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Variation in morphological traits in *Phaseolus vulgaris* L. from northern Spain

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SUMMARY

A collection of 112 accessions of common beans from the north of Spain has been evaluated for 24 characters during 2 years. A combination of cluster plus canonical discriminant analysis using morphological traits to generate canonical functions was able to separate landraces from different geographic areas of northern Spain, assigning them to germplasm groups related to their centres of origin. The accessions were clustered in three groups in both years. Two groups were related to races with South American origins (races 'Chile', 'Peru' and 'Nueva Granada'), the other group was related to races with Mesoamerican origins (races 'Mesoamerica' and 'Durango'). The variation found was higher in the west of the geographic area studied decreasing toward the east. These results will permit a more efficient management and conservation of these resources, and their efficient use in breeding programmes.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is a species of American origin with two principal areas of domestication: South America and Mesoamerica (Kaplan 1971; Gepts *et al*. 1986). The variation in both areas is very high because of the great diversity of habitats in which it is distributed and cultivated. Singh (1989) proposed six different gene pools for common beans from each of Middle America and South America, reducing them later to three races in each one of the two original areas (Singh *et al*. 1991). These races are characterized by differences in morphological and agronomic traits as well as phaseolin and allozyme patterns (Gepts *et al*. 1986; Singh 1989; Singh *et al*. 1991). Polyacrylamide gel electrophoresis (SDS/PAGE) resolved four different electrophoretic banding types for phaseolin in common bean, that were named 'H', 'C', 'S' and 'T' after the cultivars 'Huevo de Huanchaco', 'Contender', 'Sanilac' and 'Tendergreen' (Gepts *et al*. 1986).

The common bean was introduced into the Iberian Peninsula very soon after the discovery of America. Dissemination paths and new diversity areas in post-Columbian times to Europe, Africa and Asia have already been studied (Evans 1976; Martin & Adams 1987; Gepts & Bliss 1988). Gepts & Bliss (1988) found a different phaseolin variation pattern in the local cultivars grown in the Iberian Peninsula (43% type C, 30% type T, 26% type S and 1% type H) from those of the rest of Europe (principally France and the Netherlands) (72% type T, 21% type S and 7% type C). In a bean collection from the Mediterranean countries (principally Italy and Cyprus), S and C phaseolin types were more frequent than the T type (Lioi 1989). Using morphological traits and a multivariate analysis on a set of common bean landraces from the northwest of the Iberian Peninsula, Gil & de Ron (1992) and Escribano *et al*. (1998) found that they could be grouped in six and eight groups, respectively. These groups were related to the races proposed for beans in their respective regions of origin.

The bean germplasm collection at the Phytogenetic Resources Centre (CRF) of Alcalá de Henares (Madrid, Spain) includes 2344 accessions (Cuadra *et al*. 2000), most of them collected in northern Spain. This centre has the task of collecting, evaluating and conserving Spanish landraces of this species. In order to develop a more efficient management and conservation of these resources and the use of these landraces in breeding programmes, a collection of

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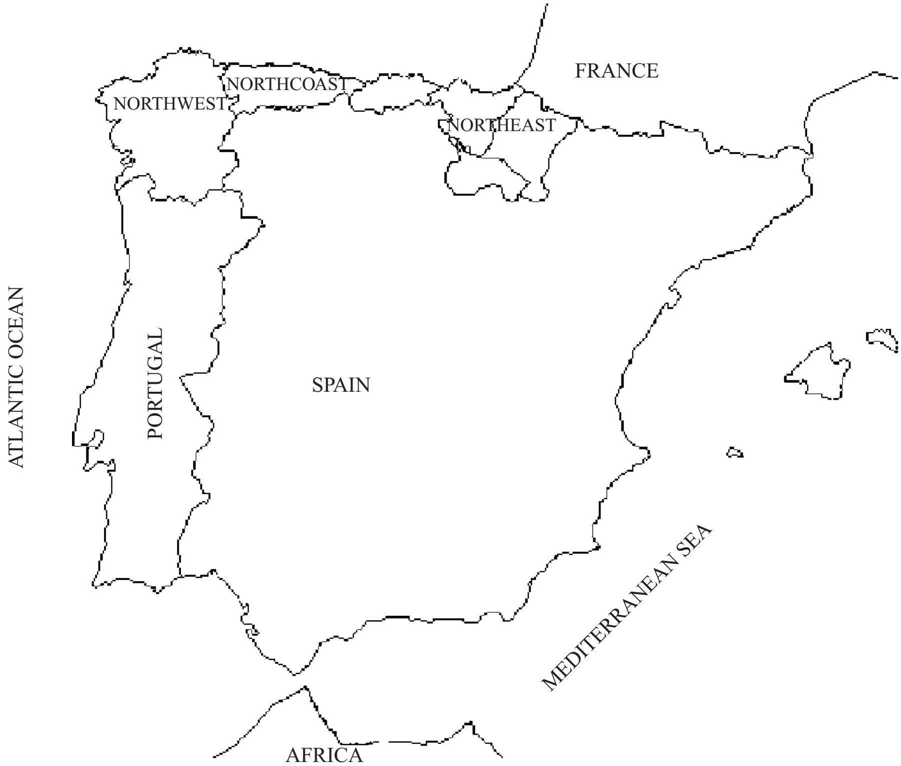

Fig. 1. Map showing the geographic origin (northwest, North Coast and northeast) of a collection of 108 common bean landraces in Spain.

common bean landraces sampled from the germplasm collection of CRF was evaluated for its diversity for morphological traits, probable origin of the accessions and geographical distribution of the diversity.

MATERIALS AND METHODS

A collection of 112 common bean accessions, randomly chosen from the bean germplasm collection maintained by the Phytogenetic Resources Centre (CRF) of Alcalá de Henares (Madrid, Spain), were sown during 2 years at the Agrarian Experiment Centre of Villaviciosa (Asturias, North Spain). 104 accessions out of the 112 were landraces from different regions of the northern Iberian Peninsula (Fig. 1) and eight accessions from the Centre for Tropical Agriculture (CIAT), Cali, Colombia.

The collection was evaluated for 27 characters following the IBPGR descriptors (1982). The unit plot consisted of a double row, 1·5 m long with 0·3 m between rows, 0·5 m between plots and 20 plants per plot. Soil fertility and moisture conditions were adequate for normal crop growth in both years. The following 27 characters were chosen for the evaluation:

Reproductive phenological plant characters

1. Days to 50% of flowering (DF).
2. Days to end of flowering (DEF).
3. Days to 50% of maturity (DM).

Leaf characters (10 plants per accession were randomly sampled)

4. Length (cm) of the terminal leaflet of the third trifoliate leaf (LTL).
5. Width (cm) of the terminal leaflet of the third trifoliate leaf (WTL).

Flower characters (10 flowers per accession were randomly sampled)

6. Length of flower (cm) (LF).
7. Length of bracteole (mm) (LBR).
8. Length of calyx (mm) (LC).
9. Length of inflorescence (cm) (LINF) (10 random plants per accession).
10. Length of pedicel (mm) (LPED).
11. Number of flowers per inflorescence (NFI); (10 random plants per accession).
12. Relation bracteole/calyx.

Pod characters (one pod per plant sampled from 10 random plants per accession)

13. Length of immature pod (cm) (LIP).

14. Length of mature pod (cm) (LMP).
15. Pod thickness (mm) (PTH).
16. Width of immature pod (mm) (WIP).
17. Width of mature pod (mm) (WMP).
18. Number of pods per plant (NPP).
19. Length of beak of the pod (mm) (LBP).
20. Pod beak position (placental and central).

Seed characters

21. Weight of 100 seeds (g) (W100S).
22. Length of seed (mm) (LS) (10 seeds per plant from 10 plants per accession).
23. Width of seed (mm) (WS) (10 seeds per plant from 10 plants per accession).
24. Seed thickness (mm) (STH) (10 seeds per plant from 10 plants per accession).
25. Number of seeds per pod (NSP) (one pod per plant from 10 plants per accession).

Structural character

26. Diameter of plant (mm) (DP) (10 random plants per accession).
27. Indeterminate or determinate growth.

All characters were examined with the Wilk–Shapiro statistic to test their normality. In all cases there was a good fit to the normal distribution.

Analysis of variance for 2 years was applied to the data according to the following model:

$$x_{ij} = \mu + A_i + Y_j + e_{ij}$$

where x_{ij} is the individual datum, μ the general mean, A_i the effect of ith accession, Y_j the effect of jth year and e_{ij} is the residual error. Variance components were used to estimate the heritability in broad sense (H) for each character (Allard 1960). Both the most significant variables for accessions ($P < 0{\cdot}1\,\%$) and the most heritable were selected to be used in the cluster analysis. When two variables were highly correlated to each other, only one of them was selected.

For cluster analysis the selected variables were standardized and a matrix of squared euclidean distances was obtained. AVERAGE, a hierarchic agglomerative method by average linkage within groups, was applied and finally a dendrogram was obtained. The existence of a qualitative jump in the fusion coefficients will allow the dendrogram to be cut at a point to produce groups that are maximally related to each other for specific variables.

The grouping obtained by cluster analysis was confirmed by Canonical Variate Analysis (CVA) also known as (linear) discriminant analysis (Mardia *et al.* 1979). Discriminant analysis was used to obtain a synthetic description of the overall variation and to assess the relative contribution of each trait.

All statistical analyses were carried out using SAS (1991).

RESULTS

Only 21 characters showed both highly significant variation between accessions and high heritability (H > 50 %) (Table 1). Number of pods per plant (NPP) showed a moderate heritability (H = 57 %) but its variation coefficients of the analysis of variance was high (30 %) (Table 1). These results suggest that the values for this character might depend on the year, therefore NPP was excluded for further analysis. The following pairs of characters: WTL and LTL, LIP and LMP, and STH and PTH were highly correlated with each other in each year ($r = 0{\cdot}87$ and 0·89, $r = 0{\cdot}93$ and 0·89, $r = 0{\cdot}87$ and 0·85, for first and second year, respectively); hence, only LTL, LIP and STH were chosen. Thus, 17 variables were used for further analysis (Table 1).

The results obtained from applying the cluster analysis to these 17 variables were very similar in both years (Figs 2 and 3); the cophenetic correlation coefficients were 0·92 and 0·89 respectively, indicating a good agreement between the matrix and the tree (respectively, the input and the output of the clustering method). The groups were formed taking into account both the existence of a qualitative jump in the fusion coefficients and similarity in the number of groups in both years.

The 104 common bean landraces plus 8 CIAT accessions clustered in three groups in both years (Figs 2 and 3). The consistency between the same groups in both years was high, as only two landraces (14 and 46) changed cluster between years. From the eight accessions from CIAT, four Mesoamerican accessions (G12952-76, G12953-87, JAMAPA-107 and DESARRURAL 1-109), were included in group III and four Southamerican (ALUBIA-85, CONTENDER-24, BUSH WHITE VENY-45 and WHITE DWARF-48), in group II.

The canonical discriminant analysis confirmed the consistency of the three groups in both years. The first two canonical discriminant functions explained 61 % and 39 % in the first year, respectively, and 60 % and 40 % in the second, of the total variation among groups. In both years, characters associated with the size and shape of seeds (WS, STH, W100S), pod (WIP, WMP, LBP, LPED) and cycle length (DF, DEF) were the most important ones in discriminating among groups.

Group I included 78 accessions that are, with one exception, of indeterminate growth habit. The seeds are coloured (black, cream, yellow and white). Some seeds showed different patterns on the seed coat, principally eye or ring patterns around the hilum and red speckling and large blotches. They are of medium to large size, often round or oval but can also be elongate. Generally speaking, for 79 % of the accessions, the flower bracteoles did not exceed the calyx length. The pod beak position was either

Table 1. *Analysis of variance and broad sense heritability* (*H*) *for 24 characters of 112 accessions of common bean grown for 2 years* (*1998–1999*)

Characters	Mean Square					CV (%)	H (%)
	Year (D.F. = 1)	*P*	Accessions (D.F. = 111)	*P*	Error (D.F. = 111)		
DF	41·1400		94·460	0·001	28·670	8·8	53·0
DEF	12434·5400	0·001	134·740	0·001	46·070	8·5	49·0
DM	14983·1400	0·001	423·110	0·001	101·800	8·2	61·0
LTL	114·7000	0·001	7·380	0·001	1·690	10·8	63·0
*WTL	30·3900	0·001	4·640	0·001	1·450	13·0	52·0
*LF	0·0034		0·050	0·01	0·026	8·0	31·0
LBR	0·4700	0·001	0·020	0·001	0·003	8·6	74·0
LC	0·0200	0·001	0·006		0·002	7·6	50·0
*LINF	31·2600	0·001	4·350	0·001	2·330	25·1	30·0
LPED	0·6500	0·001	0·110	0·001	0·010	12·8	83·0
*NFI	5·9700	0·05	1·700		1·270	23·7	15·0
LIP	26·7200	0·001	14·260	0·001	0·830	6·8	89·0
*LMP	18·7500	0·001	16·890	0·001	1·010	7·1	89·0
*PTH	0·0900	0·001	0·030	0·001	0·003	6·2	82·0
WIP	0·0500	0·01	0·040	0·001	0·006	6·3	74·0
WMP	0·0040		0·060	0·001	0·005	6·1	85·0
*NPP	2157·9100	0·001	191·520	0·001	51·860	30·6	57·0
LBP	1·8600	0·001	0·240	0·001	0·040	18·0	71·0
W100S	431·7000	0·001	353·040	0·001	26·870	10·8	86·0
LS	0·0570		0·110	0·001	0·004	4·9	93·0
WS	0·0027		0·010	0·001	0·001	3·8	82·0
STH	4·5 10–7		0·020	0·001	0·001	4·7	90·0
NSP	1·8300	0·01	1·730	0·001	0·262	8·4	74·0
DP	0·6300	0·001	0·020	0·001	0·005	10·4	60·0

* Characters excluded for further analysis. See text for list of abbreviations.
CV is the variation coefficient of the model of variance analysis.

placental (dorsal suture, 37% of the accessions) or central (between placental and ventral, 63% of the accessions). Compared with the other two groups, it had a higher mean value for seed and pod width and for days to maturity (Table 2). This group was widespread in the three regions studied.

Group II is formed by 13 accessions, all of which have a determinate growth habit and seeds of medium to large size, with kidney or cylindrical shapes. Seed colours vary among white, cream, yellow and red. Bracteoles were shorter than the calyx in 85% of the accessions. Both central and placental positions of the pod beak were found. Accessions in this group showed longer pedicels, pod beaks and seeds than those of the other two groups, as well as earlier flowering and maturity (Table 2). Four accessions from CIAT (24, 45, 48 and 85) were included in this group. Of the remaining accessions, all but two were from the Galicia region (northwest Spain).

Group III comprises 19 accessions, most of which had indeterminate growth. Seed size ranges from small to medium. The mean value for weight of 100 seeds was smaller than those of the other two groups. Seed colours are black, white, yellow and cream. This group had bracteoles longer than the calyx; all accessions show a placental pod beak. Mean values for seed width and length, pedicel length and length of pod beak were smaller than those of the other groups (Table 2). Four accessions from CIAT and the Mesoamerican origin (76, 87, 107 and 109) were included in this group.

Accessions from Galicia showed the highest variability (mean square euclidean distances among accessions was 84); they were distributed throughout the three groups (Figs 2 and 3). Almost all accessions from the North Coast region belonged to group I and only five accessions were clustered within group III (mean square euclidean distance was 62). The accessions from the northeast region were clustered principally in group I (92%); this region showed less diversity than the other two regions (mean square euclidean distance was 37).

DISCUSSION

The red colour of seed pattern and the eye or ring around the hilum seem to be virtually non-existent in the Mesoamerican Centre of Domestication, where

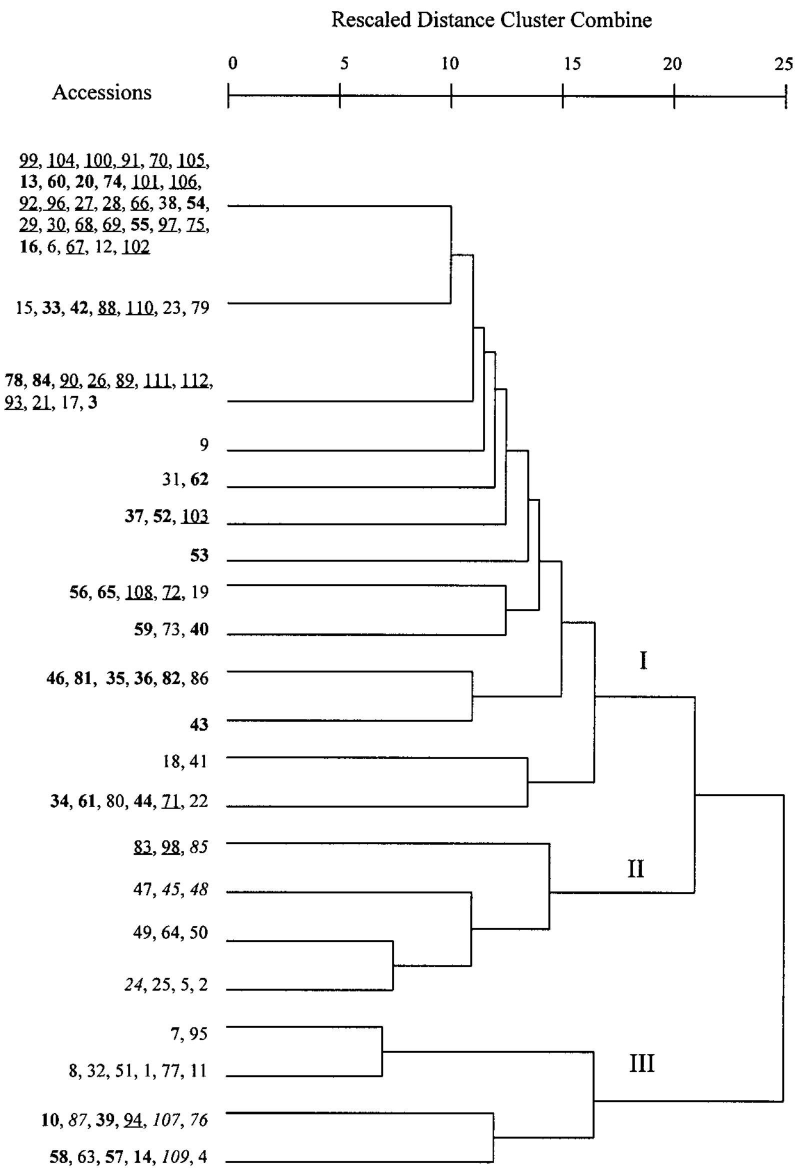

Fig. 2. Groups of common bean accessions obtained from cluster analysis in the first year. Accessions are labelled by origin; italic, bold, normal and underlined accessions are those from the CIAT, North Coast region, northwest region and northeast region, respectively. Accessions under the same branch with rescaled distance values lower than seven were grouped.

green or other colours are much more frequent than in the South American Centre (Singh 1989; Singh *et al*. 1991). Thus, groups I and II in the present study could be of South American origin and group III of Mesoamerican origin. This suggestion is supported by the fact that four accessions of Mesoamerican origin are included in group III and four South American accessions in group II. In addition, groups I and II show a high percentage of small bracteole/calyx ratio, in common with Andean races (Gentry 1969). The results of the present study suggest that most of the bean germplasm in northern Spain originated in South America as most accessions are included in Group I. These results are in agreement with Gepts & Bliss (1988) who found a high frequency of accessions with phaseolin C type (43 %) in the Iberian Peninsula. This phaseolin type is present mainly in the races from Chile and Peru.

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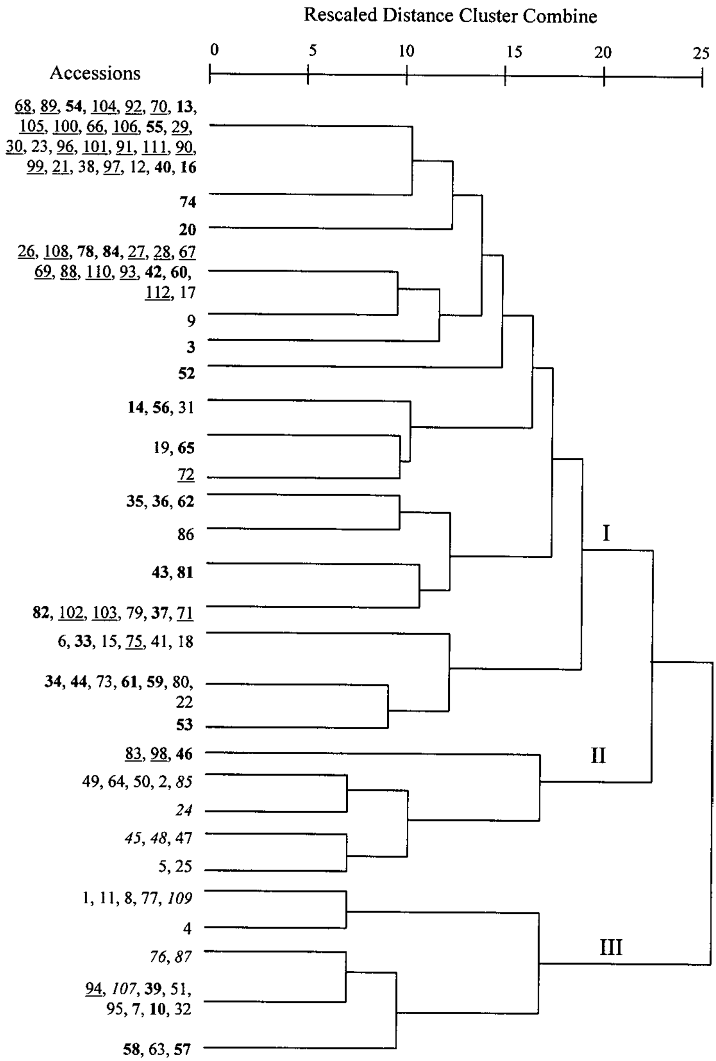

Fig. 3. Groups of common bean accessions obtained from cluster analysis in the second year. Accessions are labelled by origin; italic, bold, normal and underlined accessions are those from the CIAT, North Coast region, northwest region and northeast region, respectively. Accessions under the same branch with rescaled distance values lower than seven were grouped.

The morphology of Group I matched that of races 'Chile' and 'Peru' (Singh *et al.* 1991). Relatively small to medium leaves and round to oval seeds with a predominantly indeterminate growth habit III characterize race 'Chile'. Key morphological characteristics of germplasm belonging to race 'Peru' are the large lanceolate leaves with an indeterminate type IV climbing growth habit and its seeds are large and often round or oval (Singh *et al.* 1991). Race 'Peru' is commonly known as 'Pole beans' in Europe and the USA; landraces of this race are grown in association with maize or other crops, mostly in the warm summers of temperate countries.

Characteristics of group II match those of the race 'Nueva Granada'. This group includes an accession from CIAT ('Alubia', a typical Spanish name for common beans with elongate seeds); they have a good dry seed quality but a low yield. This landrace was introduced from Spain to Argentina where it is mainly grown for export. Insensitivity to photoperiod, early maturity, angular leaf spot and resistance to bean common mosaic virus (BCMV), halo blight and anthracnose have been found in this race (Singh *et al.* 1991).

Group III accessions show characteristics in agreement with the races 'Mesoamerica' and 'Durango'

Table 2. *Mean values and standard deviation for 17 characters used for identification of three groups of common bean from the Iberian Peninsula*

Characters	Groups		
	I (*n*=78)	II (*n*=13)	III (*n*=19)
Width of seed	0·86±0·06	0·77±0·06	0·70±0·055
Width of immature pod	1·28±0·13	1·16±0·14	1·03±0·10
Width of mature pod	1·22±0·16	1·02±0·13	0·95±0·10
Diameter of plant	0·69±0·11	0·63±0·14	0·63±0·14
Days to 50% of flowering	62·62±6·49	47·9±3·25	60·1±7·39
Days to end of flowering	82·53±10·88	63·8±6·37	77·6±10·93
Seed thickness	0·71±0·08	0·61±0·04	0·53±0·05
Length of bracteole	0·60±0·10	0·59±0·08	0·69±0·12
Length of calyx	0·59±0·06	0·65±0·06	0·55±0·04
Length of the terminal leaflet	12·53±2·11	11·21±3·35	10·5±1·88
Length of pedicel	0·75±0·17	1·21±0·24	0·57±0·09
Length of beak of the pod	1·03±0·30	1·78±0·37	0·92±0·19
Length of seed	1·29±0·21	1·5±0·18	1·1±0·20
Length of immature pod	13·74±2·80	13·62±2·12	11·2±2·12
Days to 50% of maturity	129·45±10·75	100·7±14·34	115·4±15·7
Weight 100 seeds	52·45±10·75	49·52±10·81	27·26±7·42
Number of seeds per pod	6·20±0·98	5·15±0·75	6·15±0·916

from Mesoamerica and are mainly consumed as dry seed. Resistance to BCMV, moderate tolerance to drought and poor soil fertility is exhibited by some accessions of race Mesoamerica (Singh *et al*. 1991). The 'Durango' race could also be a source of early maturity, high harvest index, general combining ability for seed yield (Nienhuis & Singh 1988) and tolerance to some viral diseases and anthracnose (Morales & Singh 1991).

The canonical discriminant analysis shows that nine characters (width, height and weight of seed, days to flowering, end of flowering, pod width, pod beak length and pedicel length), were the most prominent traits contributing to the separation among groups. Both days to flowering and seed shape and size traits have been used to characterize the different *Phaseolus vulgaris* races (Singh 1989; Singh *et al*. 1991). In the present study, pod and pedicel length also contributed to the separation among groups.

Landraces from the Galicia region (northwest Spain) showed a higher level of diversity than those of the other regions. All three groups are well represented in this region, where the greatest variation is found, in agreement with other authors (Gil & de Ron 1992; Escribano *et al*. 1998). A mountainous topography and different climatic conditions can explain this high diversity. An additional factor could be the fact that beans are mostly cultivated in small gardens, in many cases for home consumption.

Landraces from the Northern Coast clustered mainly in group I and also showed a high level of variation. In this region the climate is predominantly Atlantic. The northeastern area was the region with the lowest amount of variation. Group I accessions were the most common in this area; they showed mainly characteristics of race 'Chile'. A high proportion of its landraces showed similar seed characteristics (size, shape and colour) and clustered in a compact subgroup, suggesting a common population origin.

In conclusion, according to the results of the present study, groups I and II are made up of accessions from South American races and group III are from Middle American races. The geographical variation pattern found seems not to be distributed at random. The highest variation is found in the northwest, decreasing toward the northeast, a result to be considered in the development of a core collection and its utilization in breeding programmes.

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