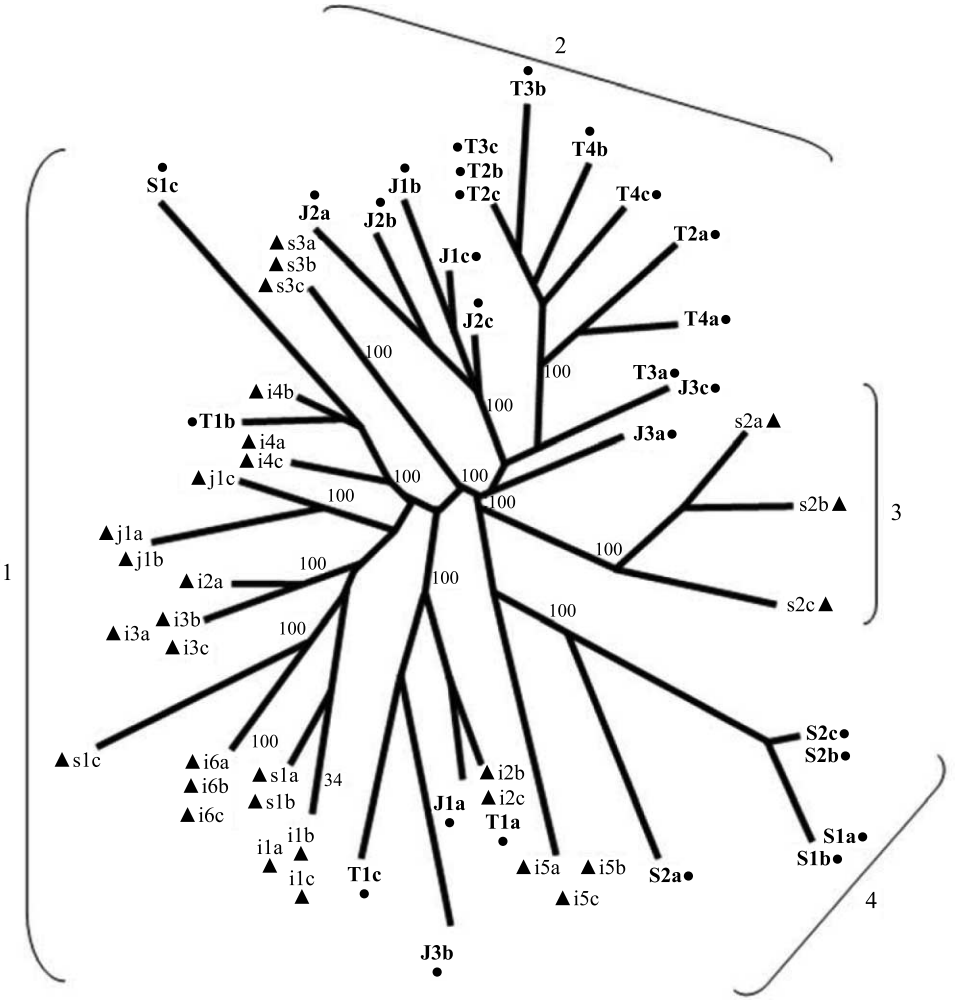

Figure 4. NJ tree of 57 individuals corresponding to wild populations and landraces of common bean from northwestern Argentina. Bootstrap values are given at the basis of branches. For nomenclature, see Table I (▲ = Landraces; • = Wild accessions).

Although morphological and biochemical data have suggested limited gene flow between wild and domesticated Argentinean beans (Santalla et al. 2004), this is unlikely since some of the wild beans analysed here were interspersed among landrace materials. Both trees (UPGMA and NJ) show that Cluster 1 consists predominantly of domesticated accessions and includes a few wild accessions. In contrast, Cluster 2 consists exclusively of wild accessions. This pattern, which was also observed in Mexico (Papa & Gepts 2003), suggests that in these regions gene flow may occur predominantly from domesticated to wild populations. This asymmetric gene flow leads to populations that are phenotypically wild but include (presumably neutral) markers originated from domesticated populations, thus explaining the inclusion of these wild accessions in a predominantly domesticated cluster.

In the UPGMA tree, landrace s2 from Santa Victoria (Salta) forms a separate cluster (Cluster 3), whereas in the NJ tree it is related to wild beans from the same site (S1 and S2). Accessions s2, S1 and S2 are highly divergent from the other materials in this study, as shown by the length of their branches in the NJ tree. Furthermore, some wild characters, such as small seeds, dehiscent pods and mottled seed colour patterns, were present in the offspring of accession s2, suggesting that hybridisation with wild beans has occurred (unpublished data). Indeed, there is evidence of variability in the reproductive system of domesticated and wild bean accessions, which, in certain areas, may have led to gene flow between sympatric populations (Ibarra-Pérez et al. 1977; Hoc & García 1999; Gepts et al. 2000; Hoc et al. 2006).

The intensity and effects of gene flow and introgression between domesticated crops and their wild progenitors depend on the species, its life history, the environment, the agro-ecosystem and human activities, among others (Papa & Gepts 2004). Even though common bean has a self-pollinated reproductive system, gene flow is sufficient to prevent genetic isolation between wild and cultivated forms (Beebe et al. 1997). In addition to this, selection appears to be a major evolutionary factor maintaining the

identity of sympatric wild and domesticated populations (Papa & Gepts 2003). In Santa Victoria (Salta), local farmers cultivate primitive landraces along with wild beans collected in inaccessible areas, such as the humid forests of Baritú National Park (Menéndez Sevillano 2002). Intermediate forms between wild and domesticated beans have been found in this area (Menéndez Sevillano 2002; Santalla et al. 2004; Hoc et al. 2006), suggesting that human intervention might facilitate gene flow between them, leading to an increase in the genetic variability of the primitive landraces.

Further studies and a more detailed sampling should be planned to determine the extent of the suggested hybridisation process and its influence on the evolution of bean landraces from northwestern Argentina.

To our knowledge, this is the first evaluation, based on DNA markers, of Argentinean landrace and wild bean diversity, which was found to be of a similar level, though structured only in wild beans. Moreover, present results suggest the existence of asymmetric gene flow from domesticated to wild beans, an aspect that requires further analysis. Breeding strategies developed to exploit the existing variation within and between wild beans and landraces could broaden the genetic base of commercial beans and might lead to healthier high-yielding cultivars.

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