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RESEARCH ARTICLE

Morphological evaluation of common bean diversity on the Island of Madeira

Gregório Freitas · José F. T. Ganança · Humberto Nóbrega · Élvio Nunes · Graça Costa · Jan J. Slaski · Miguel Â. A. Pinheiro de Carvalho

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Abstract The variability of fifty populations of common bean (*Phaseolus vulgaris* L.), representing a wide range of ecological conditions on the Island of Madeira, was evaluated based on morphological and reproductive traits. Individual data of 58 traits related to earliness, plant and tassel structure and the shape of the ear and grain were analysed using multivariate analysis. The populations belonging to two major common varieties were clustered into fifteen groups by their degree of dissimilarity, based on discriminant analysis. The dissimilarity of these groups was confirmed by one way ANOVA. The racial rank of these groups was proposed and a brief description of the common bean landraces was presented. This work represents the first morphological characterization and analysis of diversity of bean germplasm from the Archipelago of Madeira, where the traditional agricultural practices are still keeping this Portuguese region free from improved bean varieties. The description of the Madeiran bean landraces allows the preservation of the existing bean biodiversity and could be used for their registration as conservation landraces, or for conservation and breeding purposes worldwide.

Keywords Germplasm breeding · Madeira Island · Multivariate analysis · *Phaseolus vulgaris* · Variability

Introduction

Since the beginning of their cultivation history, *Phaseolus* species assumed a great importance due to its role in agriculture and nutrition, providing protein, fiber, minerals and vitamins to the human diet (Broughton et al. 2003; Ying et al. 2006). Their seeds contain also a large number of bioactive compounds (Ranilla et al. 2007), which makes them an inexpensive and abundant functional food, having known benefits to human health (Cardador-Martínez et al. 2002; Broughton et al. 2003). FAO (2009) statistics shows that the world production of beans was of 18.3×10^6 Mt in 2007, occupying an area of 27.5×10^6 ha. In Portugal, the crop occupies an area of 7,945 ha, from which 1350 ha are localized in Madeira, where it annually reaches a production of 4,230 t (Vieira and Oliveira 2007). However, the role played by Portugal in the crop globalization and germplasm transfer (Mendes Ferrão 1992), as well as

G. Freitas · J. F. T. Ganança (✉) · H. Nóbrega ·
É. Nunes · G. Costa · J. J. Slaski ·
M. Â. A. P. de Carvalho
ISOPlexis Germplasm Bank, Centre of Studies
for Macaronesia, University of Madeira,
9000-390 Funchal, Portugal
e-mail: jofitei@gmail.com

J. J. Slaski
Bioresource Technologies,
Alberta Innovates—Technology Futures,
Vegreville, AB, Canada

the traditional bean consumption, determines the existence of the present high crop biodiversity.

Phaseolus belongs to the subclass Rosidae, order Fabales, family Fabaceae, subfamily Papilionoidea (Strasburger et al. 1994) and encompasses more than 400 species (Freytag and Debouck 2002). The common bean (*P. vulgaris*) occupies more than 85% of the total crop cultivated area in the world. Common bean genetic resources evolved from two main genetic sources of wild beans, the Mesoamerican and the Andean gene pools, and one or two smaller gene pools in the northern Andes (Koenig and Gepts 1989). The poor establishment of relationships between different domesticated species, their gene pools and the crops wild relatives (cwr) makes difficult the bean germplasm evaluation and its rational use for different purposes. Beans are an extremely diverse crop in terms of cultivation methods, uses and range of environments to which they have been adapted (Broughton et al. 2003). This diversity of conditions and genetic origin (gene pool) can manifest itself in several morphological and biochemical traits (Singh et al. 1991c), and evolve in a multitude of cultivated forms, which causes much disagreement about the crop taxonomy (Freytag and Debouck 2002) and the recognition of infraspecific units. A wide range of variation in some morphological and agronomic traits have been observed in bean cultivars (Voysest and Dessert 1991), which implies the importance of germplasm evaluation for the selection of plants with traits suitable for local conditions (CIAT 1983; Araya 2003).

In Europe, the diversity of common bean germplasm is the result of adaptation to different agro-ecological conditions and multiple inputs of material from both the Andean and Mesoamerican crop domestication centres (Araya 2003). However, Graham and Ranalli (1997) advocated the theory that most European cultivars are of Andean origin, and were probably introduced to Europe through the Iberian Peninsula, after the discovery of America. Portugal and Spain, especially in their northern regions, are considered a secondary centre of genetic diversity for common bean, particularly the large white seeds cultivars of common bean (Santalla et al. 2004).

The Portuguese archipelago of Madeira, located on the Atlantic Ocean, 630 km away from the west coast of North Africa, between the latitude of 33° 10 and 32° 20 N and between the longitude of 16° 10 and 17° 20 W, where the agriculture is practiced by farmers on small plots (poios) and terraces carved into steep slopes of remote and isolated valleys, ranging from sea level to about 1,000 m.a.s.l., still keeps a large bean diversity. This crop still has an extreme importance, because it is one of the most widely used legume in local gastronomy, and it is also associated with several cultural and culinary traditions, usually based on the use of local seed production. Little is known about the introduction of beans, but there are strong indications that the crop was brought to Madeira in the seventeenth century by settlers from Brazil and South America or from mainland Portugal (Silva and Meneses 1984). The role of the Madeiran archipelago in the acclimation and agricultural experimentation of new crops, before their introduction in other regions of the world is well documented (Mendes Ferrão 1992).

The volcanic origin of the archipelago, the specific edaphic and ecological characteristics, as well as agricultural practices, have promoted the adaptation of introduced germplasm, as exemplified by the evolution of local landraces of wheat and corn (Pinheiro de Carvalho et al. 2003, 2004; Ganança et al. 2007; Pinheiro de Carvalho et al. 2008; dos Santos et al. 2009). The successful adaptation of bean crops to local conditions could explain why they are still in use by local farmers and their preferences for old regional cultivars, which are differentiated by a specific number of morphological, agronomic and nutritional traits. However, recent trends such as the decrease of rural population and the abandonment of traditional agricultural practices will lead to an irreversible genetic erosion of these cultivars in the foreseeable future.

A representative sampling of local bean resources, collected in traditional agro-systems and preserved in the ISOPlexis Germplasm Bank, at the University of Madeira, was assessed for their morpho-agronomic traits, using IPGRI (2001) descriptors. The analysis of the variability of the Madeiran bean cultivars, focusing on their morphological characterization, is of great importance for the evaluation of plant diversity and identification of local breeds. This study aims to provide an initial morphological and agronomic characterization of the Madeiran bean diversity preserved in the ISOPlexis germplasm bank, and to establish a reference collection with an identification system for the local landraces.

Materials and methods

Plant material

Fifty accessions of common bean germplasm were chosen from the collection of the ISOPlexis germplasm bank, representing the diversity of forms cultivated on the Island of Madeira (Table 1). These accessions were collected during several field missions, from farmer's field plots and traditional agro-systems. According to Santalla et al. (2001), 12 of these accessions belong to the Cranberry market class, 6 to Ojo de Cabra, 4 to Hen Eye, 3 each to Azufrado, Large Cranberry, Large Red Mottled, Large Great Northern and Red Pinto, and 1 each to Black Canellini, Bayo Gordo, Small Red, Marrow, Garbancillo and Rosada. Identification of the market class of seven accessions was impossible to perform. The selection of bean accessions for the evaluation took into consideration their origin, and the wide range of ecological conditions present on Madeira, as well as previous knowledge on their morphological and reproductive traits. For each accession evaluation, a minimum sample of 40 seeds representing variability within the accession was chosen.

Field trials design

Selected accessions were studied during two vegetative cycles (2005 and 2006) on the experimental fields of the University of Madeira, Funchal, Portugal.

Table 1 Number of accessions, their local name, geographic distribution and market class of the Madeira common bean (*Phaseolus vulgaris*)

ISOP*	Local name	Geographical origin	Market class
459	Canadiano	Ilha	Ojo de Cabra
460	Vergalheiro	Santana	Cranberry
463	Corno de Carneiro	Santana	Azufrado
478	Filipe	Arco S. Jorge	Hen Eye
480	Preto	Arco S. Jorge	Black Canellini
489	Rasteiro	Arco S. Jorge	Large Cranberry
492	Fava	S. Jorge	Cranberry
497	Touquinho	S. Jorge	Hen Eye
505	Corno de Carneiro	S. Jorge	Large Red Mottled
508	Milheiro	Ilha	Cranberry
514	Algarve	Ilha	Large Great Northern
519	Touquinho	Santana	Cranberry
521	Faial	Santana	Cranberry
528	Faial	Santana	Ojo de Cabra
534	Vaginha	Santana	Large Great Northern
541	Manteiga	Faial	Bayo Gordo
668	Preto	S. Vicente	UD
670	Branco Rasteiro	S. Vicente	Large Great Northern
679	Vassoura Rasteiro	Santa	UD
712	Vaginha	S. Vicente	Ojo de Cabra
713	Vaginha Grossa	S. Vicente	UD
719	Feijão	S. Vicente	Small Red
722	Açores	S. Vicente	Ojo de Cabra
724	Vermelho	S. Vicente	Large Red Mottled
726	Vassoura Rasteiro	S. Vicente	UD
730	Boneco	Ribeira Brava	Hen Eye

Table 1 continued

ISOP*	Local name	Geographical origin	Market class
731	Rasteiro (Vassoura)	Ribeira Brava	UD
732	Vaginha	S. Vicente	Large Red Mottled
743	Feijão	S. Vicente	Red Pinto
744	Feijão	S. Vicente	Large Cranberry
748	Rajado	S. Vicente	Ojo de Cabra
749	Vaginha	S. Vicente	Azufrado
755	Valinho	Santa	Hen Eye
757	Feijão	Santa	Cranberry
760	Vaginha	Ribeira Janela	UD
761	Alfarroba	Ribeira Janela	Ojo de Cabra
764	Rasteiro	Ribeira Janela	Red Pinto
770	Vermelho	Santa	Cranberry
773	Gordo	Santa	Cranberry
777	Catarino	Ribeira Brava	Large Cranberry
778	Vagem Vermelha	Ribeira Brava	Cranberry
798	Riscado de Vara	Ponta Pargo	Cranberry
800	Vara	Ponta Pargo	Cranberry
806	Corno de Carneiro	Boaventura	Cranberry
809	Vagem Vermelha	Pta Delgada	UD
822	Feijão de Pé	S. Vicente	Marrow
824	Rajado	Pta Delgada	Garbancillo
828	Amarelo	Boaventura	Azufrado
829	Feijão de Pé	Boaventura	Red Pinto
849	Corno de Carneiro	S. Vicente	Rosada

UD Unidentified class

* Code given to each accession in the ISOPlexis Germplasm Bank

Plants were sown, at the same time, in March and harvested during August. The plots were cultivated according to traditional methods used by local farmers, with high amounts of organic manure applied before planting (5 kg m^{-2}), and mineral fertilizers applied during the experiment (1 kg m^{-2}). All plots were irrigated once a week during the vegetative period with an average of 40 l m^{-2}. The "hill-plot" (Rodiño 2001a) experimental design was used, which allows characterizing a large number of plants in a small space. The experiments were performed in a completely randomized design, in which each experimental unit consisted of 2.5 m^2 with 10 planting holes. In each hole, four seeds were placed, making a total of 40 seeds sown per experimental plot. The spacing between the plots was 90 cm. Thus, the maximum theoretical density of plants in the experiments was 75,000 plants ha^{-1}.

Morphological evaluation

Data for 58 morphological traits were taken from 10 randomly chosen plants from each plot. These characteristics were related to plant architecture (9), vegetative cycle (6), morphology of the leaf (5), morphology of the flower (12), morphology of the pod (14) and morphology of the seed (12) (Table 2). Traits related to pigmentation and coloration were observed between 08:00 and 09:00, using the colour chart from The Royal Horticultural Society (2001).

Data from the two vegetative cycles were compiled and the resulting mean values, standard deviation and variation indices were calculated for each parameter, independently and for all accessions. The Principal Components Analysis (PCA) was performed as described by Pinheiro de Carvalho et al. (2008), and used to summarize variability of the 50

Table 2 Morphological traits considered in the study of Madeira common bean germplasm

Abbreviations	Trait
D1F	Days to first flower
NDF	Days to flowering
NDFF	Days until the end of flowering
DF	Duration of flowering (days)
NDM	Days to maturity
ND1VS	Days until first dry pod
CH	Hypocotyl length (cm)
PH	Hypocotyl pigmentation
CCE	Emerging cotyledon colour
HC	Plant type
TCCF	Leaf colour of chlorophyll
AP	Plant height (cm)
PF	Leaf persistence
NNC	Node number at harvest
DC	Stem diameter (mm)
CF	Leaflet length (cm)
LF	Leaflet width (cm)
FF	Leaf shape
CP	Pedicel length (mm)
NN1I	Node number until first inflorescence
NGFI	Flower buds per inflorescence
NC	Racemes per plant
TB	Flower bud size
CDB	Bracteole length (mm)
LB	Bracteole width (mm)
FB	Shape of bracteole
CB	Colour of bracteole
AA	Wing opening
CAS	Colour of wings
CE	Colour of standard
CV	Pod length (mm)
LV	Pod width (mm)
AV	Pod height (mm)
STV	Pod cross-section
CUV	Pod curvature
CVI	Immature pod colour
CVM	Mature pod colour
CVS	Dry pod colour
PAV	Pod beak position
OAV	Pod beak orientation
CAV	Pod beak length (mm)
PVP	Position of pods
LOV	Locules per pod
FPV	Pod wall fibre
NVP	Pods per plant
NSV	Seeds per pod
PTS	Seed coat patterns
CME	Seed coat darker colour
CC	Seed coat lighter colour
NS	Apparent seed veining
CA	Hilar ring colour
BS	Brilliance of seed
CS	Length of seed (mm)
LS	Width of seed (mm)
AS	Height of seed (mm)
FS	Seed shape
PS	Seed weight (g 100 seed^{-1})
VS	Seed volume (cm^3 100 seed^{-1})

accessions. Factor analysis of average values based on Eigenvalues was performed using SPSS version 16.0 for Windows. The Principal Coordinates Analysis (PCO) using the Gower general similarity coefficient was conducted to summarize variation and discriminate against the weight of the traits using MVSP version 3.13 d for Windows. Accession clusters were compared by one way ANOVA and discriminant analysis to assess their independence, to examine the relationships between groups and to identify the subset of features and variables that best distinguish populations and groups. *t*-Student tests were performed for each parameter to evaluate the existence of differences between the two vegetative cycles. One way ANOVA, discriminant analysis and t-Student tests were performed using SPSS version 16.0 for Windows.

Results

The results of the quantitative morphological traits for all populations, as well as for the dwarf and climbing bean groups separately, are presented in Table 3. Traits showed a wide range of variability, especially in plant height (AP), days until the end of flowering (NDFF), node number at harvest (NNC), pod length (CV), pods per plant (NPP), weight of 100 seeds (PS) and volume of 100 seeds (VS).

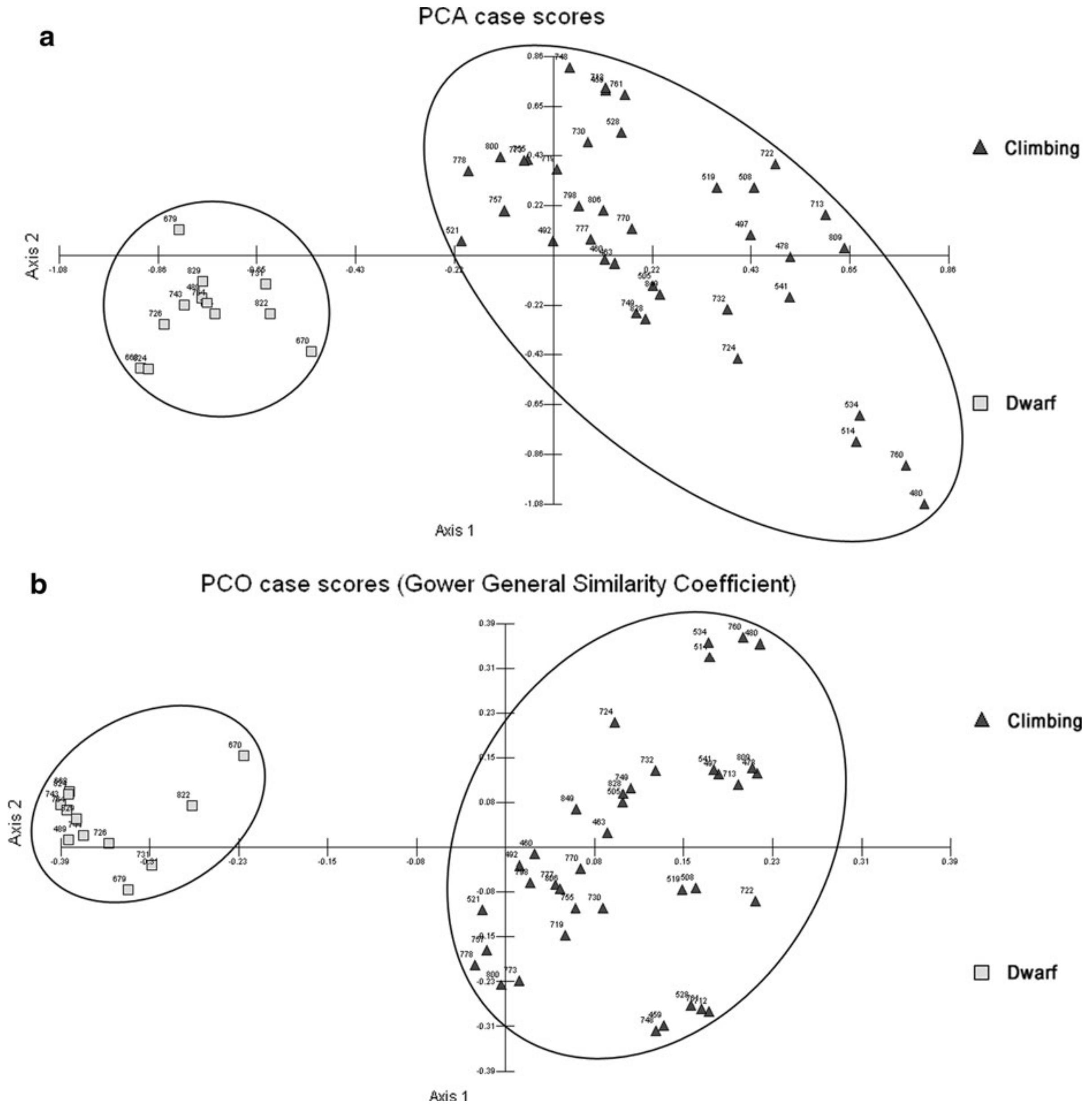
Table 3 Morphological traits variability of the Madeiran beans

Traits	Total			Dwarf			Climbing		
	N	Average ± SD	Min–max	*N*	Average ± SD	Min–max	*N*	Average ± SD	Min–max
D1F	50	49.2 ± 4.4	42.0–61.0	12	45.3 ± 1.3	43.0–48.0	38	50.5 ± 4.2	42.0–61.0
NDF	50	52.6 ± 4.6	43.0–62.0	12	47.8 ± 1.4	45.0–49.0	38	54.1 ± 4.2	43.0–62.0
NDFF	50	77.5 ± 7.9	64.0–94.0	12	66.3 ± 2.5	64.0–73.0	38	81.0 ± 5.4	74.0–94.0
DF	50	24.9 ± 5.5	15.0–36.0	12	18.4 ± 2.5	15.0–25.0	38	26.9 ± 4.5	19.0–36.0
NDM	50	95.3 ± 4.8	89.0–108.0	12	92.0 ± 2.3	89.0–95.0	38	96.3 ± 4.9	90.0–108.0
ND1VS	50	94.8 ± 4.6	82.0–104.0	12	91.3 ± 3.9	85.0–96.0	38	95.9 ± 4.3	82.0–104.0
CH	50	4.2 ± 1.2	1.2–7.5	12	3.3 ± 1.1	1.2–6.9	38	4.5 ± 1.1	1.7–7.5
AP	50	192.2 ± 108.7	13.8–448.1	12	25.7 ± 5.5	13.8–38.2	38	244.8 ± 63.2	108.6–448.1
NNC	50	15.3 ± 7.2	3.0–37.0	12	4.2 ± 0.6	3.0–5.0	38	18.8 ± 4.0	10.0–37.0
DC	50	5.6 ± 1.2	2.9–9.9	12	6.3 ± 1.3	4.0–9.9	38	5.3 ± 1.0	2.9–8.4
CF	50	11.8 ± 2.1	6.2–18.4	12	11.6 ± 2.1	7.2–18.4	38	11.9 ± 2.0	6.2–17.3
LF	50	8.3 ± 1.7	4.5–15.1	12	9.3 ± 2.0	5.2–15.1	38	8.0 ± 1.5	4.5–12.0
CP	50	10.3 ± 1.9	5.2–16.3	12	11.8 ± 1.9	8.3–16.3	38	9.8 ± 1.6	5.2–15.9
NN1I	50	2.9 ± 1.1	1.0–7.0	12	3.8 ± 1.1	2.0–7.0	38	2.6 ± 0.9	1.0–5.0
NGFI	50	3.5 ± 1.0	2.0–7.0	12	4.3 ± 0.7	3.0–6.0	38	3.2 ± 0.9	2.0–7.0
NC	50	11.2 ± 6.4	3.0–42.0	12	4.9 ± 1.2	3.0–8.0	38	13.3 ± 6.1	3.0–42.0
CDB	50	7.0 ± 1.0	4.2–10.6	12	6.7 ± 1.0	4.2–9.0	38	7.1 ± 1.0	4.8–10.6
LB	50	4.7 ± 1.0	2.3–10.7	12	4.2 ± 0.7	2.3–5.7	38	4.8 ± 1.0	2.9–10.7
CV	50	160.0 ± 37.8	12.4–309.1	12	142.7 ± 21.4	12.4–195.1	38	165.5 ± 40.1	87.5–309.1
LV	50	11.4 ± 1.1	7.8–16.7	12	11.4 ± 1.0	8.8–13.9	38	11.4 ± 1.2	7.8–16.7
AV	50	15.9 ± 2.5	10.6–25.9	12	14.4 ± 1.3	11.2–16.9	38	16.4 ± 2.6	10.6–25.9
CAV	50	18.6 ± 4.3	6.4–33.8	12	21.1 ± 5.6	6.4–33.8	38	17.9 ± 3.5	7.6–31.9
LOV	50	5.9 ± 1.6	3.0–21.0	12	5.2 ± 0.8	3.0–8.0	38	6.1 ± 1.7	3.0–21.0
NVP	50	11.2 ± 7.1	2.0–56.0	12	6.7 ± 3.2	2.0–22.0	38	12.7 ± 7.4	3.0–56.0
NSV	50	5.6 ± 1.2	3.0–9.0	12	5.0 ± 0.9	3.0–8.0	38	5.9 ± 1.3	3.0–9.0
CS	50	14.4 ± 2.1	8.3–20.8	12	13.8 ± 1.9	9.2–18.6	38	14.6 ± 2.2	8.3–20.8
LS	50	6.9 ± 1.2	4.0–69.6	12	6.5 ± 0.8	4.0–8.9	38	7.0 ± 1.2	4.0–69.6
AS	50	8.9 ± 1.0	4.9–16.4	12	8.3 ± 0.7	4.9–11.0	38	9.1 ± 1.1	4.9–16.4
PS	50	60.2 ± 12.7	30.8–91.7	12	52.1 ± 7.0	39.8–62.9	38	62.8 ± 13.1	30.8–91.7
VS	50	49.0 ± 11.4	26.0–76.0	12	43.0 ± 8.8	27.0–56.0	38	50.9 ± 11.5	26.0–76.0

The table shows average values and standard deviation (SD), minimum (Min) and maximum (Max) for the 50 accessions (12 dwarf and 38 climbing)

The comparison of the evaluation for morphological traits in climbing and dwarf common bean populations (Table 3) revealed that the most significant differences were related to the NDF, NNC, AP, CV, NVP, NSV, PS, and VS parameters. When climbing bean samples were compared with dwarf beans there was a significant difference in plant height. Climbing beans also had a higher number of pods, of larger size, and heavier and larger seeds, which makes them more productive.

A Student *t* test was performed to analyze morphological trait variation between the two vegetative growth cycles. Results indicate that variation was not significant except for two parameters (data not shown). These results allowed us to use an average for the two vegetative cycles in the following multivariate statistical analysis. The analysis divided the accessions along the first and second PCA axes, explaining 36.42% of the total variability (Fig. 1a). The PCO analysis using the Gower general similarity



PCA case scores

PCO case scores (Gower General Similarity Coefficient)

Fig. 1 **a** Principal Component Analysis of Madeira bean populations. The analysis allowed us to identify two groups of common beans (Dwarf and Climbing). **b** Principal Coordinates Analysis of Madeira bean populations, using the Gower similarity coefficient. The analysis allowed us to delimit two groups (Dwarf and Climbing)

coefficient to discriminate qualitative and quantitative traits showed an increase of the discontinuity of the accessions (Fig 1b). The separation along both PCO axes explains 26.85% of the observed variability.

This analysis permitted to pool bean accessions into two main groups according to the growth behaviour, the dwarf cluster, with 12 accessions, and the climbing one, including 38 accessions (Fig. 1). The discriminant analysis revealed that 100% of the accessions were properly classified. However, the spatial distribution of the accessions inside these groups allowed hypothesizing the existence of several different clusters inside the dwarf and climbing bean groups. To clarify further

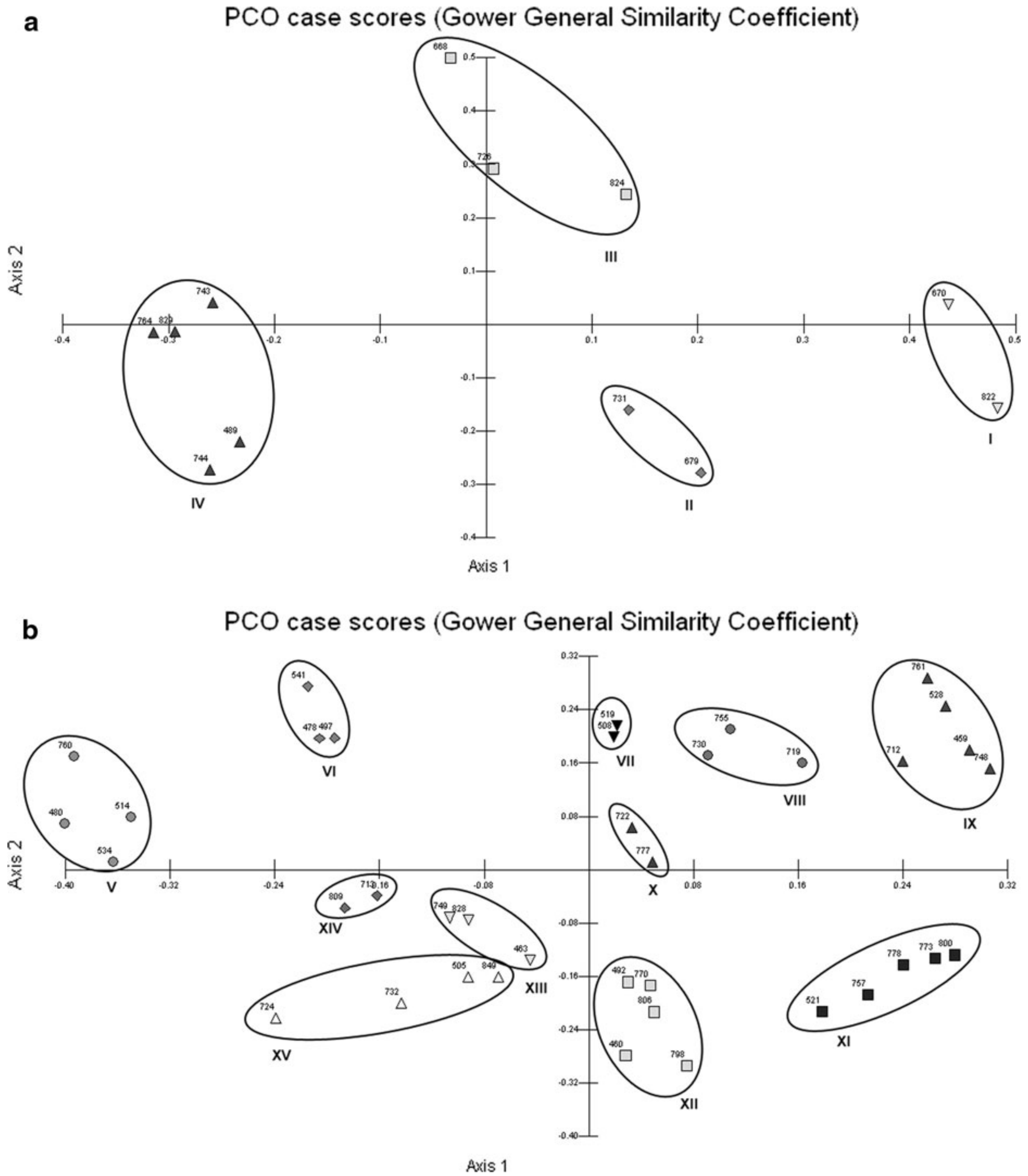


Fig. 2 **a** Principal Coordinates Analysis of Madeira dwarf beans populations, using the Gower similarity coefficient. The identified clusters are marked by a circle and marked from I to IV. **b** Principal Coordinates Analysis of Madeira climbing bean populations, using the Gower similarity coefficient. The identified clusters are marked by a circle and marked V–XV

the existence of sub-clusters, a separate and independent analysis was made for the dwarf and climbing beans groups.

Figure 2a illustrates the PCO analysis performed on dwarf bean accessions, which grouped them into four clusters. In the PCO analysis using the

Gower general similarity coefficient, both coordinates explained 41.69% of the total variability among the dwarf bean accessions. The spatial distribution of the accessions identified four clusters: I (ISOPs 670 and 822), II (ISOPs 679 and 731), III (ISOPs 668, 726 and 824) and IV (ISOPs 489, 743, 744, 764 and 829) (Fig 2a). This classification was subjected to a discriminant analysis, considering only quantitative traits, which revealed that 100% of the accessions have been properly classified. A group cross-validation confirmed that 83.3% of the cases were still correctly classified. The maximum degree of dissimilarity was determined using the generalized Mahalanobis distance, considering that the first two canonical variables accounted for 98.93% of the existing variability. The one way ANOVA procedure was used to confirm the existence of differences between the identified groups. Significantly different traits ($P \leq 0.05$, $\alpha \leq 0.05$) were determined using the Tukey test. Mean values for these significant traits are summarised in Table 4. More detailed information about the traits that discriminate between individual groups can be found on Online Resource 1.

The obtained results confirmed the existence of the dissimilarity between clusters and shown that they could be distinguished by several significant morphological and reproductive traits. The greatest differences were observed between clusters II and III, while the smallest difference was observed between clusters III and IV. The distance between each of the groups is suggested by the value of F obtained in the discriminant analysis. The lowest value of *F* (303.25) confirmed that clusters III and IV are closer to each other, while clusters II and III were confirmed as the most distant, with the highest value of F (3,723.03).

The same set of analysis was performed with the climbing beans. Figure 2b illustrates the PCO analysis for climbing bean accessions, which grouped them into 11 clusters. In the PCO analysis using the Gower general similarity coefficient, the principal coordinate associated to axis 1 explained 15.76% of the total variation observed, while the one associated with axis 2 explained 11.74%. The spatial distribution of the accessions allowed to identify eleven clusters: V (ISOPs 480, 514, 534, 760), VI (ISOPs 478, 497 and 541), VII (ISOPs 508 and 519), VIII (ISOPs 719, 730; 755), IX (ISOPs 459, 528, 712, 748 and 761), X (ISOPs 722 e777), XI (ISOPs 521, 757, 773, 778 and 800), XII (ISOPs 460, 492, 770, 798 and 806), XIII (ISOPs 463, 749 and 828), XIV (ISOPs 713 and 809) and XV (ISOPs 505, 724, 732 and 849) (Fig 2b). This classification was submitted to discriminant analysis, using the same methodology as above, which revealed that 100% of the accessions were correctly classified, and correctly grouped in 84.20% of cases after cross-validation. For this purpose, the maximum degree of dissimilarity was determined using the generalized Mahalanobis distance, considering that the first three canonical variables accounted for 96.73% of the existing variability. Results of ANOVA confirm the existence of differences between the identified groups. Significantly different traits ($P \leq 0.05$, $\alpha \leq 0.05$) were determined using the Tukey test. Mean values for these significant traits are summarised in Tables 5 and 6. More detailed information about the traits that discriminate between individual groups can be found on Online Resources 2 and 3. These results show that

Table 4 Mean parameter values for clusters I–IV (dwarf beans)

Traits	*N*	Cluster I	*N*	Cluster II	*N*	Cluster III	*N*°	Cluster IV
D1F	2	47.5 ± 0.0	2	45.5 ± 0.0	3	44.5 ± 1.0	5	44.7 ± 0.3
NDM	2	90.5 ± 0.0	2	90.3 ± 1.1	3	90.0 ± 0.0	5	94.6 ± 0.4
DC	2	5.2 ± 0.6	2	5.4 ± 0.6	3	5.6 ± 0.2	5	7.5 ± 0.6
NGFI	2	3.2 ± 0.3	2	3.2 ± 0.2	3	5.1 ± 0.5	5	3.6 ± 0.1
CDB	2	6.0 ± 0.4	2	5.9 ± 0.1	3	6.7 ± 0.4	5	7.4 ± 0.5
LV	2	10.5 ± 0.2	2	11.5 ± 0.7	3	10.6 ± 0.6	5	12.1 ± 0.3
AV	2	14.0 ± 1.1	2	14.4 ± 1.0	3	13.3 ± 0.8	5	15.3 ± 0.3
LS	2	6.3 ± 0.6	2	6.9 ± 0.4	3	5.7 ± 0.4	5	6.8 ± 0.2
AS	2	7.9 ± 0.4	2	8.8 ± 0.2	3	7.7 ± 0.0	5	8.7 ± 0.2

Table 5 Mean parameter values for clusters V–IX (climbing beans)

Traits	*N*	Cluster V	*N*	Cluster VI	*N*	Cluster VII	*N*	Cluster VIII	*N*	Cluster IX
D1F	4	55.3 ± 5.0	3	55.5 ± 1.7	2	53.0 ± 0.7	3	48.8 ± 2.9	5	52.3 ± 0.5
NDFF	4	83.1 ± 5.6	3	91.0 ± 2.2	2	86.5 ± 0.0	3	78.5 ± 0.0	5	78.0 ± 0.0
NDM	4	98.3 ± 3.6	3	103.2 ± 2.0	2	101.8 ± 1.1	3	91.7 ± 1.8	5	94.8 ± 2.7
ND1VS	4	96.0 ± 4.4	3	99.3 ± 0.8	2	97.5 ± 5.0	3	94.0 ± 2.2	5	98.2 ± 2
CH	4	3.6 ± 0.4	3	4.3 ± 0.9	2	4.7 ± 0.3	3	3.8 ± 0.5	5	5.4 ± 0.6
AP	4	298.0 ± 13.2	3	329.0 ± 42.7	2	319.1 ± 16.8	3	189.4 ± 25.5	5	194.4 ± 6.5
NNC	4	19.8 ± 1.8	3	25.7 ± 3.6	2	23.5 ± 0.4	3	17.8 ± 1.8	5	16.5 ± 0.5
DC	4	5.9 ± 0.7	3	5.3 ± 0.4	2	5.6 ± 0.1	3	4.5 ± 0.7	5	4.6 ± 0.1
CF	4	11.7 ± 0.8	3	11.2 ± 0.6	2	11.3 ± 0.4	3	10.5 ± 1.4	5	10.1 ± 0.6
LF	4	9.1 ± 0.7	3	7.3 ± 0.5	2	8.0 ± 0.3	3	6.7 ± 0.8	5	6.6 ± 0.6
CP	4	8.2 ± 0.9	3	9.8 ± 0.6	2	10.0 ± 0.3	3	9.2 ± 0.4	5	8.9 ± 1.0
NN1I	4	3.2 ± 0.6	3	4.4 ± 1.1	2	4.1 ± 0.1	3	3.1 ± 1.2	5	2.9 ± 0.1
NGFI	4	3.5 ± 1.0	3	3.1 ± 0.5	2	3.7 ± 0.1	3	1.8 ± 0.5	5	2.5 ± 0.1
NC	4	13.9 ± 3.7	3	19.7 ± 6.0	2	22.5 ± 1.5	3	12.7 ± 5.8	5	10.3 ± 1.1
CDB	4	8.0 ± 1.0	3	6.7 ± 0.7	2	7.0 ± 0.1	3	6.5 ± 0.3	5	6.3 ± 0.4
Lb	4	6.7 ± 1.2	3	3.8 ± 0.3	2	4.4 ± 0.5	3	4.6 ± 0.3	5	4.6 ± 0.5
CV	4	239.7 ± 31.4	3	154.7 ± 8.4	2	132.0 ± 15.2	3	103.1 ± 8.9	5	134.1 ± 8.9
LV	4	10.3 ± 0.3	3	10.4 ± 0.6	2	11.9 ± 0.3	3	11.9 ± 0.8	5	12.3 ± 0.7
CAV	4	13.6 ± 2.1	3	15.5 ± 2.0	2	13.6 ± 3.0	3	16.9 ± 1.7	5	16.8 ± 1.0
LOV	4	8.1 ± 0.7	3	6.5 ± 0.4	2	5.7 ± 0.6	3	5.4 ± 0.3	5	5.2 ± 0.8
NSV	4	7.8 ± 0.5	3	6.3 ± 0.2	2	5.5 ± 0.5	3	5.2 ± 0.1	5	5.0 ± 0.7
CS	4	14.0 ± 0.8	3	13.8 ± 0.1	2	11.7 ± 0.7	3	10.5 ± 0.1	5	13.8 ± 1.1
LS	4	5.4 ± 0.3	3	6.9 ± 0.2	2	7.6 ± 0.2	3	7.8 ± 0.3	5	7.8 ± 0.2
AS	4	7.5 ± 0.6	3	8.5 ± 0.3	2	9.1 ± 0.1	3	9.3 ± 0.1	5	10.2 ± 0.7
PS	4	44.2 ± 9.9	3	54.6 ± 3.8	2	58.9 ± 1.7	3	52.7 ± 2.2	5	72.0 ± 15.4
VS	4	35.1 ± 7.6	3	44.0 ± 1.7	2	46.3 ± 1.8	3	40.3 ± 1.4	5	59.4 ± 12.7

the groups can be distinguished by several significant morphological and reproductive traits. This classification was confirmed by the values of F obtained by discriminant analysis. The greatest differences were observed between clusters XI and XIV (1,546.21). The smallest difference was observed between the clusters XII and XV (16.70).

Discussion

The accessions used in this study were representative of observed field diversity, local names and plant, pod and seed traits. We were able to observe a great variability in the majority of the studied traits among the 50 accessions. These results are consistent with Gepts and Bliss (1988), Rodiño et al. (2003) and Rodiño et al. (2006), who studied the morphology of the Iberian Peninsula common bean germplasm. They concluded that the samples showed a high variability of the analysed morphological characters and this reflects the wide range of environments, in which crop resources evolved (Singh 1989; Singh et al. 1991b). This great variability has fuelled intensive discussions on the taxonomy and taxonomical structure of this species. Several authors have proposed classification systems to explain the intraspecific structure of common bean, but the classification used by Singh et al. (1991a) is the one that generates greater consensus giving a high taxonomic value to traits such as size, colour and seed shape in comparison to the vegetative characteristics of the plant.

Nevertheless, Smith and Smith (1989) emphasized the importance of the discriminating value of

Table 6 Mean parameter values for clusters X–XV (climbing beans)

Traits	*N*	Cluster X	*N*	Cluster XI	*N*	Cluster XII	*N*	Cluster XIII	*N*	Cluster XIV	*N*	Cluster XV
D1F	2	50.5 ± 7.1	5	45.7 ± 1.9	5	46.5 ± 2.0	3	51.3 ± 3.3	2	54.0 ± 3.5	4	48.1 ± 1.1
NDFF	2	85.3 ± 3.9	5	74.5 ± 0.0	5	79.3 ± 1.6	3	77.8 ± 1.2	2	90.8 ± 0.4	4	80.0 ± 2.3
NDM	2	101.0 ± 7.8	5	91.3 ± 1.7	5	95.1 ± 0.7	3	91.5 ± 0.5	2	105.3 ± 0.4	4	96.0 ± 0.4
ND1VS	2	98.8 ± 2.5	5	91.1 ± 4.0	5	94.3 ± 1.4	3	95.0 ± 0.5	2	103.5 ± 0.0	4	94.9 ± 3.5
CH	2	4.2 ± 0.7	5	5.3 ± 0.6	5	4.6 ± 0.6	3	4.6 ± 0.7	2	3.9 ± 0.1	4	4.3 ± 0.6
AP	2	246.5 ± 9.0	5	223.7 ± 30.3	5	222.4 ± 18.9	3	195.2 ± 32.7	2	248.8 ± 9.7	4	284.6 ± 55.0
NNC	2	22.1 ± 3.6	5	15.6 ± 0.6	5	18.2 ± 0.8	3	15.6 ± 0.7	2	18.4 ± 0.1	4	19.6 ± 1.7
DC	2	5.1 ± 0.2	5	4.8 ± 0.3	5	6.3 ± 0.6	3	5.8 ± 0.6	2	5.3 ± 0.1	4	5.7 ± 0.7
CF	2	10.9 ± 1.4	5	12.6 ± 1.3	5	13.2 ± 1.2	3	13.0 ± 0.4	2	12.3 ± 0.8	4	13.1 ± 1.3
LF	2	7.8 ± 1.4	5	8.2 ± 0.6	5	8.8 ± 1.0	3	8.2 ± 0.6	2	8.1 ± 0.9	4	8.7 ± 1.2
CP	2	9.5 ± 0.4	5	11.0 ± 0.8	5	10.4 ± 1.7	3	9.8 ± 0.3	2	8.1 ± 0.5	4	11.1 ± 1.0
NN1I	2	3.9 ± 1.2	5	3.0 ± 0.1	5	2.7 ± 0.2	3	3.0 ± 0.2	2	4.1 ± 0.3	4	2.6 ± 0.3
NGFI	2	2.7 ± 0.1	5	2.1 ± 0.2	5	2.2 ± 0.4	3	2.8 ± 0.2	2	2.6 ± 0.1	4	2.9 ± 0.7
NC	2	15.2 ± 2.4	5	7.7 ± 0.8	5	12.7 ± 1.8	3	10.2 ± 3.4	2	15.5 ± 0.3	4	15.3 ± 4.7
CDB	2	6.3 ± 0.3	5	7.1 ± 0.9	5	7.5 ± 0.7	3	7.4 ± 0.7	2	7.0 ± 0.1	4	7.5 ± 0.6
Lb	2	4.6 ± 0.1	5	4.2 ± 0.4	5	4.8 ± 0.5	3	4.9 ± 0.2	2	5.2 ± 0.2	4	5.2 ± 0.4
CV	2	158.3 ± 22.7	5	150.6 ± 16.1	5	171.5 ± 14.5	3	175.8 ± 2.8	2	197.0 ± 12.8	4	193.4 ± 9.1
LV	2	11.1 ± 0.0	5	11.7 ± 0.4	5	11.3 ± 0.6	3	11.0 ± 0.9	2	12.6 ± 1.0	4	10.6 ± 0.4
CAV	2	17.6 ± 0.8	5	19.2 ± 1.2	5	21.3 ± 2.3	3	20.1 ± 2.5	2	19.3 ± 0.9	4	19.7 ± 0.8
LOV	2	5.9 ± 0.3	5	5.1 ± 0.3	5	6.5 ± 1.8	3	6.2 ± 0.6	2	6.4 ± 0.1	4	6.6 ± 0.2
NSV	2	5.7 ± 0.4	5	5.0 ± 0.2	5	5.7 ± 0.3	3	6.2 ± 0.6	2	6.2 ± 0.0	4	6.4 ± 0.2
CS	2	14.9 ± 2.7	5	14.8 ± 0.7	5	16.5 ± 0.7	3	16.8 ± 0.2	2	17.5 ± 0.3	4	15.1 ± 0.3
LS	2	7.3 ± 0.7	5	7.2 ± 0.6	5	7.0 ± 0.6	3	6.9 ± 0.4	2	7.7 ± 0.2	4	6.3 ± 0.2
AS	2	9.2 ± 0.6	5	9.7 ± 0.6	5	9.6 ± 0.5	3	8.7 ± 0.5	2	9.2 ± 0.1	4	8.5 ± 0.1
PS	2	71.1 ± 23.7	5	63.1 ± 5.3	5	70.7 ± 8.8	3	64.6 ± 10.2	2	82.2 ± 8.9	4	60.0 ± 5.3
VS	2	57.0 ± 20.5	5	52.3 ± 3.1	5	56.1 ± 10.0	3	55.7 ± 6.5	2	70.0 ± 6.4	4	47.3 ± 4.1

morphological traits, which depends of the historical origin and the genetic pool of the studied accessions. According to Voysest (1983) large seeds (>40 g 100 seeds^{-1}) correspond to bean of Andean origin. In our study, it is notorious the dominance of bean accessions with large seeds, indicating their possible Andean origin. This hypothesis is supported by the data of phaseolin pattern electrophoresis analysis of the Madeiran beans (da Silva et al. 2010). Our data are consistent with the results of the analysis of 88 germplasm accessions from northern Portugal performed by Rodiño et al. (2001b), who also observed the predominance of the Andean germplasm (seed size) over germplasm of Mesoamerican origin. The same result was observed by Igrejas et al. (2009). To ensure thorough morphological characterization, during the evaluation of the diversity of this crop on Madeira, we analysed the results of field trials by multivariate statistics. The results of Student's *t* test illustrate that, from all the characters studied, only the number of locules and seeds per pod shown significant differences ($P < 0.05$) between both vegetative periods. These results demonstrate that the samples had a great phenotypic stability and were well adapted to the agro-ecological conditions of Madeira. The successful adaptation to local edapho-climatic conditions explains why the bean is still used by local farmers and their preferences for old regional cultivars, which are differentiated by a specific number of morphological and agronomic traits. The verification of this stability allowed us to use average data from the 2 years for each parameter, for further statistical procedures (PCA, PCO, discriminant analysis and one-way Anova). Using the Principal Coordinates

(PCO) analysis we were able to group the accessions according to their morphological variability (Fig. 1b). Two major morphological groups were distinguished among the common bean accessions by their growth behaviour, corresponding to the two varieties reported by Lowe (1862) to Madeira, namely Nana and Procerae varieties, which are homologous to the two subspecies defined by Maroto (1989), ssp. nanus (Kapri) Grad. (dwarf bean) and ssp. volubilis (Kapri) Grad (climbing beans).

The separated analysis of these groups using the PCA and PCO analysis detected 15 clusters (four dwarf beans and 11 climbing beans) (Fig. 2a and Fig. 2b), which according to the values of F could be classified as racial groups. However, the Singh classification (Singh et al. 1991a) defines the existence of only three Andean races, which are the Chile, Nueva Granada and Peru races. Probably we are dealing with local varieties or landraces belonging to one or more of these Andean racial groups. Martins et al. (2006) proved that Portuguese white bean landraces were genetically very diverse, and we hypothesize that the same diversity occurs within Madeira landraces. This last hypothesis needs to be tested. Rodiño et al. (2001a) identified the existence of several landraces for the Iberian Peninsula common bean, e.a. groups of cultivars sharing the same morphological and genetic structure. Madeira varietal groups were described according to the identification of significant traits. Similar approach was used by Santalla et al. (1994), Rodiño (2001a, b), (2003) to define bean groups. The majority of these traits were associated with productivity or the characteristics of vegetative cycle, and significantly ($P \leq 0.05$) differentiated the obtained clusters (Pinheiro de Carvalho et al. 2008). Thus, cluster III and IV were characterized by their early flowering precocity (44.5 ± 1.0 and 44.7 ± 0.3), while cluster VI showed the longest vegetative cycle. The clusters with indeterminate growth habit, indeterminate arbustive and indeterminate climber, achieved physiological maturity later compared with the first four clusters having a determinate growth. The clusters V and X with indeterminate growth produced on average more pods per plant (18.00 ± 1.8 and 17.3 ± 7.2), and with the highest average number of seeds per pod (5.7 ± 0.4 and 7.8 ± 0.5). These observations are in agreement with studies conducted by García et al. (1997) who demonstrated that for the plants the increasing climbing ability determines the increase of the time needed for achieving physiological maturity, and that the plants with indeterminate growth are more productive than plants with determinate growth habit. According to Kinnear and Gray (2000), the *F* values in a discriminant analyses with a magnitude higher than six values permit group validation, hence, we can conclude that the groups are spaced sufficiently to constitute distinct taxonomic units. The highest value of F observed for the dwarf bean clusters occurred between groups II and III, while the lowest recorded value of F was between groups III and IV, indicating that the first two groups are more spaced and the latter are those that share a greater number of morphological features. For the climbing beans clusters, the highest value of F was observed between groups XI and XIV. Groups VII and XV shared the largest number of features with the lowest value of F. A discriminant analysis exceeding proper classification of 80% of the cases is considered to be statistically reliable by Kinnear and Gray (2000). Thus, our results confirm that the groups defined by the PCO analysis were accurately classified, since 100% of the populations were correctly grouped including the dwarf and climbing beans.

On Madeira, the effect of artificial selection made by the local farmers and the adaptation of the varieties to different agro-climatic conditions could explain the balance achieved between the reproductive traits (productive) and the vegetative traits of the studied populations. This balance demonstrates that the combination of the two types of parameters allows a better characterization of the bean resources. This is consistent with the results reported by Santalla et al. (1994), Rodiño (2001a) and Rodiño et al. (2003), who concluded that the best descriptors should combine the production parameters with the vegetative parameters. Overall, for the traits used in the morphological evaluation of the common bean germplasm, the ones of particular importance are directly related with the growth cycle and reproduction. Regarding the Madeira beans, cultivars (ISOPs) 00521 and 00668 are characterized by an early flowering and pod maturation stage. These traits contribute to the reduction and incidence of pests and diseases (Rodiño et al. 2003). Yet, an early physiological maturity is negatively related to plant productivity (White and Singh 1991), therefore, a better assessment of these accessions for a posterior identification of their genotypes will be required. From an agronomical point of view, there are interesting cultivars with good quality of immature pods for fresh consumption, such as green beans (Rodiño et al. 2003). Madeiran ISOPs 00480, 00514, 00534 and 00760, characterized by a greater pod length, may exhibit such potential. The ISOPs accessions 00480, 00519, 00534, 00724, 00760 and 00849 stand out by having a high grain yield per plant.

Scarcity of studies aiming to evaluate the Portuguese bean resources prevents the comparison of our results with a national reference. Rodiño et al. (2001b) and Igrejas et al. (2009) also present the Andean gene pool as dominant in Portugal. Our results seem to add to the hypothesis that Madeira bean germplasm was brought from mainland Portugal or followed the same introduction routes. There is evidence that Spanish bean germplasm does not share the same origin that the Portuguese one. Our research of the Madeiran germplasm suggests the existence of 15 groups of common beans, which could include landraces or hybrid varieties. Portuguese landraces were shown to be genetically diverse (Martins et al. 2006). It is probable that the same happens in Madeira. The high level of heterogeneity could be the result of different ecological adaptations, since the island mountainous terrain and steep valleys promoted the isolation of populations for centuries. Due to this isolation, genetic drift and low population size probably also contributed to the bean heterogeneity in Madeira. However, to confirm this hypothesis an array of detailed studies of the bean resources on Madeira, particularly involving further molecular and biochemical analyses, are warranted. The anticipated results would assist in trustworthy grouping of these accessions in the racial and landraces classification systems, proposed by Singh et al. (1991a) and by Rodiño et al. (2003), respectively. The evaluation and identification of local common bean landraces will be useful for the future development and implementation of research and breeding programmes. They could be a source of new genes, considering the fact that the Madeiran beans are well adapted to low-input sustainable farming systems and intercropping, frequently used on islands for bean production.

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