

Genetic diversity and phaseolin variation in Portuguese common bean landraces

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Abstract

A collection of 18 landraces of common bean (*Phaseolus vulgaris* L.) from northern Portugal was evaluated for both phaseolin pattern and a set of qualitative and quantitative traits. The phaseolin Contender and Tendergreen types were the most commonly occurring, with Boyaca and Huevo de Huanchaco present, but rare. The colour of the flower standard and wing, and seed shape, colour and size were all highly variable. On the basis of morphological traits, the landraces and testers clustered into two main groups. No relationship was found between phaseolin profile and any of the quantitative traits measured. Based on phaseolin patterns, the origin of Portuguese common bean populations was probably the Andean region of South America.

Keywords: germplasm; phaseolin; *Phaseolus vulgaris*; Portugal; variation

Introduction

Legumes play a critical role in agriculture, since they are major contributors both to the human and animal diet and to the maintenance of soil fertility. The level of protein in the seed of a grain legume can be as much as three times that in a cereal grain, and a significant proportion of the human nutritional requirement for protein is supplied by legumes, especially in the developing world (Gepts *et al.*, 2005). The common bean (*Phaseolus vulgaris*) is one of the world's most important grain legumes. It originates from Latin America, and was introduced to Europe shortly after the Spanish discovery of the Americas in the 15th century (Zeven, 1997; Rodiño *et al.*, 2001). On the basis of variation in morphology (Singh *et al.*, 1991a), allozymes (Singh *et al.*, 1991b), seed storage proteins (Gepts and Bliss, 1986) and DNA markers (Nodari *et al.*, 1993; Beccerra-Velásquez and Gepts, 1994), it has been established that common bean

germplasm falls into two major gene pools, the 'Mesoamerican' and the 'Andean', within each of which three races can be distinguished (Gepts *et al.*, 1986; Singh *et al.*, 1991c; Chacon *et al.*, 2005). Two further distinct gene pools have been located, centred on the northern Andes and Colombia (Tohme *et al.*, 1996; Debouck, 1999). Genetic evidence suggests that most Spanish bean cultivars originated from Chilean populations (Gepts and Bliss, 1988), but the origin of Portuguese bean germplasm is unclear, because there is little evidence for seed exchange with Spain (Rodiño *et al.*, 2001). In northern Portugal, highly variable landraces persist, maintained by mass selection. However, the existence of two major groups of germplasm in the cultivated common bean landraces from Galicia, Mesoamerican versus Andean American is reported (Gil and De Ron, 1992; Rodiño *et al.*, 2006).

The genetic diversity of wild and cultivated beans has been assessed by documenting variation at the level of morphology and phenotype (García *et al.*, 1997; Singh, 2001), seed proteins (predominantly phaseolins; Brown *et al.*, 1982; Gepts *et al.*, 1986; Gepts, 1991;

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Limongelli *et al.*, 1996), allozymes (Debouck *et al.*, 1993) and DNA markers (Beccerra-Velásquez and Gepts, 1994; Maciel *et al.*, 2001; Masi and Zeuli, 2003; Martins *et al.*, 2006). This has proved to be a useful tool for evolutionary and taxonomic studies (Ladizinsky & Hymowitz, 1979; Ladizinsky, 1983), for establishing the genetic relationships between accessions within a species (Limongelli *et al.*, 1996), and as an additional approach for species identification (Ladizinsky, 1979). Wild species and local cultivars of the genus *Phaseolus* are still used for food, medicine, forage ornament, fermentation, etc. (Nabhan *et al.*, 1986). The seed storage proteins of the common bean comprise 46–81% globulins, one of which, phaseolin, contributes up to half of the total seed protein (Chagas and Santoro, 1997), and is encoded by a small gene family of tightly linked genes (Brown *et al.*, 1981a; Talbot *et al.*, 1984; Nodari *et al.*, 1993). Phaseolin profiling has allowed the definition of a number of biotypes, with certain patterns predominating in wild germplasm, and others being common among Mesoamerican and Andean landraces (Gepts and Bliss, 1986; Gepts *et al.*, 1986; Koenig and Gepts, 1989). Phaseolin pattern variation has proved to be a particularly important biochemical marker for the assessment of genetic variability in germplasm collections. The present study was carried out to evaluate the variation in phaseolin content and morphology among a collection of Portuguese common bean landraces. We also intended to evaluate whether phaseolin profiling could be used to discriminate between closely related landraces.

Material and methods

Plant material

The bean populations studied consisted of seven landraces collected from the Minho region (North Atlantic part of Portugal) and 11 from the Trás-os-Montes region (north inner part of the country), along with three tester lines, one from Canada (T1), one from Argentina (T2) and the commercial variety Big Bolotto (T3). The landraces from the Minho region belong to the market classes of large red mottled (three populations), mulatinho (three populations) and chumbinho (one population). The 11 populations from the Trás-os-Montes region are distributed by the market classes red pinto (four populations), large red mottled and rosado (two populations in each class), and canela, hook and cranberry (one population per class). The tester T1 belongs to the market class large cranberry, the T2 to the large great northern market class, while the T3 tester to the mulatinho market class. Phaseolin patterns were assessed by comparison with those of a standard panel of cultivars, Ayacucho (A), Boyaca (B), Contender (C), Chibcha (CH), Huevo

de Huanchaco (H), Middle America (M), Sanilac (S) and Tendergreen (T), kindly provided by the Genetic Resources Unit CIAT (Colombia).

Morphological traits

The landraces and testers were sown in a randomized complete block design with three replications. Each plot contained 30 plants arranged in two 3 m long rows, each separated by 0.4 m. The interplot distance was 0.8 m. A set of 25 characters was evaluated, following IBPGR (1982) descriptors, on 20 plants per accession; these characters were: growth habit, hypocotyl pigmentation and length, emerging cotyledon colour, leaf anthocyanin, colour of standard and wings of the flower, and number of nodes per plant on the main stem, the colour of the immature and dry pod, pod curvature, pod beak position and orientation, pod wall fibre, number of pods per plant, number of locules per pod, seed brilliance, seed shape, pattern, colour of seed-coat, number of seeds per pod, seed length, width and thickness and 100-seed weight.

A standard analysis of variance was applied for the quantitative traits and frequency for the qualitative ones. Two multivariate analyses were performed, using SAS software (SAS Institute, 1991). A cluster analysis was applied, by standardizing the variables and generating a matrix of square Euclidean distances. COMPLET, a hierarchic agglomerative method, was applied to produce a dendrogram, and a principal component analysis was performed using standardized values for all quantitative traits and the six most variable qualitative traits.

Phaseolin profiling

The testa and embryo were manually removed from the cotyledons before grinding. Phaseolin was extracted from 15 single seeds of each accession following Brown *et al.* (1981b). Briefly, 50 mg of cotyledon flour was suspended in 0.5 M NaCl (1/10 w/v). After shaking three times with a 5 min interval, the mixture was centrifuged at 12 krpm for 15 min. The supernatant was mixed with an equal volume of 0.625 M Tris-HCl, 2 mM EDTA, 2% (w/v) SDS, 10% (w/v) glycerol, 1% (v/v) 2-mercaptoethanol and 0.01% (w/v) bromophenol blue, held at 100°C for 5 min, and finally centrifuged at 14 krpm for 15 min. Proteins were separated by one-dimensional SDS-PAGE (Laemmli, 1970), modified according to Ma and Bliss (1978). The gels were 1.5 mm thick, with the running gel made to 14% and the stacking gel to 3.5% polyacrylamide. A 3 µl protein sample was loaded

Table 1. Mean values with standard deviation for nine quantitative traits in a collection of Portuguese common bean landraces and testers

| Landraces/testers | Hypocotyl length (cm) | Number (nodes/plant) | Number (pods/plant) | Number (locules/pod) | Number (seeds/pod) | Length seed (mm) | Width seed (mm) | Thickness seed (mm) | 100-seed weight (g) |
|--|-----------------------|----------------------|---------------------|----------------------|--------------------|------------------|-----------------|---------------------|---------------------|
| Minho landraces (F1 to F7) | 3.295 ± 0.295 | 20.000 ± 7.821 | 24.500 ± 14.582 | 5.840 ± 1.080 | 5.321 ± 1.315 | 15.219 ± 1.821 | 7.784 ± 1.050 | 6.661 ± 1.200 | 69.966 ± 30.513 |
| Trás-os-Montes landraces (PH 8 to PH 49) | 3.356 ± 0.967 | 19.923 ± 7.672 | 24.123 ± 12.448 | 5.091 ± 1.180 | 4.359 ± 1.568 | 16.421 ± 2.436 | 8.399 ± 1.397 | 6.630 ± 1.245 | 40.034 ± 20.622 |
| Total landraces | 3.332 ± 0.952 | 19.957 ± 7.680 | 24.271 ± 13.217 | 5.401 ± 1.196 | 4.733 ± 1.546 | 15.215 ± 2.710 | 8.399 ± 1.397 | 6.265 ± 1.312 | 52.089 ± 26.358 |
| Canadian accession (T1) | 2.561 ± 0.442 | 6.368 ± 1.300 | 9.200 ± 3.968 | 5.053 ± 1.311 | 4.250 ± 1.482 | 15.689 ± 1.957 | 9.056 ± 1.014 | 6.944 ± 0.933 | 25.500 ± 6.908 |
| Argentina accession (T2) | 2.862 ± 0.533 | 10.667 ± 4.726 | 25.800 ± 9.426 | 4.474 ± 0.841 | 4.300 ± 0.923 | 17.779 ± 1.317 | 8.789 ± 0.713 | 6.389 ± 0.856 | 38.632 ± 20.397 |
| Big Bolotto variety (T3) | 3.350 ± 0.920 | 11.750 ± 2.217 | 11.778 ± 2.539 | 3.812 ± 1.109 | 3.600 ± 1.188 | 15.363 ± 1.446 | 9.275 ± 0.759 | 8.200 ± 1.052 | 24.250 ± 7.126 |
| Total | 3.277 ± 0.936 | 16.600 ± 8.631 | 22.335 ± 13.109 | 5.310 ± 1.242 | 4.636 ± 1.524 | 15.478 ± 2.641 | 8.498 ± 1.329 | 6.382 ± 1.307 | 49.982 ± 32.573 |

in each well and electrophoresed for 7 h at 40 mA. The gels were stained with Coomassie Blue.

Results

There was no variation for hypocotyl pigmentation (all purple), anthocyanin in the leaves (none present) or pod wall fibre (all leathery podded). Nine of the quantitative traits were highly variable (Table 1). Some variability was noted for emerging cotyledon colour and immature and dry pod colour, pod curvature, pod beak position, and seed orientation and brilliance. Growth habit, colour of the flower standard and wings, seed shape, seed-coat pattern and seed-coat colour were more variable (Table 2). For all the quantitative traits, the accessions differed significantly ($P < 0.05$) from one another. Compared with the landraces, in general, displayed more nodes per plant, locules and seeds per pod and a greater 100-seed weight. The landraces and testers clustered into two main groups, with the first divided into three and the second into two subclusters (Fig. 1).

Cluster 1

Subcluster I.1 included eight landraces, all with an indeterminate growth habit, green emerging cotyledons, a high hypocotyl length and a high node number on the main stem between the base and the first inflorescence. Most had carmine red flower standards, with wings strongly veined in red to dark lilac. Immature pod colour was mostly green, with some displaying a carmine stripe, while the dry pods were pale yellow to white, with a few carmine red pods. Pod beak position, margined or non-margined, appeared in the same proportion and pod orientation for all accessions was straight. Seeds were large, cuboid or oval in shape. The seed-coat pattern was mostly rhomboid spotted, although some had stripes and a few no coloration. Subcluster I.2 consisted of two landraces and two testers. Accessions were uniform with respect to hypocotyl length (short), dry pod colour (pale yellow to white), pod beak orientation (straight) and brilliance of seed (medium). The length/width and length/height relation was high, which confirm the cuboid shape predominant in the seeds of landraces and testers of this subcluster. The flower wing colour was predominantly lilac, the pods slightly curved and the pod beak marginal and straight. Subcluster I.3 consisted of one landrace and one tester. Both had dark lilac (with purplish spots) coloured flower standards, strongly veined in red to dark lilac wings, deep yellow dry pods with straight beaks, medium brilliance and

Table 2. Variation for 14 morphological traits among a collection of Portuguese common bean landraces and testers

| Growth habit | Determinate bush (Type I) 23.8 | Indeterminate bush (Type III) 28.6 | Indeterminate climber (Type IV) 47.6 |
|-------------------------|-----------------------------------|---|---|
| Cotyledon colour | Green 38.1 | Pale green 61.9 | |
| Standard flower colour | Lilac 14.3 | White with lilac edge 28.6 | Carmine red 14.3 Purple 9.5 |
| Wing flower colour | Lilac 23.8 | Strongly veined in red to dark lilac 76.2 | |
| Pod colour | Carmine stripe on green 42.9 | Green 57.1 | |
| Dry pod colour | Carmine red 9.5 | Deep yellow 33.3 | Pale yellow to white 57.2 |
| Pod curvature | Slightly curved 61.9 | Curved 38.1 | |
| Pod beak position | Marginal 70.0 | Non-marginal 30.0 | |
| Pod beak orientation | Straight 71.4 | Downward 28.6 | |
| Seed shape | Oval 28.6 | Cuboid 38.1 | Kidney 9.5 |
| Seed brilliance | Matt 14.3 | Medium 76.2 | Shiny 9.5 |
| Seed-coat pattern | Absent 28.6 | Striped 42.8 | Rhomboid spotted 19.0 |
| Seed-coat darker colour | Maroon 19.0 | Yellow to greenish yellow 14.3 | Pale cream 9.5 |
| Seed-coat darker colour | Yellow to greenish yellow 9.5 | Pale cream 52.4 | Whitish 4.8 |

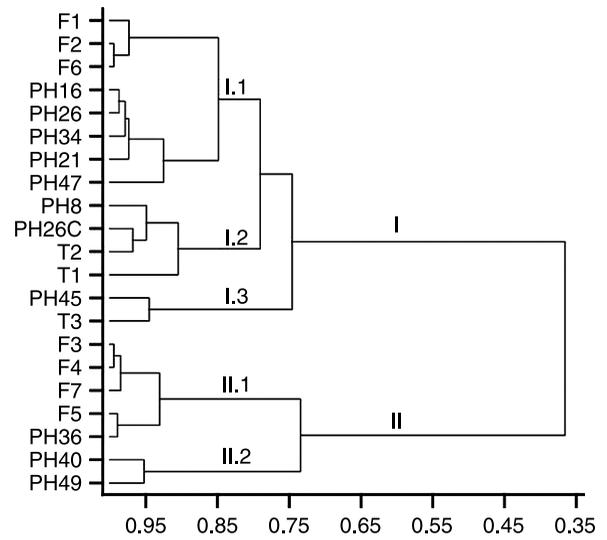


Fig. 1. Genetic relationships among a collection of Portuguese common bean landraces and testers, based on morphological and agronomic traits.

oval seeds, few pods per plant, locules and seeds per pod and a low 100-seed weight.

Cluster II

Subcluster II.1 included five landraces. The four landraces from the Minho region had semi-climbing main stem and branches, while PH36 from the Trás-os-Montes region was an indeterminate type. All landraces had pale green cotyledons, a short hypocotyl, white with lilac edge standards (except PH36 which had a purple standard), strongly veined in red to dark lilac wings, green immature pods (PH36 had pale red striped or green immature pods), pale yellow dry pods, slightly curved pods with a marginal and downward beak. The four Minho landraces had oval seeds, while PH36 had cuboid ones, which was confirmed by the low length/width relation. All had a large number of pods per plant, locules and seeds per pod and a high 100-seed weight. Subcluster II.2 comprised two landraces, with determinate bush-type plants having few nodes on the main stem. Cotyledons were pale green, the hypocotyl short, the standard either dark lilac with purplish spots or lilac, the wings lilac, the immature pod green with or without a carmine stripe, the dry pod deep yellow, the pods slightly curved or curved with a marginal and straight beak, and the seeds cuboid or kidney-shaped. The number of seeds per pod was low, and the seeds had a low 100-seed weight. The cluster analysis showed that each subcluster included landraces and testers from different locations, but all from the same origin centre means that the parameters selected are good and enough to separate the material from the two main origin centres.

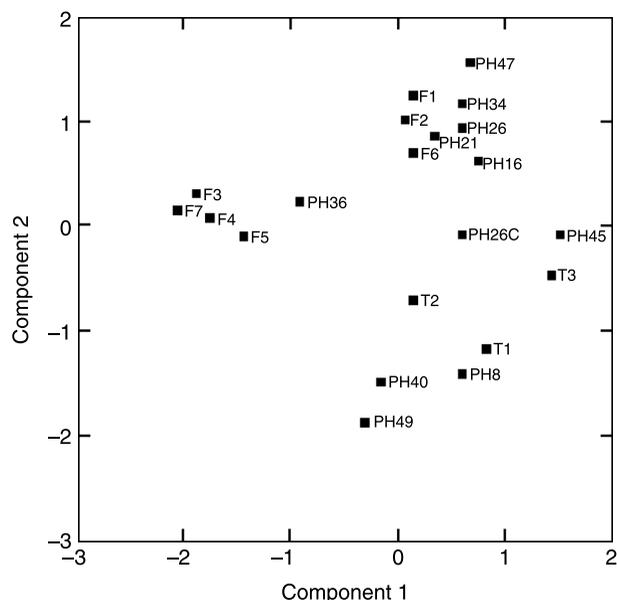


Fig. 2. Principal component analysis for nine quantitative and six qualitative traits in a collection of Portuguese common bean landraces and testers.

Hypocotyl length, number of nodes/plant, and width and thickness of seed were the main parameters responsible for the Mesoamerican landraces in the negative axis of component 2 (Fig. 2). The first three principal components accounted, in turn, for 43, 19 and 15% of the total variation. The major traits responsible for separation along the first principal component axis (loading in parentheses) were seed thickness (0.888), locule number per pod (-0.857), 100-seed weight (-0.845), seed number per pod (-0.837) and seed width (0.757). The equivalent traits for the second axis were the number of nodes per plant (0.825) and hypocotyl length (0.774), and for the third axis, standard colour (-0.846) and immature pod colour (0.781).

Four phaseolin patterns were identified among the 21 accessions: C, T, B and H (Fig. 3). The most frequent pattern was C (52%), followed by T (34%).

Discussion

While historical information provides little evidence concerning the origin and introduction of common bean to Europe, analysis of phaseolin profiles has been informative in determining the dissemination pathways from Latin America to the rest of the world (Gepts *et al.*, 1986). The C type, thought to be a hybrid of S and T, is associated with optimal adaptation to photoperiods which prevail between 36° and 42° N (Gepts and Bliss, 1988). The predominance of C and T types among the Portuguese landraces makes it likely that they were derived

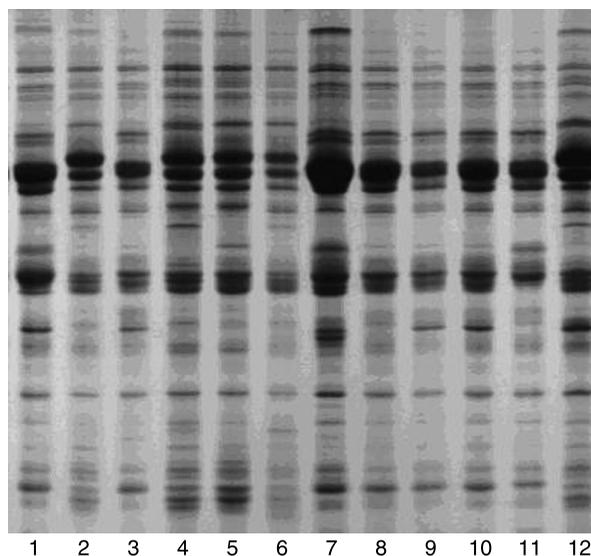


Fig. 3. SDS-PAGE-generated phaseolin patterns among Portuguese common bean landraces. Lanes: 1, F5 (B); 2, F7 (T); 3, F5 (H); 4, Tendergreen (T); 5 and 6, PH47 and Commercial (T); 7, G10994 (M); 8, F3 (B); 9, F4 (H); 10, F6 (B); 11, PH26C (C); 12, Commercial (T).

from the Andean type. The distribution of phaseolin types among the Minho landraces (59% C, 24% T, 13% B and 4% H) differs markedly from that among the Trás-os-Montes landraces (82% T and 18% C). This variation in protein bands elaborates the relationship among the collection from the various geographical northern regions and could help to distinguish their origin. However, in both groups, the C type was more frequent than the H type, in contrast to the distribution of phaseolin types in material from northern Spain (Escribano *et al.*, 1998; Rodiño *et al.*, 2006). Thus, it appears that Portuguese and Spanish germplasm does not share the same origin, as also suggested by Gepts and Bliss (1988) and Gil and Ron (1992), who proposed two gene pools in the northern part of the Iberian Peninsula. These results can suggest the importation of seeds in the north region of the farmers conducted separately by the immigration of people from Spain and Portugal (Zeven 1999).

Spanish common bean landraces, such as the Portuguese ones, are highly variable with respect to both qualitative and quantitative traits (Gil and Ron, 1992; Rodiño *et al.*, 2003, 2006). This diversity contrasts with the uniformity of modern varieties. Characterization of the genetic variation within unimproved germplasm can facilitate the effective utilization of these materials in genetics and breeding (Rodiño *et al.*, 2003; Logozzo *et al.*, 2007). Even though there was a great deal of apparent uniformity at the morphological level, a collection of Portuguese white bean landraces proved to be genetically very diverse at the DNA marker level (Martins *et al.*, 2006). The increasing reliance on modern varieties,

since they tend to yield more than landraces, is inevitably reducing the genetic variability within Portuguese common bean germplasm (Carnide *et al.*, 1997).

Gepts *et al.* (1986) have described a relationship between phaseolin patterns and certain aspects of seed and plant morphology, stress adaptation and disease resistance. Thus, for example, seed weight was correlated with phaseolin type, although Blair *et al.* (2003) found both T-type and C-type phaseolin patterns among large seeded accessions, which are characteristic of the Andean type (Singh *et al.*, 1991a). No correlation between phaseolin type and seed-coat pattern or colour was evident among the 18 Portuguese landraces analysed here. Most of these (>72%) are large seeded, with the Minho landraces having a 100-seed weight at least three times that of Big Bolotto or the Canadian tester T1, and reflecting consumer preference.

North Portugal, the area in which the landraces were collected, is a mountainous region characterized by a diversity of microenvironments. Thus, it was expected that the landraces would be diverse, as they would have been selected to match rather specific growing conditions. As Portuguese farmers grow common bean on its own, or in conjunction with either maize or maize and cucurbits, and frequently as a mixture of several types covering small areas, this is an interesting germplasm that is important to conserve.

The preservation and the evaluation of landraces and local cultivars constitute a major challenge. For this reason, on-farm conservation is being stimulated in some regions of Portugal. The common bean landraces still retain much as yet largely untapped genetic variation of relevance for crop improvement. Their genetic structure remains similar to that of the wild species. Thus, they represent a significant resource in the continuing process of limiting the vulnerability of modern varieties to biotic and abiotic stresses.

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