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RESEARCH

Genetic Diversity in a Core Collection Established from the Main Bean Genebank in Spain

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ABSTRACT

Common bean (*Phaseolus vulgaris* L.) is a traditional crop in many Spanish regions. A major collection of Spanish common bean landraces is maintained at the National Genebank in the Center for Plant Genetic Resources (CRF), Alcala de Henares, Madrid, Spain. A core collection including 200 common bean accessions was established from the CRF collection. We sought to analyze the genetic diversity of this CRF core collection using morpho-agronomic traits, phaseolin seed protein, and a set of 11 molecular markers. Accessions were classified in 65 groups according to their seed phenotype. Seventy-one accessions have appropriate qualities for culinary use as green or snap beans. The four bean growth habits were present among the accessions included in the CRF core collection, with the indeterminate climbing habit (Type IV; 113 accessions) being the most common. Five different phaseolin patterns were found, the most common being type C (86 accessions), followed by types T (59 accessions) and S (42 accessions). With the set of molecular markers used, an average number of 6.18 alleles marker^{-1} and an average polymorphism information content marker^{-1} of 0.66 were found. The dendrogram and the principal components analysis developed using the molecular marker data revealed the existence of two main groups of accessions corresponding to the Middle American and the Andean gene pools, respectively, and suggested the existence of some intermediate forms. The possible origin of these putative intermediate forms is discussed. Knowledge of the genetic diversity in the CRF core collection will contribute to improved use and conservation of Spanish bean genetic resources.

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Abbreviations: CRF, Center for Plant Genetic Resources; CRF collection, National Spanish common bean collection; MDRK, 'Michigan Dark Red Kidney'; PCA, principal components analysis; PIC, polymorphic information content; SCAR, sequence characterized amplified region; UPGMA, unweighted pair group method with arithmetical averages.

Common bean (*Phaseolus vulgaris* L.) is one of the most important grain legumes for direct human consumption in the world (Broughton et al., 2003). Common bean is mainly harvested fresh as snap bean (pods harvested before the seed development phase) or as dry bean (seeds harvested at complete maturity). The species was domesticated by Middle American and South American cultures (Gepts et al., 1986), and from these regions the species was progressively dispersed worldwide. Two gene pools, Middle American and Andean, have been identified in wild and cultivated common bean associated with these two geographical areas. Variation in the major seed protein phaseolin has contributed significantly to differentiating between these two gene pools. In the Middle American gene pool, the phaseolin type S ('Sanilac') predominates, while the Andean gene pool expresses primarily the phaseolin type T ('Tendergreen'), followed by the C and H types (Gepts et al., 1986; Islam et al., 2002). Morphological differences between the two gene pools were described in domesticated common bean by Singh et al. (1991). Differences between the two gene pools have

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also been revealed using different molecular markers such as random amplified polymorphic DNA (Johns et al., 1997; Beebe et al., 2000), amplified fragment length polymorphisms (Tohme et al., 1996; Beebe et al., 2001), or microsatellites (Blair et al., 2006), which explore genome-wide variation, including linkage group B7 where the phaseolin has been mapped (Freyre et al., 1998).

Since introduction to Europe in the 16th century, common bean has been traditionally grown in many Spanish regions. A considerable diversity has been described among the landraces collected in Spain, suggesting that this area could be considered a secondary center of genetic diversity of common bean. Puerta Romero (1961) described wide morphological variation among 296 local varieties and found that those having white seed and dry culinary use were the most common. From phaseolin data, Gepts and Bliss (1988) suggested the presence of the two gene pools in the Iberian Peninsula. Using morphological traits, allozyme markers, and phaseolin variants, Rodiño et al. (2001, 2006) and Santalla et al. (2002) described local bean germplasm from the Iberian Peninsula as Middle American or Andean in origin, with the majority of materials having Andean origin. A few inter–gene pool recombinants also were observed.

The National Spanish common bean collection (CRF collection) is maintained at the National Genebank in the Center for Plant Genetic Resources (CRF), Alcalá de Henares, Madrid, Spain. At present, the CRF collection is formed by 2900 accessions (active collection) from which 1250 constitute the base collection. Most of these accessions derive from collecting missions performed in Spain since 1978 or from acquiring accessions from regional genebanks included in the Network of the Spanish Program of Conservation and Utilization of Plant Genetic Resources for Food and Agriculture.

A core collection of 200 bean accessions, established from the CRF collection considering geographical criteria and seed phenotype (De la Rosa et al., 2000), was formed to represent the genetic diversity and genetic structure maintained in the larger germplasm collection (Brown, 1989; van Hintum et al., 2000). Core collections have been proposed as useful tools for the study, utilization, and management of genetic diversity maintained in large germplasm collections (van Hintum et al., 2000). The objective of the present study was to characterize the genetic diversity of the CRF core collection using morpho-agronomic traits, phaseolin seed protein patterns, and DNA markers. Extensive knowledge of this core collection will contribute to improved use and conservation of bean genetic resources from Spain.

MATERIALS AND METHODS

Plant Germplasm

The 200 accessions included in the CRF core collection (De la Rosa et al., 2000) are listed in Table 1. Passport data can be found in the Spanish Plant Genetic Resources Inventory (http://wwwx.inia.es/webcrf/CRFing/PaginaPrincipal.asp [verified 29 Mar. 2009]). The international cultivars Sanilac, Michelite, TU, AB136, G2333, Tendergreen, and Michigan Dark Red Kidney (MDRK), and the wild *Phaseolus vulgaris* accessions G13004 and G23415 were included as reference materials for analysis of the phaseolin and DNA markers. G13004, Sanilac, Michelite, TU, AB136, and G2333 belong to the Middle American gene pool. G23415, Tendergreen, and MDRK belong to the Andean gene pool. The *Phaseolus coccineus* L. accession V215 was also included in this study.

To characterize morpho-agronomic traits, the 200 accessions were planted in the greenhouse at Villaviciosa, Asturias, Spain, during the spring. One replication, consisting of 10 or 12 plants in a meter-long row for each accession, was planted in a single year. There were two replications (2004 and 2005) in a randomized complete block design.

Morpho-Agronomic Traits

Core collection accessions were characterized for i) seed phenotype, recorded following the *Phaseolus vulgaris* descriptor list from IBPGR (1982), including seed coat color (white, cream, yellow, brown, pink, red, purple, black, and gray), pattern (absent, bicolor, constant mottled, speckled, and striped), shape (kidney, oblong, oval, and round), cross-section (flat, oval, and round), size (g 100 weight^{-1}; an average of the 2 yr), and market class (Voysest, 2000; Santalla et al., 2001); ii) growth habit, according to Singh (1982) as determinate (Type I), indeterminate erect (Type II), indeterminate prostrate (Type III), and indeterminate climbing (Type IV); and iii) pod color (green, yellow, and mottled) and potential culinary use as snap bean, considering pod phenotype (length, fiber amount, fleshiness, and softness) at the R8 stage (Fernández et al., 1985).

Phaseolin Determination

Phaseolin protein pattern was analyzed in five individual seeds accession^{-1} using the sodium dodecyl sulfate polyacrylamide gel electrophoresis system of Laemmli (1970) as modified by Ferreira et al. (2000). Proteins were extracted from flour samples (0.01 to 0.02 g) taken from the raphe end of each seed placed in a buffer solution (62 mM Tris-HCl pH 8.8, 2% (w/v) sodium dodecyl sulfate, 10% (v/v) glycerol, and 0.005% (w/v) bromphenol blue) for 6 h at room temperature. The extracts were later reduced with one drop of 2-mercaptoethanol. The mixture was heat treated (100°C) for 5 min, centrifuged, and electrophoresis of the supernatant was performed using 1-mm-thick slab gels of 12 or 17% (w/v) polyacrylamide. The proteins were visualized using Coomassie Brilliant Blue R.

Molecular Marker Analyses

Genomic DNA was isolated from bulked young leaves from five to 10 plants accession^{-1}, using the Nucleon PhytoPure Genomic DNA Extraction Kit (Amersham Biosciences, Fairfield, CT) following the supplier's instructions. To select a set of molecular markers showing a high polymorphism level, the cultivars TU, MDRK, Michelite, and AB136 were analyzed for 35 microsatellite and sequence characterized amplified region (SCAR) markers previously mapped in the common bean genetic map (Miklas et al., 2000; Blair et al., 2003; Larsen and

Miklas, 2004). Based on the criteria of at least one marker linkage group^{-1}, high polymorphism level, and easy allele identification, 11 markers were selected: SCAR markers SW12 (Singh et al., 2000) and SAP6 (Miklas et al., 2000), and microsatellite markers BMd17, BM184, BM151, BMd45, BM170, BM210, BM172, BM141, and BM175 (Gaitán-Solís et al., 2002; Blair et al., 2003). Amplification of these markers was performed as described by the respective authors. Polymerase chain reaction products corresponding to the SCAR markers were resolved on 2% agarose gels, stained with ethidium bromide, and visualized under ultraviolet (UV) light. Polymerase chain reaction products corresponding to the microsatellite markers were resolved on 8% polyacrylamide gels, stained with ethidium bromide, and visualized under UV light. A 100-bp ladder (Amersham Biosciences, Fairfield, CT) and the software PhotoCaptMw (Vilber Lourmat, Marne-la-Vallée, France) were used to measure the size of the amplification products.

Data Analysis

Molecular marker data were scored as presence (1) or absence (0) of amplified bands. Diversity for each marker was calculated using the polymorphic information content (PIC) according to Anderson et al. (1993): PIC = $1 - \sum p_{ij}^2$, where p_{ij} is the frequency of the patterns (j) for each marker (i). The data matrix obtained for presence or absence of bands was analyzed by two methods to group the accessions. Principal components analysis (PCA) was performed with NTSYSpc software version 2.11V (Rohlf, 2002) and the two principal components were used to visualize the dispersion of the accessions in a graphic. Cluster analysis was performed using unweighted pair group method with arithmetical averages (UPGMA) with NTSYSpc software version 2.11V. A dendrogram was created with the TREE program of NTSYSpc software version 2.11V from the similarity matrix obtained using Jaccard's similarity coefficient (Jaccard, 1928). Significant associations between germplasm groups and morpho-agronomic traits, phaseolin types, and DNA marker variants were identified by means of contingency tests.

RESULTS AND DISCUSSION

Morpho-Agronomic Traits and Phaseolin Diversity

According to seed phenotype, accessions of the CRF core collection were classified in 65 groups (Table 2), 43 of which corresponded to market classes previously described by Voysest (2000) and Santalla et al. (2001). The identified market classes, small white, great northern, canella, cranberry, white kidney, cannellini, hook, or fabada, have high preference and an established reputation among consumers and are traditionally cultivated in several regions in Spain. The remaining 22 groups were designated CRF01 to CRF22. From the green pod characterization it was concluded that 71 accessions (59 green, 9 yellow, and 3 striped) have appropriate qualities for culinary use as snap bean. These accessions were included in 33 seed phenotype groups. With respect to growth habit, indeterminate climbing was the most common (Type IV; 113 accessions),

Table 1. List of common bean accessions included in the Center for Plant Genetic Resources core collection described by De la Rosa et al. (2000). Passport data can be found at http://wwwx.inia.es/webcrf/CRFing/PaginaPrincipal.asp (verified 29 Mar. 2009).

BGE000993	BGE003079	BGE003550	BGE004434	BGE011016	BGE020003	BGE023679	BGE026196
BGE001144	BGE003121	BGE003554	BGE004435	BGE011021	BGE020030	BGE024024	BGE026211
BGE001145	BGE003122	BGE003555	BGE004445	BGE011023	BGE020048	BGE024038	BGE026222
BGE001452	BGE003128	BGE003559	BGE004452	BGE011026	BGE020119	BGE024699	BGE027076
BGE001472	BGE003138	BGE003562	BGE004453	BGE011030	BGE022070	BGE025069	BGE027085
BGE001539	BGE003139	BGE003568	BGE004454	BGE011037	BGE022106	BGE025080	BGE027961
BGE001856	BGE003161	BGE003626	BGE004459	BGE011058	BGE022120	BGE025085	BGE027962
BGE002016	BGE003164	BGE003645	BGE004469	BGE011060	BGE022129	BGE025124	BGE028939
BGE002108	BGE003165	BGE003654	BGE004489	BGE011062	BGE022366	BGE025130	BGE028940
BGE002116	BGE003168	BGE003693	BGE004496	BGE011731	BGE022378	BGE025142	BGE028947
BGE002132	BGE003203	BGE003696	BGE004673	BGE011732	BGE022476	BGE025180	BGE028953
BGE002134	BGE003208	BGE003700	BGE004813	BGE011735	BGE022480	BGE025330	BGE028958
BGE002152	BGE003246	BGE003705	BGE005439	BGE011736	BGE022494	BGE025739	BGE028960
BGE002188	BGE003254	BGE003746	BGE005440	BGE011753	BGE022504	BGE025740	BGE028964
BGE002189	BGE003261	BGE003955	BGE005475	BGE011758	BGE022508	BGE025745	BGE029568
BGE002196	BGE003266	BGE003966	BGE005484	BGE011762	BGE022510	BGE026146	BGE029569
BGE002201	BGE003274	BGE003997	BGE005487	BGE013952	BGE022512	BGE026151	BGE029581
BGE002204	BGE003283	BGE004000	BGE008273	BGE013953	BGE022519	BGE026155	BGE029586
BGE002207	BGE003293	BGE004005	BGE008274	BGE013962	BGE022827	BGE026158	BGE029593
BGE002209	BGE003298	BGE004010	BGE008987	BGE013964	BGE022831	BGE026163	BGE029604
BGE003029	BGE003404	BGE004025	BGE009979	BGE013965	BGE022832	BGE026166	BGE029629
BGE003037	BGE003482	BGE004026	BGE010387	BGE013972	BGE022836	BGE026169	BGE029705
BGE003043	BGE003483	BGE004031	BGE010548	BGE013980	BGE022837	BGE026172	BGE030143
BGE003073	BGE003484	BGE004034	BGE010549	BGE013981	BGE023180	BGE026173	BGE030453
BGE003074	BGE003487	BGE004432	BGE010957	BGE019991	BGE023190	BGE026186	BGE030893

Table 2. Morpho-agronomic characterization of the common bean accessions included in the Center for Plant Genetic Resources core collection. Data for all accessions are available on request.

Market class or group	No. of accessions	Seed phenotype					Growth habit				Pod phenotype				Phaseolin type					Gene pool†	
											Color										
		Coat color‡	Coat pattern	Shape	Cross-section	Size§	I	II	III	IV	Green	Yellow	Mottled	Snap¶	S	T	C	A	H	A	M
Black cannellini	6	bl	Absent	Oval	Oval	Small	3	–	1	2	5	1	–	2	1	3	2	–	–	5	1
Black mottled	1	bl, c	Constant mottled	Oblong	Round	Medium	1	–	–	–	1	–	–	1	–	1	–	–	–	1	
Black turtle	2	bl	Absent	Round	Oval	Small	–	–	2	–	2	–	–	1	2	–	–	–	–		2
CRF07	2	bl	Absent	Kidney	Oval	Medium	–	–	–	2	2	–	–	–	1	–	1	–	–		2
CRF08	1	bl	Absent	Oval	Round	Medium	–	–	–	1	1	–	–	1	–	1	–	–	–	1	
CRF19	1	bl	Absent	Oval	Round	Large	–	–	–	1	1	–	–	–	–	–	1	–	–	1	
Negro brillante	8	bl	Absent	Oval	Round	Medium	–	–	–	8	5	3	–	2	–	–	6	1	1	8	
Brown garbanzo	6	br	Absent	Oval	Oval	Medium	–	–	–	6	6	–	–	4	4	–	2	–	–	2	4
Brown marrow	2	br	Absent	Oval	Oval	Medium	–	–	–	2	1	1	–	2	–	–	2	–	–	2	
Brown mottled	7	br	Absent	Oblong	Flat	Large	–	–	–	7	5	2	–	4	4	2	–	1	–	1	6
CRF03	1	br	Absent	Kidney	Round	Large	–	–	–	1	1	–	–	1	–	–	1	–	–	1	
CRF04	2	br	Absent	Kidney	Round	Extra large	–	–	–	2	2	–	–	2	–	–	2	–	–	2	
CRF05	2	br	Absent	Kidney	Flat	Small	–	–	–	2	–	2	–	1	–	–	2	–	–	1	1
CRF14	1	br, c	Constant mottled	Oval	Oval	Medium	1	–	–	–	1	–	–	–	–	1	–	–	–	1	
CRF18	1	br	Absent	Oval	Oval	Extra small	1	–	–	–	–	1	–	–	–	1	–	–	–	1	
CRF20	1	br	Absent	Kidney	Flat	Small	–	–	1	–	1	–	–	–	1	–	–	–	–		1
CRF21	1	br	Absent	Kidney	Oval	Large	–	–	–	1	1	–	–	–	–	–	1	–	–	1	
Dark garbanzo	3	br	Absent	Oval	Round	Medium	1	–	–	2	–	3	–	–	–	–	3	–	–	3	
Manteca	3	br	Absent	Kidney	Flat	Medium	1	–	–	2	2	1	–	1	1	1	1	–	–	1	2
Mulatinho	2	br	Absent	Kidney	Round	Small	2	–	–	–	–	2	–	1	–	2	–	–	–	2	
Bayo gordo	6	c	Absent	Oval	Round	Large	–	–	–	6	6	–	–	1	–	2	4	–	–	6	
Bico de Ouro	2	c	Absent	Oval	Oval	Small	–	–	2	–	2	–	–	–	2	–	–	–	–		2
Canela	6	c	Absent	Kidney	Oval	Large	2	–	–	4	6	–	–	2	–	2	3	1	–	6	
Carioca	1	c, br, g	Striped	Oval	Oval	Extra small	–	–	1	–	–	–	1	–	1	–	–	–	–		1
Cranberry	3	c, pu	Striped	Oval	Oval	Large	–	–	1	2	3	–	–	1	–	–	1	2	–	3	
CRF02	4	c, br	Constant mottled	Kidney	Round	Medium	4	–	–	–	3	–	1	4	–	–	4	–	–	4	
CRF11	1	c	Absent	Kidney	Flat	Small	–	1	–	–	1	–	–	–	1	–	–	–	–		1
CRF13	1	c, y	Striped	Kidney	Flat	Small	–	–	–	1	1	–	–	1	1	–	–	–	–		1
Large cranberry	8	c, pu	Striped	Kidney	Oval	Large	1	–	1	6	4	–	4	3	–	2	4	1	–	8	
Ojo de cabra	2	c, g, br	Striped	Oval	Oval	Large	–	–	–	2	2	–	–	1	–	–	2	–	–	2	
CRF16	1	g	Absent	Kidney	Oval	Medium	–	–	1	–	1	–	–	–	1	–	–	–	–		1
CRF17	1	g	Absent	Round	Round	Medium	–	–	1	–	1	–	–	–	–	–	1	–	–	1	
Light red kidney	1	p	Absent	Oblong	Round	Medium	–	–	–	1	1	–	–	–	–	–	1	–	–	1	
Miss Kelly	1	p, pu	Striped	Kidney	Oval	Small	1	–	–	–	1	–	–	–	–	1	–	–	–	1	

Market class or group	No. of accessions	Seed phenotype					Growth habit				Pod phenotype				Phaseolin type					Gene pool†	
											Color										
		Coat color‡	Coat pattern	Shape	Cross-section	Size§	I	II	III	IV	Green	Yellow	Mottled	Snap¶	S	T	C	A	H	A	M
Rosada	20	p	Absent	Oval	Round	Large	4	–	4	12	19	1	–	10	2	–	14	2	1	18	2
Rosinha	1	p	Absent	Kidney	Round	Small	–	–	1	–	1	–	–	–	1	–	–	–	–		1
CRF09	1	pu	Absent	Oblong	Oval	Large	–	–	–	1	1	–	–	–	–	–	1	–	–	1	
CRF10	1	pu	Absent	Kidney	Oval	Small	–	–	1	–	–	1	–	–	–	–	1	–	–	1	
CRF15	1	pu, c	Speckled	Round	Round	Small	1	–	–	–	1	–	–	–	–	–	1	–	–	1	
Red caparron	2	pu, w	Bicolor	Oval	Round	Medium	–	–	1	1	2	–	–	–	–	–	2	–	–	2	
Dark red kidney	2	r	Absent	Oblong	Oval	Medium	2	–	–	–	2	–	–	–	–	2	–	–	–	2	
Gernikesa	1	r	Absent	Round	Round	Small	–	–	–	1	1	–	–	–	–	–	1	–	–	1	
Large red mottled	1	r, c	Constant mottled	Kidney	Flat	Medium	–	1	–	–	1	–	–	–	–	–	1	–	–	1	
Red pinto	4	r, c	Constant mottled	Kidney	Flat	Large	–	–	2	2	4	–	–	1	–	1	3	–	–	4	
Sangretoro	5	r	Absent	Round	Round	Medium	–	–	–	5	4	1	–	2	–	–	5	–	–	4	1
Small red	1	r	Absent	Round	Round	Small	–	–	–	1	1	–	–	–	–	–	1	–	–	1	
Cannellini	2	w	Absent	Oblong	Round	Large	–	–	1	1	2	–	–	–	–	2	–	–	–	2	
CRF01	2	w	Absent	Kidney	Flat	Small	2	–	–	–	2	–	–	–	1	1	–	–	–	1	1
CRF12	2	w, pu, c	Bicolor	Kidney	Round	Large	1	–	1	–	2	–	–	–	–	1	1	–	–	2	
CRF22	1	w	Absent	Kidney	Round	Small	–	–	–	1	1	–	–	1	1	–	–	–	–		1
Fabada	3	w	Absent	Oblong	Oval	Extra large	–	–	–	3	3	–	–	–	–	3	–	–	–		3
Fabada pinto	1	w, pu	Bicolor	Oblong	Round	Extra large	–	–	–	1	1	–	–	–	–	1	–	–	–	1	
Great northern	9	w	Absent	Kidney	Oval	Medium	2	–	4	3	9	–	–	3	5	4	–	–	–	1	8
Hook	1	w	Absent	Kidney	Oval	Medium	–	–	–	1	1	–	–	–	1	–	–	–	–		1
Kidney caparron	1	w, pu, c	Bicolor	Oblong	Oval	Large	–	–	1	–	1	–	–	–	–	–	1	–	–	1	
Large great northern	4	w	Absent	Kidney	Oval	Large	1	–	–	3	4	–	–	1	3	1	–	–	–	1	3
Marrow	11	w	Absent	Oval	Round	Medium	3	2	3	3	11	–	–	7	1	10	–	–	–	9	2
Rounded caparron	7	w, c, pu	Bicolor	Oval	Round	Medium	–	–	1	6	7	–	–	3	–	–	7	–	–	7	
Small white	3	w	Absent	Oval	Oval	Extra small	1	–	1	1	3	–	–	1	3	–	–	–	–		3
White kidney	6	w	Absent	Kidney	Round	Large	4	–	2	–	5	1	–	2	–	6	–	–	–	6	
Azufrado	4	y	Absent	Kidney	Round	Large	1	–	3	–	4	–	–	–	–	3	–	–	1	4	
Canario bola	2	y	Absent	Oval	Oval	Large	–	–	1	1	2	–	–	1	–	1	1	–	–	2	
CRF06	1	y	Absent	Kidney	Round	Small	–	–	1	–	–	1	–	1	–	1	–	–	–	1	
Dorado	3	y	Absent	Kidney	Flat	Large	1	–	–	2	3	–	–	–	–	2	1	–	–	3	
Small yellow	6	y	Absent	Oval	Round	Small	2	–	1	3	6	–	–	2	4	1	1	–	–	3	3

†A, Andean; M, Middle American. Based on the dendrogram constructed using unweighted pair group method with arithmetical averages clustering of Fig. 1.

‡Primary, secondary, and tertiary seed colors are separated by commas and designated as bl, black; br, brown; c, cream; g, gray; p, pink; pu, purple; r, red; w, white; y, yellow.

§Extra small (<25 g 100 seeds^{-1}), small (25–40 g 100 seeds^{-1}), medium (40–55 g 100 seeds^{-1}), large (55–70 g 100 seeds^{-1}), extra large (>70 g 100 seeds^{-1}).

¶Potential culinary use as snap bean.

followed by determinate (Type I; 43 accessions) and indeterminate prostrate (Type III; 40 accessions). The indeterminate erect growth habit was relatively scarce (Type II; four accessions). Within-group variation for growth habit was present in 21 seed phenotype groups.

Similar wide diversity for seed phenotype was observed in other bean collections from Spain. Puerta Romero (1961) characterized 296 bean accessions, with white, oblong, and large-seed phenotypes the most common. Santalla et al. (2002) classified 343 accessions in 48 market classes, and Rodiño et al. (2003) grouped 388 accessions in 50 market classes and proposed a core collection with 52 accessions containing 31 market classes. These studies described a higher ratio of white-seeded to color-seeded accessions (163 white:198 colored and 185 white:226 colored, respectively) than observed in the CRF core collection (42 white:158 colored). The potential culinary use of the accessions as snap bean was not evaluated in the previous two studies. For the CRF core collection, 71 of 200 accessions had pod traits corresponding to snap or green bean (Table 2). Puerta Romero (1961) also described a high proportion of snap bean in a Spanish germplasm collection (77 out of 296) and, in many cases (49 out of 296), accessions exhibited dual use as either a snap or dry bean.

Five different phaseolin patterns were observed (Table 2). The most common pattern was phaseolin type C (86 accessions), followed by types T, S, A, and H with 59, 42, 8, and 3 accessions, respectively. Within-group variation in phaseolin type was present in 21 seed phenotype groups, of which 11 had phaseolin patterns of both the Middle American and Andean gene pools. Previous phaseolin studies on bean accessions collected in the Iberian Peninsula (Gepts and Bliss, 1988; Santalla et al., 2002) similarly found the C and T phaseolin patterns as the most frequent.

Molecular Marker Diversity and Cluster Analysis

The number of alleles and the PIC values for the nine microsatellite and two SCAR markers are presented in Table 3. Molecular markers SAP6, SW12, BM170, and BMd17 presented null allelles (lack of amplification). All markers had a maximum of one amplified band accession^{-1} except the SCAR marker SW12, which had a maximum of three bands. Rodríguez-Suárez et al. (2007) observed that at least two of the SW12 bands represented closely linked loci. Evidences of heterogeneous accessions were not obtained. The number of alleles varied between two and nine, with an average number of 6.2 alleles marker^{-1}. An average PIC value marker^{-1} of 0.66 was found with this set of molecular markers. Although the number of alleles and PIC values for the microsatellites, in most cases, were smaller than those reported by Blair et al. (2006), the level of polymorphism for the markers was relatively high, confirming the wide diversity contained in the CRF core collection. With the set of markers used, 196 different genotypes were identified among the 200 accessions. For other species, such as grapevine (*Vitis vinifera* L.) cultivars (This et al., 2004) or tomato (*Solanum lycopersicum* L.) (Bredemeijer et al., 2002), a set of reference cultivars and a minimal standard markers set were proposed for investigating genetic diversity and identifying specific cultivars. Our work similarly describes a set of well-known cultivars and molecular markers whose variants or alleles are easily identified.

The UPGMA cluster analysis differentiated two major groups of accessions (Fig. 1). The group considered Middle American (designated as M) is formed by 60 accessions and includes the Middle American reference materials Sanilac, Michelite, TU, AB136, and G2333, and wild accession G23415. The group considered Andean (designated as A) is formed by 149 accessions and includes the Andean reference materials Tendergreen, MDRK, and wild accession G13004. The number of accessions assigned to each gene pool within each market class or group according to seed phenotype is indicated in Table 2. Among the 71 accessions having snap bean pod characteristics, 20 were Middle American and 51 were Andean gene pool. In Fig. 1, the presence of four pairs of accessions having an identical combination of marker alleles (Jaccard's genetic distance = 1) is indicated. However, these pairs of accessions showed

Table 3. Number of alleles and polymorphism information content (PIC) of the DNA markers used to analyze 200 common bean core accessions and 10 reference materials.

Marker	Marker type†	Linkage group	Amplification patterns									PIC
			1	2	3	4	5	6	7	8	9	
			bp									
BM141	SSR	B9	185	190	195	218	240	243	245	248	260	0.81
BM151	SSR	B8	136	142	145	148	150	153	–	–	–	0.71
BM170	SSR	B6	Null	200	210	220	230	240	250	–	–	0.79
BM172	SSR	B3	84	87	89	92	94	96	98	110	119	0.75
BM175	SSR	B5	162	170	173	180	185	190	195	198	200	0.76
BM184	SSR	B11	150	155	157	160	163	–	–	–	–	0.76
BM210	SSR	B7	160	165	170	178	183	188	195	–	–	0.70
BMd17	SSR	B2	Null	94	105	120	–	–	–	–	–	0.56
BMd45	SSR	B1	150	200	–	–	–	–	–	–	–	0.47
SAP6	SCAR	B10	Null	800	–	–	–	–	–	–	–	0.44
SW12‡	SCAR	B4	Null	475	550	700	725	750	600/725	550/725/750	–	0.56

†SCAR, sequence characterized amplified region; SSR, simple sequence repeat.

‡The SCAR marker SW12 had a maximum of three amplified bands.

different seed phenotypes: BGE002108 (small yellow) and BGE008273 (brown mottled); BGE022508 (CRF20) and BGE022512 (rosada); BGE003283 (negro brillante) and BGE003645 (dark garbanzo); BGE022476 (rounded caparron) and BGE022494 (brown marrow). Subgroups were not distinguished within the two gene pools, and accessions did not cluster by site of collection.

Table 4 shows the comparison (contingency chi-square tests) between the frequencies of the different morpho-agronomic phenotypes (seed and pod phenotype and growth habit) and phaseolin types in the two groups established by the UPGMA dendrogram. Differences between observed and expected values were highly significant in all cases except for seed shape and culinary use (snap and/or dry vs. only dry). The Andean group possessed a higher number of round-section, large-seeded, and determinate accessions than expected under a random hypothesis. Conversely, a higher number of oval-flat section, small-seeded, and indeterminate accessions were present in the Middle American group. The frequencies of phaseolin types T and C were significantly higher in the Andean group, whereas the frequency of type S phaseolin was significantly higher in the Middle American group.

Variation among the three principal components accounted for 25.9, 11.3, and 5.1% variation, respectively. Figure 2 depicts distribution of the 209 *P. vulgaris* accessions (the *P. coccineus* accession was not included in this analysis) obtained from the first two principal components (Dim-1 and Dim-2). The two groups established by the cluster analysis are not clearly separated by PCA. Although Andean accessions are located mainly top left, and the Middle American accessions bottom right, 16 accessions showed an intermediate position. These accessions belong to 10 seed phenotypes including the following market classes: brown mottled (BGE002204, BGE026172), canario bola (BGE005439), fabada (BGE010957, BGE011016, BGE027962), negro brillante (BGE003559, BGE022378), red pinto (BGE028939), sangretoro (BGE003138), small red (BGE011058), small yellow (BGE004031, BGE004435, BGE020030), white kidney (BGE022129), and the group CRF1 (BGE024024). Five of them have pods with appropriate characteristic for culinary use as snap bean.

SUMMARY

The UPGMA and the principal components analyses using molecular marker data revealed the existence of two main groups of accessions in the CRF core collection. The two groups correspond to the Middle American and the Andean gene pools, as supported by the reference materials included in the study, phaseolin types, and different frequencies for growth habit and seed size. The occurrence of two gene pools and characterization

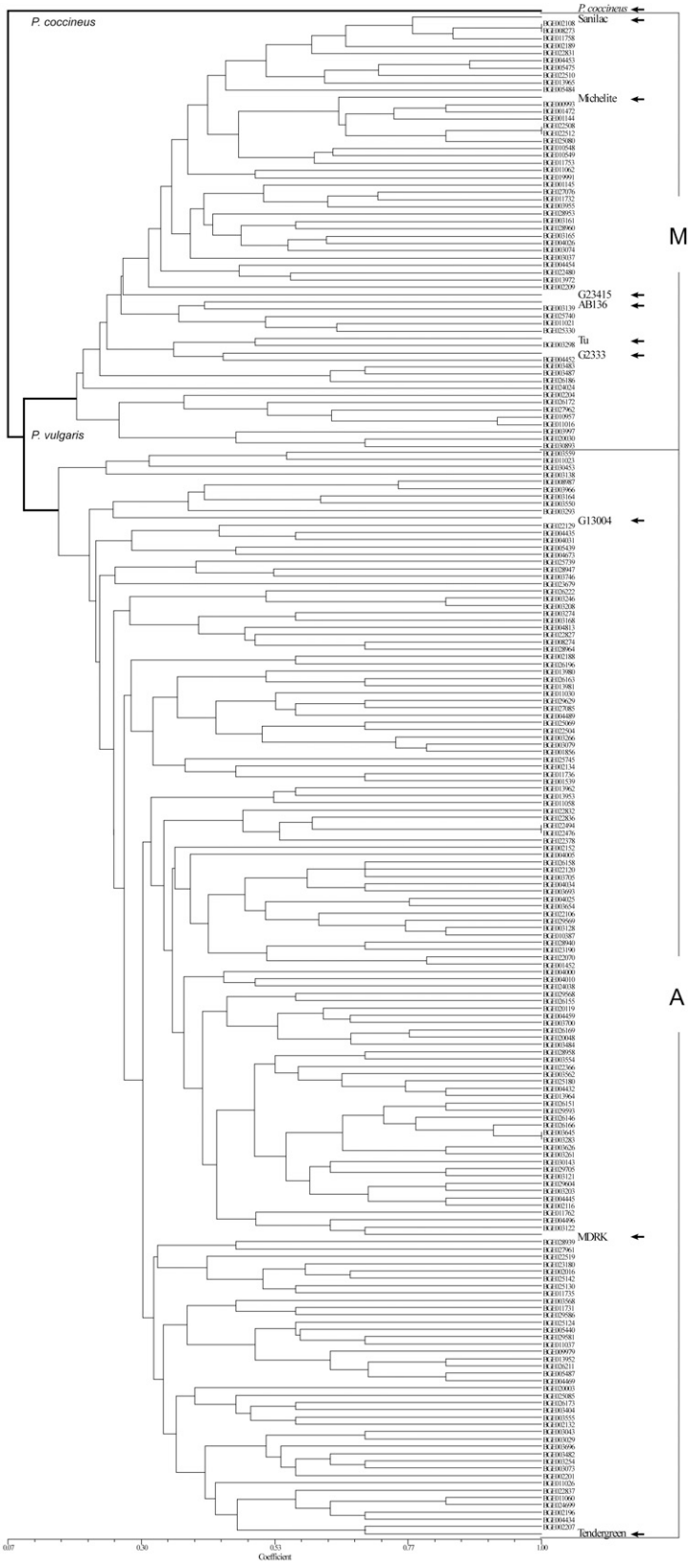

Figure 1. Dendrogram constructed using unweighted pair group method with arithmetical averages clustering, based on Jaccard's distance analysis of a data set of 11 molecular markers, performed with 210 *Phaseolus vulgaris* accessions (200 accessions of the Center for Plant Genetic Resources core collection and 10 reference cultivars). The Middle American (M) and the Andean (A) reference materials, and the *Phaseolus coccineus* accession are indicated. MDRK = 'Michigan Dark Red Kidney'.

Table 4. Comparison between the frequencies of the different morpho-agronomic common bean phenotypes (seed and pod phenotype and growth habit) and phaseolin types in the Middle American and Andean gene pools established by a dendrogram constructed using unweighted pair group method with arithmetical averages clustering.

Trait	Main type	Andean		Middle American		χ^2	*P*
		Observed	Expected	Observed	Expected		
Seed shape	Round	10	11	5	4.1		
	Oval	54	54	20	20		
	Oblong	17	15.3	4	5.7		
	Kidney	65	65.7	25	24.3	1.80	ns
Seed cross-section	Round	88	73.7	13	27.3		
	Oval	48	57.7	31	21.3		
	Flat	10	14.6	10	5.4	21.60	***
Seed size	Extra large	10	9.5	3	3.5		
	Large	59	44.5	2	16.5		
	Medium	56	53.3	17	19.7		
	Small	20	35.8	29	13.2		
	Extra small	1	2.9	3	1.1	48.45	***
Growth habit	Determinate	40	31.4	3	11.6		
	Indeterminate	106	114.6	51	42.4	11.14	***
Culinary use	Snap and/or dry	51	51.8	20	19.2		
	Only dry	95	94.2	34	34.8	0.07	ns
Phaseolin type	S	2	30.5	40	11.5		
	T	49	42.9	10	16.1		
	C	82	62.5	4	23.5		
	A	8	5.8	0	2.2		
	H	3	2.2	0	0.8	127.29	***

***Significant at 0.001 probability level.

thereof based on phaseolin and morpho-agronomic traits agrees with previous observations for bean germplasm collections in Spain and elsewhere (Gepts and Bliss 1988; Singh et al., 1991; Santalla et al., 2002; Rodiño et al., 2003, 2006).

The intermediate positions for some accessions in the PCA suggest possible derivation from recombination between the two gene pools. Santalla et al. (2002) and Rodiño et al. (2003, 2006) suggested the existence of recombinants between the Middle American and Andean gene pools in accessions from Spain and Portugal. They mainly attributed the presence of such recombinants to natural cross-pollination between common bean of different origins, and concluded that the Iberian Peninsula is a secondary center of genetic diversity for common bean. However, the majority of these putative recombinant materials could have a different origin and the natural occurrence of genetic diversity in the Iberian Peninsula could be lower than indicated by these authors. Accessions maintained in the Network of Spanish Bean Genebanks were obtained in different collecting missions, performed since 1970, including areas where small farmers selected and maintained their own cultivars, considering specific criteria in each area such as seed or pod phenotype, culinary tradition, and cropping system. The cultivation of mixed bean materials (mixed populations for morphological traits) in such areas is practically nonexistent, and the outcrossing rate in the local conditions is extremely low (Ferreira et al., 2000). As indicated by Doré and Varoquaux (2006), the presence of elite cultivars (mainly snap beans) derived from bean breeding programs in Europe, especially in France, began at the end of the 19th century. In Spain the commercialization and distribution of snap bean cultivars has been traditionally more common than those of dry beans. The exchange of dry bean seeds between farmers is very common due to the limited availability of commercial dry bean cultivars. For these reasons, although most accessions present in the Spanish genebanks could be considered as landraces or local germplasm, the possibility for some accessions being originally derived from elite cultivars obtained in breeding programs, including crosses between different gene pools, especially in the case of snap beans, cannot be excluded.

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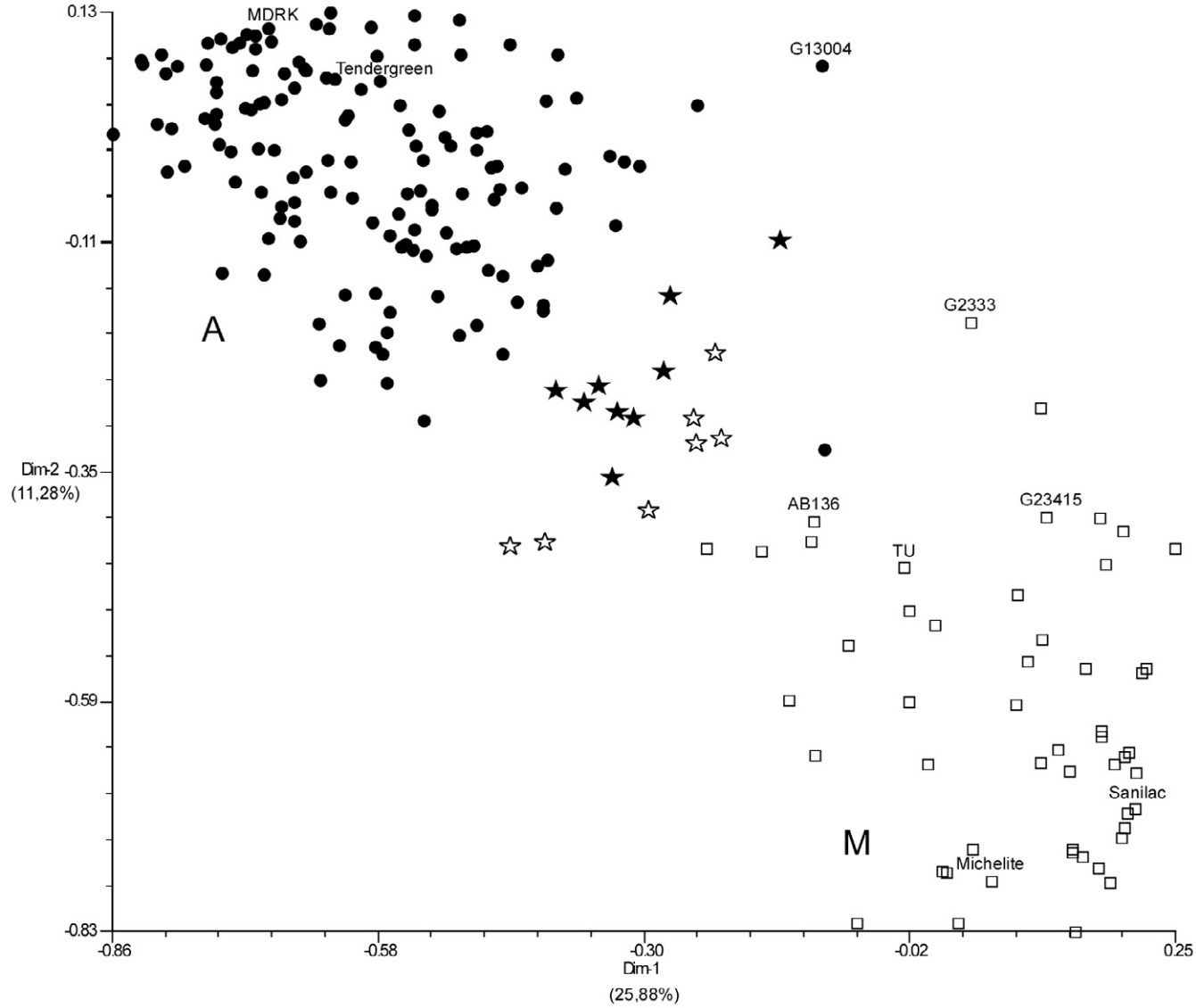

Figure 2. Plot of distribution of 209 *Phaseolus vulgaris* accessions obtained from Dimensions 1 and 2 (Dim-1 and Dim-2) of a principal components analysis performed with a data set of 11 molecular markers. Middle American (M) and Andean (A) reference materials are indicated. The accessions discriminated in the dendrogram constructed using unweighted pair group method with arithmetical averages clustering as belonging to the Andean or the Middle American gene pools are indicated as filled circles or stars and open squares or stars, respectively. The putative recombinant accessions are indicated by stars. MDRK = 'Michigan Dark Red Kidney'.

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