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RESEARCH ARTICLE

Genetic diversity and population structure of a common bean (*Phaseolus vulgaris* L.) collection from Calabria (Italy)

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Abstract Eighty-seven *Phaseolus vulgaris* landraces, still cultivated in Calabria (Italy), were investigated in order to study the patterns of common bean genetic diversity in this region, to better understand the evolutionary development of beans in Europe and to properly manage these genetic resources. Four American accessions and five Italian varieties were also included. Different markers, such as 12 microsatellites, seed traits, phaseolins and 100-seed weight were combined with different statistical approaches. For each microsatellite, expected (H_e) and observed (H_o) heterozygosities, polymorphism information content (PIC), probability of identity (PI) and homozygosity were calculated. Furthermore, in Calabrian group of bean landraces, total (N_a) and private (N_{pa}) number of alleles, observed (H_o), expected heterozygosities (H_e) and allelic richness (AR) were calculated. Genetic distances among landraces were estimated using Nei's coefficient and a cluster analysis using the UPGMA algorithm was performed. The results clearly indicated that: (1) Calabrian germplasm showed a high level of diversity ($H_e = 0.595$); (2) Mesoamerican and Andean gene pools were clearly distinguished in Calabrian germplasm, with the Andean gene pool predominating (83 %); (3) Calabrian landraces were largely hybridized within and between the gene pools. A model-based approach, using the STRUCTURE software, was adopted. Six groups, including 4 of Andean origin and one of Mesoamerican origin were identified. Even more interesting, a small group (8 %) showed a distinct genetic structure, in which interspecific hybridizations with runner bean (*Phaseolus coccineus* L.) could have occurred. Nevertheless, a relatively high proportion of Calabrian bean landraces (12.6 %) was derived from intra and interspecific hybridizations.

Keywords Cluster analysis · Intra-and inter-specific hybridization · Microsatellites–Simple Sequence Repeat (SSR) · *Phaseolus coccineus* L.

Introduction

The common bean (*Phaseolus vulgaris* L.), originally from Latin America, is an important food legume that provides a source of protein in the diet of many people around the world (Broughton et al. 2003) and is mainly cultivated in eastern and southern Africa and Latin America. Europe has only 1 % of the worldwide land that is committed to bean production (FAOSTAT 2009).

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Common bean was introduced into Europe from two centers of domestication, Central and South America, where the Mesoamerican and the Andean cultivated gene pools were originated. Gene pool diversity is based on seed size, phaseolin seed protein patterns, morphological traits, allozymes and DNA markers (Gepts et al. 1986; Koenig and Gepts 1989; Singh et al. 1991a, b; Singh et al. 1991c; Tohme et al. 1996; Beebe et al. 2000; Beebe et al. 2001). Within each gene pool, different groups of landraces or 'races' have been identified according to their morphological traits and agro-ecological adaptations (Singh et al. 1991a, b; Gepts 1988). The dissemination and evolution of the common bean in Europe from both centers of domestication have not been completely clarified yet due to a wide geographic diffusion among different climatic environments and the divergent selection criteria for agronomic traits chosen by farmers (Piergiovanni et al. 2006). In addition, the spread of the crop from South America into Europe has caused a genetic erosion of European common bean germplasm, whose incidence has to be further verified as reported by Angioi et al. (2010). Recently, using phaseolin and molecular markers, the highest incidence ever of the Andean gene pool (around 70 %) in common bean has been found across all European countries (Logozzo et al. 2007; Angioi et al. 2010) and at the regional level (Limongelli et al. 1996; Escribano et al. 1998; Sicard et al. 2005; Angioi et al. 2009).

In addition, there has been a high percentage (44 %) of hybridization between the Andean and Mesoamerican gene pools reported, with different frequencies being found in Central and Southern Europe (Angioi et al. 2010). In Spain and Italy, for example, this hybridization seems to be rare, although recently, some evidence for hybridization has been shown in Sardinia, Italy (Angioi et al. 2009). Furthermore, interspecific cross hybridizations between common and runner beans (*Phaseolus coccineus* L.) could have taken place (Sicard et al. 2005; Papa et al. 2006; Spataro et al. 2011). The runner bean, introduced into Europe along with the common bean (Gepts and Debouck 1991; Santalla et al. 2004), is a perennial outcrossing crop often cultivated in small gardens in Western and Southern Europe (Acampora et al. 2007; Spataro et al. 2011).

In Italy, many farmers maintained their old local landraces, populations or varieties which were well adapted to the pedoclimatic conditions of their limited geographical areas, and exchanged their seeds with surrounding areas, mainly in local markets (Piergiovanni et al. 2000b). Protecting this germplasm, from regions where agriculture still maintains a traditional structure and where the fixation of local ecotypes has taken place, should be considered a high priority and needs to be collected, characterized and evaluated for potential utilization.

In recent years, molecular markers have developed into an important tool in the analysis of genetic diversity (Pallottini et al. 2004; Kwak and Gepts 2009; Burle et al. 2010). Furthermore, the knowledge of genetic distances among the landraces has become determinant in improving the productivity of bean varieties by molecular assisted breeding programs.

With the aim of reducing genetic erosion, the focus of the present study was to collect and characterize a large collection of common bean landraces that are still cultivated in Calabria (Italy) in order to clarify the origin and the genetic diversity of this germplasm. These results have allowed to: (1) clarify the bean dissemination process in Europe; (2) identify the original gene pool (Andean and Mesoamerican) of the cultivated Calabrian common bean; (3) evaluate the cross hybridization frequency between the two gene pools of origin in the Calabrian common bean and (4) identify rare events of natural interspecific hybridization among *Phaseolus* species in Europe.

Materials and methods

Plant material

Eighty-seven bean landraces (each seed lot obtained from a single farmer) from different areas of Calabria (Fig. 1) along with five Italian commercial varieties and four American accessions were analyzed. The American genotypes and the Calabrian landraces were kindly provided by Centro Internacional de Agricoltura Tropical (CIAT, Colombia) and by Agenzia Regionale Sperimentazione e Servizi in Agricoltura (ARSSA—Calabria, Italy), respectively. The collection was grouped according to the type and the site of origin (Table 1).

Morpho-phenotypic seed analysis

The following seed traits on twenty seeds for each landrace were measured with a millimeter (mm)

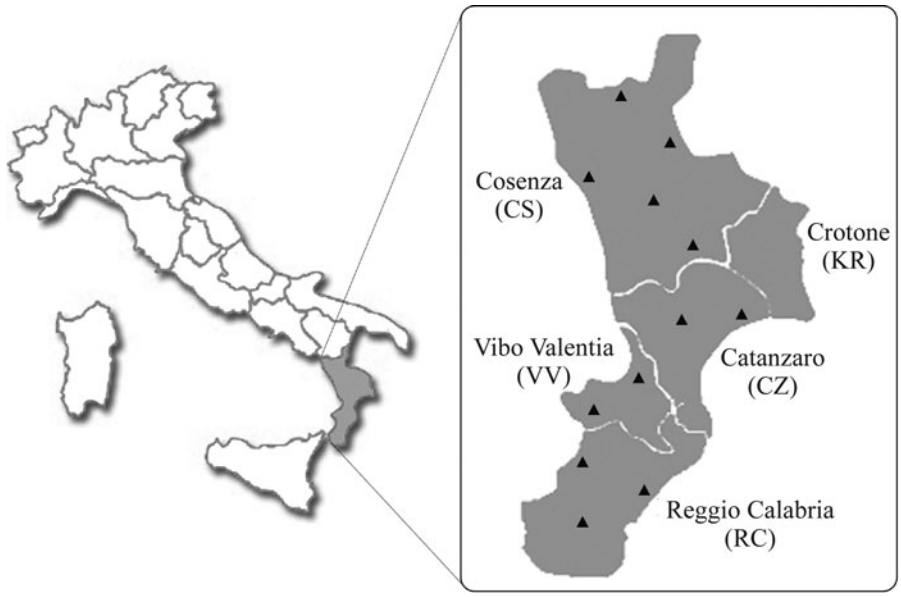


Fig. 1 The Calabria region and its provinces: *Cosenza* (CS), *Crotone* (KR), *Catanzaro* (CZ), *Vibo Valentia* (VV) and *Reggio Calabria* (RC); the *triangles* indicate the different areas where the bean germplasm was collected

adjustment caliper: width, end to end length and side to side height at the hilum. Seed color, coat pattern and shape were scored for all landraces according to the IBPGR/IPGRI (1982) descriptors list. According to Angioi et al. (2009), a trait called 'prevalent' was adopted for seed classification, for which 3 states are possible: (1) darker color as background and lighter color as stripes; (2) darker color and lighter color equally distributed and (3) lighter color as background and darker color as stripes. In addition, the 100-seed weight (g) was also measured. The American genotypes and the Italian varieties were not analyzed for morpho-phenotypic seed traits due to the limited number of seeds available.

Phaseolin analysis

Five seeds of each landrace were analyzed. The seed coats were manually removed before grinding and phaseolin was extracted according to Limongelli et al. (1996). One-dimensional SDS-PAGE of the extracts was performed, as reported by Ma and Bliss (1978), using 17 % (w/v) polyacrylamide slab gels.

DNA extraction and microsatellite analysis

Total genomic DNA was isolated from young fresh leaves of each bean landrace (one plant/landrace) and, after lyophilization, stored at −80 °C until required. The CTAB (cetyl-trimethyl-ammonium bromide) extraction method was used (Lodhi et al. 1994) with some modifications. Briefly, the extract was treated with DNase-free RNase (Roche Diagnostics, Germany) and quantified in agarose gels (1 %) using standard lambda DNA as a comparison.

Twelve microsatellite (SSR) loci from several linkage groups, chosen from among the most utilized bean, were selected based on their dispersal map location (Yu et al. 2000; Blair et al. 2003; Guerra-Sanz 2004) and on the evaluation of 150 microsatellites previously tested for polymorphism and PCR conditions (Blair et al. 2006). The genetic linkage map location can be found in the original publications (Blair et al. 2003; Yu et al. 2000; Gaitàn-Solìs et al. 2002), while repeat motif, primer sequences and annealing temperatures are reported in Table 2.

The PCR amplifications were carried out with a GeneAmp 2700 Thermal Cycler (Applied Biosystems) under varying annealing temperatures (T*a*), depending on the primer pairs. One of each pair was fluorescently labelled with FAM, JOE or TAMRA. PCR reactions were carried out in reaction volumes of 15 µl containing 20 ng of genomic DNA and 0.2 µM each of forward and reverse primers, in a 2X Qiagen multiplex Master Mix (Qiagen GmbH, Hilden, Germany). Reactions were performed under the following conditions: 15 min at 95 °C; 35 cycles of 30 s at 94 °C, 1 min 30 s at specific T*a*, 1 min at 72 °C, followed by

Table 1 List of samples analyzed, their code (ID), site of origin (CS Cosenza, CZ Catanzaro, VV Vibo Valentia, RC Reggio Calabria) and type

ID	Name	Province	Type	ID	Name	Province	Type
1	Fagiolo uncino	CS	Climbing	49	Piani corona	RC	Climbing
2	Crune	CS	Climbing	50	Sangue di porco	CZ	Climbing
3	Quarantino70	CS	Climbing	51	Milanese	CZ	Climbing
4	Fagiolo a unghia	CS	Climbing	52	Vovolaci	CZ	Climbing
5	Serpiata	CS	Climbing	53	Core di gesù	CZ	Climbing
6	Serpente	CS	Climbing	54	Sarrisa	CZ	Climbing
7	Ammerulla	CS	Climbing	55	Colostrigna spuria	CZ	Climbing
8	Quarantino	CS	Climbing	56	Colostrigna	CZ	Climbing
9	A fava	CS	Climbing	57	Favu	CZ	Climbing
10	A ciota	CS	Climbing	58	Vravalacu	CZ	Climbing
11	Merde e palummu	CS	Climbing	59	Quarantino du bombu	CZ	Climbing
12	Ciota serpiata	CS	Climbing	60	Posa nigra	CZ	Climbing
13	Mangiatutto a granella	CS	Climbing	61	Povarella	CZ	Climbing
14	Azzicca grande	CS	Climbing	62	Riccia	CZ	Climbing
15	San francischino bianco	CS	Climbing	63	Russa janca	CZ	Climbing
16	Franceschino nero	CS	Climbing	64	Povaredda i cambugi	CZ	Climbing
17	Paulitana	CS	Climbing	65	Chiumbina	CZ	Climbing
18	Fagiolo pisello	CS	Climbing	66	Nicolisa	CZ	Climbing
19	Bombina	CS	Climbing	67	Monacune	CZ	Climbing
20	Menza luna	CS	Climbing	68	Monachella	CZ	Climbing
21	Azzicca	CS	Climbing	69	Cocò bianco	CZ	Climbing
22	A caciumbalo	CS	Climbing	70	Borlotto paesano	CZ	Dwarf
23	Azzicca quarantinu	CS	Climbing	71	Cocò gialla	CZ	Dwarf
24	Quartu e luna	CS	Climbing	72	Borlotto locale	CZ	Dwarf
25	Cannellino	CS	Climbing	73	Suriaca burro di caria	CZ	Unknown
26	Azzicca a caciumbalo	CS	Climbing	74	Fasola pijella	VV	Climbing
27	Cervineddu	CS	Climbing	75	Ziccarija	VV	Climbing
28	Favarula nera	CS	Climbing	76	Paesano	VV	Climbing
29	Merulla a vaianeddra	CS	Climbing	77	Muriscia	VV	Climbing
30	Poverello di Mormanno	CS	Climbing	78	Cannellina	VV	Dwarf
31	Bianco	CS	Climbing	79	Gallinesa	VV	Dwarf
32	Occhialina	CS	Climbing	80	Regineja niura	Unknown	Climbing
33	Fasolu vasciu	CS	Dwarf	81	Pappaluni bianco	Unknown	Climbing
34	Serpe	CS	Dwarf	82	Facciuzza	Unknown	Climbing
35	Cannellino bianco	CS	Dwarf	83	Fagiolo bianco di dama	Unknown	Climbing
36	Fasolu quarantino	CS	Dwarf	84	Suraca larga	Unknown	Climbing
37	Borlotto spineto	CS	Dwarf	85	A cavolo	Unknown	Dwarf
38	Borlotto spaddrera	CS	Dwarf	86	Fasolu nero	Unknown	Dwarf
39	Fagiolo ciuncu	CS	Dwarf	87	Nera di montagna	Unknow	Dwarf
40	Posa di montagna	RC	Climbing	88	Lingua di fuoco (tester)	Italy	Dwarf
41	Rama	RC	Climbing	89	Lingua di fuoco (tester)	Italy	Climbing
42	Zicca	RC	Climbing	90	Marconi (tester)	Italy	Climbing
43	Lupinella	RC	Climbing	91	Marconi (tester)	Italy	Dwarf
44	Fagiolino	RC	Climbing	92	Cannellino nano (tester)	Italy	Dwarf

Table 1 continued

ID	Name	Province	Type	ID	Name	Province	Type
45	Posa bianca di settembre	RC	Climbing	93	Midas (tester)	USA	Dwarf
46	Fagiolo bianco piccolo	RC	Climbing	94	Jalo (tester)	Brasil	Climbing
47	Posa rossa di settembre	RC	Climbing	95	BAT93 (tester)	Colombia	Climbing
48	Sbraca pasta	RC	Dwarf	96	G1287 (tester)	Mexico	Climbing

Table 2 Microsatellite primer pairs (SSR), motif and annealing temperatures (T_a) used for genetic characterization of common bean germplasm collection

SSR	Genbank entry	Motif	T_a (°C)	Forward primer (5′–3′)	Reverse primer (5′–3′)
PV-AG003	X04001	$(AG)_8$	49	TCACGTACGAGTTGAATCTCAGGAT	GGTGTCGGAGAGGTTAAGGTTG
PV-AG004	X04660	$(AG)_8$	49	TTGATGACGTGGATGCATTGC	AAAGGGCTAGGGAGAGTAAGTTGG
PV-AT007	X80051	$(AT)_{12}$	49	AGTTAAATTATACGAGGTTAGCCTAAATC	CATTCCCTTCACACATTCACCG
BM210	AF483902	$(CT)_{15}$	52	ACCACTGCAATCCTCATCTTTG	CCCTCATCCTCCATTCTTATCG
BM157	AF483873	$(GA)_{16}$	52	ACTTAACAAGGAATAGCCACACA	GTTAATTGTTTCCAATATCAACCTG
BM160	AF483876	$(GA)_{15}(GAA)_5$	52	CGTGCTTGGCGAATAGCTTTG	CGCGGTTCTGATCGTGACTTC
BM172	AF483884	$(GA)_{23}$	50	CTGTAGCTCAAACAGGGCACT	GCAATACCGCCATGAGAGAT
BM212	AF483904	$(CA)_{13}$	50	AGGAAGGGATCCAAAGTCACTC	TGAACTTTCAGGTATTGATGAATGAAG
BM151	AF483867	$(TC)_{14}$	50	CACAACAAGAAAGACCTCCT	TTATGTATTAGACCACATTACTTCC
BMd-1	X96999	$(AT)_9$	50	CAAATCGCAACACCTCACAA	GTCGGAGCCATCATCTGTTT
BMd-18	X59469	$(TGAA)_3$	52	AAAGTTGGACGCACTGTGATT	TCGTGAGGTAGGAGTTTGGTG
BMd-51	AF128454	$(CT)_5$	52	CGCCAATTCTTCAACCCTAA	GTAGTTCGCCCGAGGACTG

10 min at 72 °C. Amplification products were checked and quantified by electrophoresis on 3 % agarose using Low DNA mass Ladder (Invitrogen). The fragments were separated by capillary electrophoresis and genotyped with an ABI PRISM 3500 Genetic Analyzer.

Data analysis

Alleles in base pairs were estimated by comparing the fragment peaks with the internal size standard using the default method for band calling with SSR, and the expected repeat size. Electropherograms were verified visually using Gene Mapper version 4.1 software to ensure that the proper selection of multiplex markers had been made. The pairwise genetic distances for phylogenetic relationships among landraces were estimated using Nei's (1973) coefficient, cluster analysis was performed according to the UPGMA (Unweighted Pair-Group Method with Arithmetical Averages) algorithm and a dendrogram was generated (PowerMarker version 3.25; Liu and Muse 2005). A consensus tree was created in nexus format for viewing in Tree-View (Page 1996), the nodes being supported by bootstrap analysis (1,000 replicates).

Genetic diversity was estimated by comparing the number of alleles per locus (N_a), number of effective alleles (N_e), allele size range, number of private alleles (N_{pa}), allelic richness (AR), expected (H_e) and observed heterozygosities (H_o) using GeneALex 6 (Peakall and Smouse 2006) and FSTAT version 2.9.3.2 (Goudet 2002) software.

Microsatellite screening ability (MSA) was calculated using the probability of identity (PI) (Paetkau et al. 1995) and the polymorphic information content (PIC) (Weber 1990) as follows:

$$PI = \sum_{i=1}^{n} p_i^4 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2p_i^2 p_j^2$$

$$PIC = 1 - \left(\sum_{i=1}^{n} p_i^2 \right) - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2p_i^2 p_j^2$$

where p_i and p_j are the frequencies of the ith and jth allele and n is the number of alleles.

These indices, ranging from 0.0 to 1.0, were calculated using the IDENTITY software (Wagner and Sefc 1999 version 1.0; Centre for Applied Genetics, University of Agricultural Sciences, Vienna, Austria).

To identify the genetic groups within the Calabrian bean collection and the relationships with American and Italian genotypes, STRUCTURE version 2.2 software (Pritchard and Wen 2003) was utilized. This Bayesian approach to analysis uses no a priori classification and assigns samples to *K* populations based on the allele frequencies at each locus. The estimate of the most likely number of genetic groups (*K,* ranged from 1 to 10) was performed following the procedure of Evanno et al. (2005), which proposed an *ad hoc* statistic, ΔK. Program settings used the admixture ancestry and correlated marker frequency models. Alpha was inferred from the data and lambda was set to 1 (Pritchard and Wen 2003; Evanno et al. 2005). For each *K*, 20 independent runs (500,000 burn-in, 1,000,000 Marchov Chain Monte Carlo) were carried out.

Results

Seed traits and phaseolin

According to the IBPGR/IPGRI (1982) descriptor list, 34 diverse seed morphotypes were described (Table S1). The frequency of morphotypes ranged from 1.3 to 13.8 % (Fig. 2). Eight out of 34 morphotypes (23.5 %) showed a higher that 5.0 % frequency, 55 % of analyzed seeds were grouped as 00712 'white-oval' (13.8 %), 00714 'white reniform' (7.5 %), 00313 'maroon cuboid' (7.5 %), 2(12)613 'pale-cream, red striped, cuboid' (6.3 %), 00113 'black cuboid' (5.1 %), 00713 'white cuboid' (5.1 %), 21314 'maroon, black striped, reniform' (5.1 %) and 72313 'maroon, broad striped, cuboid' (5.1 %) (Fig. 3). Twenty-four landraces were included in the morphotypes 00711 (3), 00712 (11), 00713 (4) and 00714 (6), which were all characterized by a white coat color. Moreover, according to the seed trait analysis, 77 landraces out of 87 (88.5 %) were homogeneous while

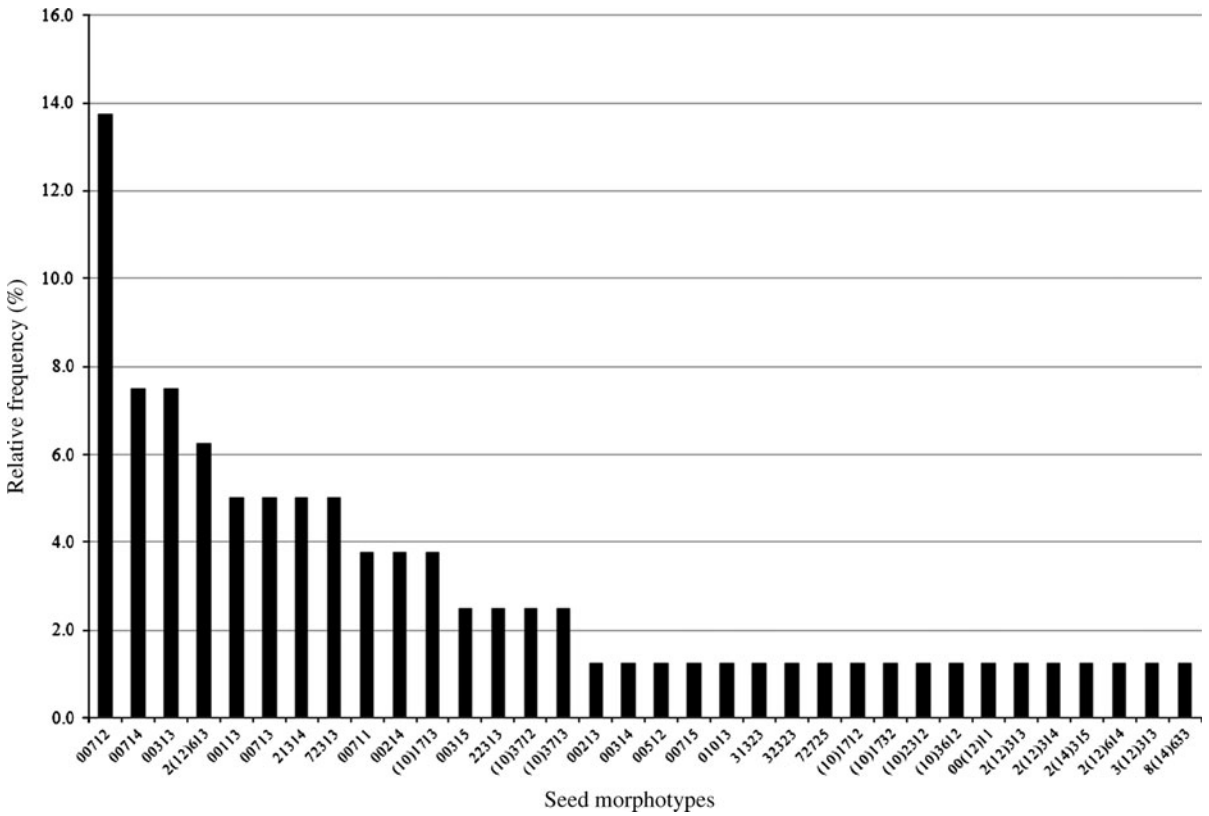


Fig. 2 Frequency distribution of the seed morphotypes within the 80 *Phaseolus vulgaris* landraces collected from different areas of Calabria

Fig. 3 The eight out of the 34 most frequent morphotypes. 00712 = *white oval*; 00714 = *white reniform*; 00313 = *maroon cuboid*; 2(12)613 = *pale-cream, red striped, cuboid*; 00113 = *black cuboid*; 00713 = *white cuboid*; 21314 *maroon, black striped, reniform;* 72313 = *maroon, broad striped, cuboid*

only ten (11.5 %) were heterogeneous, generally showing 2 morphotypes, one of which was considered predominant and selected as representative for frequency data counting.

The landraces showing the greatest spread of seed-morphotype (00712 'white oval'), such as "Posa di montagna", "Sbraca pasta" and "Bianco", were frequently indistinguishable from Cannellino nano, an Italian commercial variety. According to the seed trait analysis, differentiation among Calabrian landraces was high ($F_{ST} = 0.557$), especially considering that some morphotypes (19) consisted of a single landrace.

The phaseolin pattern was analyzed for all bean germplasm (Table S1, Fig. S1). Phaseolin C (56.3 %) and T (18.4 %) types, typical of the Andean gene pool, were found in most landraces, while the S type (11.5 %), from the Mesoamerican gene pool, was present in a limited number of landraces. Unexpectedly, in a few landraces (6.9 %), an unknown phaseolin pattern similar to that observed in wild runner bean by Gepts et al. (1986), never reported in the common bean, was observed. On the basis of these results, since *P. coccineus* and *P. vulgaris* differ in germination method, being hypogeal and epigeal, respectively (CIAT 1986), germination type for all landraces was analyzed. Only five ("Ciota serpiata", "A fava", "Favarula nera", "Favu" and "Quarantino") out of 87 landraces showed hypogeal germination (data not shown), the first evidence that these landraces, cultivated as common bean in Calabria, might be instead assigned to *P. coccineus* or to interspecific hybridization between the 2 *Phaseolus* species. At the flowering stage, red flowers were observed in "Ciota serpiata" and "Favarula nera" landraces, flower color reported as typical from *P. coccineus* (CIAT 1986). The 100-seed weight was also analyzed to further distinguish among landraces and their provenances. A significant difference (*t* test: $P = 0.001$) between 100-seed weight means (31 vs. 48 g) was observed in landraces from Mesoamerican and Andean origin, respectively (Table S1).

Taking into account seed traits and biochemical results, seven landraces showed an average 100-seed weight of 85 g, further, 6 out of the same 7 landraces harbored the not classified phaseolin type, while only one ("Ciota Serpiata") clearly showed a T type pattern.

SSRs based genetic diversity

A genetic diversity analysis on all germplasm using 12 widespread SSR loci was carried out. Four SSRs (PV-AG004, BMd-1, BMd-18 and BMd-51) were monomorphic, thus the statistical analyses were performed on the eight polymorphic SSRs of which the main genetic parameters are reported in Table 3. A total of 57 alleles were detected with an average of 7.1 alleles per locus, ranging from 73 to 260 bp. The expected heterozygosity (H_e) ranged from 0.181 to 0.827 (mean = 0.625) while H_o ranged from 0.162 to 0.483 (mean = 0.326), which was expected for an autogamous species. According to previous studies (Kwak and Gepts 2009), the PIC, which defines the ability of each SSR locus to distinguish among landraces, ranged from 0.167 to 0.793. The PI, which indicates the probability of the identity between 2 landraces harbouring different alleles at an analyzed locus, ranged from 0.103 to 0.710, while the PI value calculated for overall loci was 9.14×10^{-6} (Table 3). Since the high level of PIC, along with lower PI values, indicated a greater SSR ability to discriminate among landraces, the results indicated that the BM160 locus was the best SSR for discriminating among bean landraces (PIC = 0.793 and PI = 0.103). The percentage of homozygosity (Hom) in each locus, as expected for an autogamous species, ranged from 53.1 to 85.4 % (Table 3). The data, including the number of alleles, shows that the heterozygotic profiles for each locus ranged from 2 to 15, with an average of 8 (Table 3).

The genetic parameters already analyzed in Table 3 were recalculated for overall polymorphic SSRs and across each group of "genotypes". The H_e of the Calabrian common bean group was 0.595, the total number of alleles (N_a) ranged from 11 to 49

Fig. 4 **a** Dendrogram of genetic relationships among the 96 genotypes generated with Nei's coefficient (Nei 1973) and UPGMA cluster analysis. Origin of Calabrian common bean landraces: (CS) *Cosenza*, (CZ) *Catanzaro*, (VV) *Vibo Valentia*, (RC) *Reggio Calabria*, (unk) unknown. In *bold italics* landraces of putative hybrid origin. *Underlined* Italian and American tester; (c) climbing, (d) dwarf. **b** Hierarchical organization of genetic relatedness of 96 bean genotypes analyzed by the STRUCTURE program. Each color represents one population (Group 1 *white dashed*; Group 2 *dark gray*; Group 3 *light gray*; Group 4 *white*; Group 5 *black*; Group 6 *white pointed*) and the length of the *colored segment* shows the estimated membership proportion of each sample to designed group. **c** Phaseolin types for each landrace are reported (C and T phaseolin types are from the Andes, S type is from Mesoamerica, nc-not classified)

Table 3 Main genetic parameters from the 8 polymorphic SSR loci

SSR	No alleles	Allele size range (bp)	H_e[a]	H_o[a]	PI[b]	PIC[c]	Hom.[d]	No Het.[e]
PV-AG003	3	147–161	0.181	0.203	0.710	0.167	80.2	2
PV-AT007	11	190–218	0.673	0.162	0.130	0.654	85.4	11
BM210	9	158–184	0.715	0.246	0.199	0.676	76.0	8
BM157	6	88–110	0.691	0.335	0.240	0.639	69.7	6
BM160	8	180–260	0.827	0.405	0.103	0.793	60.4	15
BM172	9	73–107	0.664	0.483	0.262	0.609	53.1	11
BM212	6	191–223	0.651	0.389	0.209	0.601	64.6	8
BM151	5	135–145	0.609	0.411	0.362	0.531	59.4	3
All loci	57	–	–	–	9.14×10^{-6}	–	–	–
Mean	7.1	–	0.625	0.326	0.277	0.584	68.6	8.0

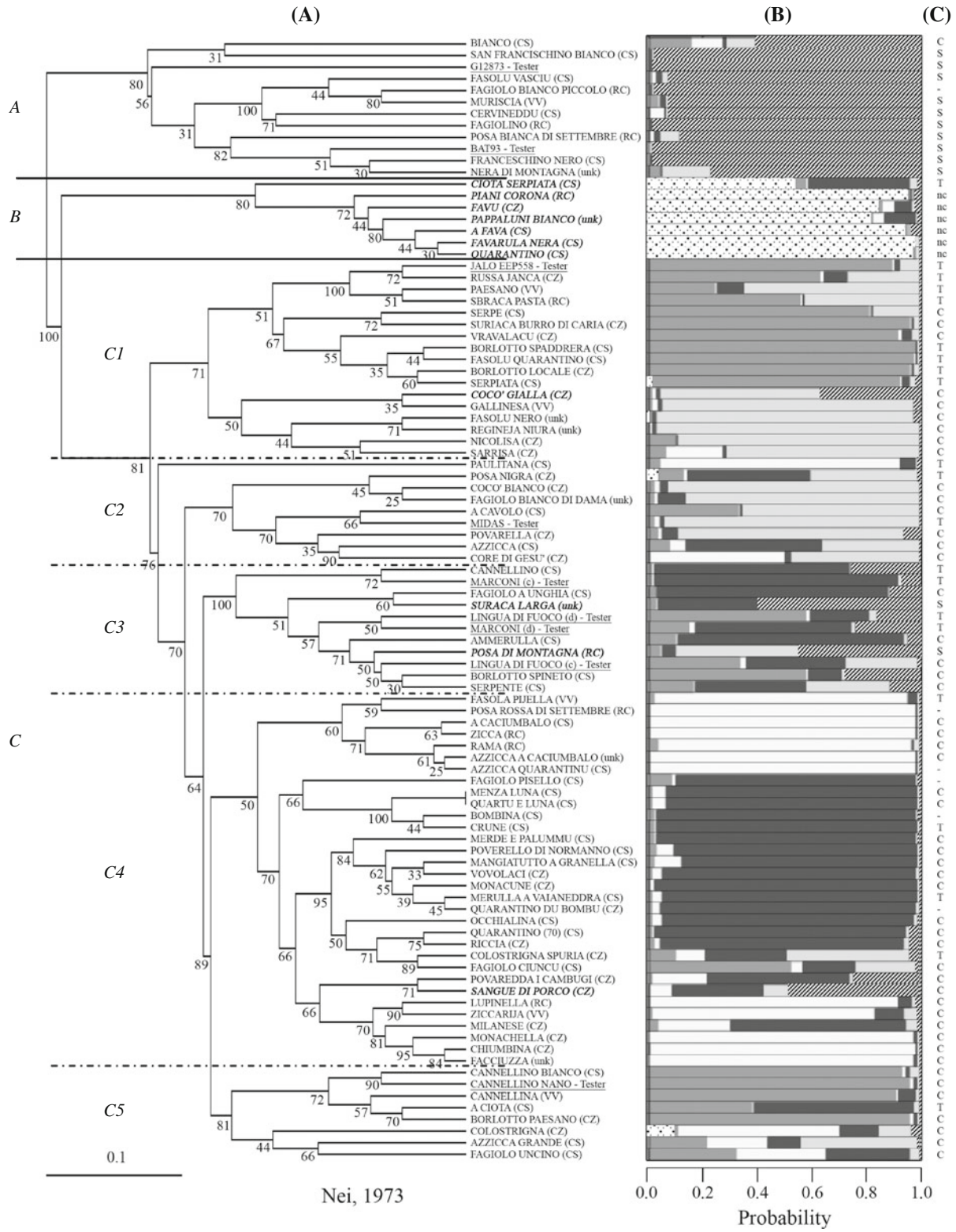
[a] Genetic diversity (average H_e, Nei 1987) and observed (H_o) heterozygosity

[b] Probability of identity (PI)

[c] polymorphic information content (PIC)

[d] Percentage of homozygosity at each locus

[e] Number of heterozygotic profiles


(A)
(B)
(C)
A
B
C
C1
C2
C3
C4
C5
BIANCO (CS)
SAN FRANCISCHINO BIANCO (CS)
G12873 - Tester
FASOLU VASCIU (CS)
FAGIOLO BIANCO PICCOLO (RC)
MURISCIA (VV)
CERVINEDDU (CS)
FAGIOLINO (RC)
POSA BIANCA DI SETTEMBRE (RC)
BAT93 - Tester
FRANCESCHINO NERO (CS)
NERA DI MONTAGNA (unk)
CIOTA SERPIATA (CS)
PIANI CORONA (RC)
FAVU (CZ)
PAPPALUNI BIANCO (unk)
A FAVA (CS)
FAVARULA NERA (CS)
QUARANTINO (CS)
JALO EEP558 - Tester
RUSSA JANCA (CZ)
PAESANO (VV)
SBRACA PASTA (RC)
SERPE (CS)
SURIACA BURRO DI CARIA (CZ)
VRAVALACU (CZ)
BORLOTTO SPADDRERA (CS)
FASOLU QUARANTINO (CS)
BORLOTTO LOCALE (CZ)
SERPIATA (CS)
COCO' GIALLA (CZ)
GALLINESA (VV)
FASOLU NERO (unk)
REGINEJA NIURA (unk)
NICOLISA (CZ)
SARRISA (CZ)
PAULITANA (CS)
POSA NIGRA (CZ)
COCO' BIANCO (CZ)
FAGIOLO BIANCO DI DAMA (unk)
A CAVOLO (CS)
MIDAS - Tester
POVARELLA (CZ)
AZZICCA (CS)
CORE DI GESU' (CZ)
CANNELLINO (CS)
MARCONI (c) - Tester
FAGIOLO A UNGHIA (CS)
SURACA LARGA (unk)
LINGUA DI FUOCO (d) - Tester
MARCONI (d) - Tester
AMMERULLA (CS)
POSA DI MONTAGNA (RC)
LINGUA DI FUOCO (c) - Tester
BORLOTTO SPINETO (CS)
SERPENTE (CS)
FASOLA PIJELLA (VV)
POSA ROSSA DI SETTEMBRE (RC)
A CACIUMBALO (CS)
ZICCA (RC)
RAMA (RC)
AZZICCA A CACIUMBALO (unk)
AZZICCA QUARANTINU (CS)
FAGIOLO PISELLO (CS)
MENZA LUNA (CS)
QUARTU E LUNA (CS)
BOMBINA (CS)
CRUNE (CS)
MERDE E PALUMMU (CS)
POVERELLO DI NORMANNO (CS)
MANGIATUTTO A GRANELLA (CS)
VOVOLACI (CZ)
MONACUNE (CZ)
MERULLA A VAIANEDDRA (CS)
QUARANTINO DU BOMBU (CZ)
OCCHIALINA (CS)
QUARANTINO (70) (CS)
RICCIA (CZ)
COLOSTRIGNA SPURIA (CZ)
FAGIOLO CIUNCU (CS)
POVAREDDA I CAMBUGI (CZ)
SANGUE DI PORCO (CZ)
LUPINELLA (RC)
ZICCARIJA (VV)
MILANESE (CZ)
MONACHELLA (CZ)
CHIUMBINA (CZ)
FACCIUZZA (unk)
CANNELLINO BIANCO (CS)
CANNELLINO NANO - Tester
CANNELLINA (VV)
A CIOTA (CS)
BORLOTTO PAESANO (CZ)
COLOSTRIGNA (CZ)
AZZICCA GRANDE (CS)
FAGIOLO UNCINO (CS)
0.1
Nei, 1973
0.0
0.2
0.4
0.6
0.8
1.0
Probability

(landraces), and the number of private alleles ranged from 0 to 19 (landraces). Allelic richness (AR) showed values ranging from 1.5 to 2.5 (landraces), confirming the high rate of Calabrian germplasm diversity.

A dendrogram that included all bean landraces by cluster analysis (Nei 1973) and UPGMA algorithm was generated (Fig. 4a). Considering the large number of landraces investigated, based on the SSR analyzed only one putative synonym ("Quartu e luna" – "Menza luna") was found. On the other hand, several couple or groups of landraces ("Cocò Bianca" and "Cocò gialla"; "Borlotto paesano", "Borlotto locale", "Borlotto spaddrera" and "Borlotto spineto"; "Cannellino", "Cannellino bianco" and "Cannellina"), with similar names but different genetic profiles were also found.

According to genetic distances, 3 discrete clusters, (A), (B) and (C), were obtained (Fig. 4a). Cluster (A) included the Mesoamerican genotypes (BAT93 and G12783) together with 10 landraces from Calabria, most of which (50 %) came from Cosenza province. Cluster B included most (4) of the seven landraces that were taken from Cosenza which, in terms of morphological traits (seed and plant), phaseolin type and mode of germination, could be classified as runner beans (*P. coccineus*) or as hybrids between common and runner beans. Finally, the larger cluster (C) included the Andean genotypes (Jalo EEP558 and Midas) together with all the Italian varieties and 70 Calabrian landraces. On the basis of Nei's (1973) genetic distances, this latter large cluster could be further subdivided in 5 sub-clusters, *C1*, *C2*, *C3*, *C4* and *C5* (Fig. 4a). In the first sub-cluster (*C1*) a large number of landraces (11) had come from the border areas between Cosenza and Catanzaro provinces, and the "Paulitana" landrace (as a zipper between sub-clusters *C1* and *C2*) were present. The sub-cluster *C3* included four out of the five Italian varieties, while 40 % of the landraces from Reggio Calabria were included in sub-cluster *C4* (Fig. 4a). In summary, the Calabrian landraces were distributed between all the clusters identified, which meant that the area of sampling was not distinguishable by clustering.

Structure of the genetic groups

To ascertain the likely number of genetic groups (*K*) within the collection, STRUCTURE software was utilized. The distribution of all genotypes across different groups was determined on the assumption that (i) the Mesoamerican genotype BAT93 (Colombia) and the wild accessions G12783 (Morelos, Mexico) belonged to group I and (ii) the Andean genotypes Jalo EEP558 (Brazil) and Midas (USA) were included in group II.

The number of genetic groups (*K*) showed a clear peak at 6, where 6 main groups (I, II, III, IV, V and VI) were distinguished, including all Calabrian landraces. Group I consisted of 11 individuals, group II-14, group III-25, group IV-16, group V-14 and group VI-7 (Table 4, Table S2). Group I was considered representative of the putative Mesoamerican gene pool, groups II to V represented the putative Andean gene pool, while group VI could represent the genetic structure of the landraces derived from putative hybridizations between common (*P. vulgaris*) and runner bean (*P. coccineus*). The landraces were colored based on the STRUCTURE assignments at $K = 6$ (Fig. 4b). These results closely mirrored the pattern of diversity described in the UPGMA dendrogram (Fig. 4a).

Based on their mixed genetic structure (Fig. 4b), some landraces of Andean gene pool origin seemed to

Table 4 Distribution of the individuals of the 96 genotypes analyzed, across the six genetic groups identified by the STRUCTURE software

Original groups	Genetic groups based on the SSR polymorphism						Total
	Group I	Group II	Group III	Group IV	Group V	Group VI	
Calabria	11 (12.6)	14 (16.1)	25 (28.7)	16 (18.4)	14 (16.1)	7 (8.1)	87
Central America	2	0	0	0	0	0	2
South American	0	1	0	0	1	0	2
Italy	0	0	3	0	2	0	5
Total	13	15	28	16	17	7	96

In parentheses the percentage of landraces assigned to the group

be derived from hybridization events. Furthermore, rare events of hybridization between landraces from both Andean and Mesoamerican gene pools were observed in "Cocò gialla" (sub-cluster *C1*), "Suraca larga", "Posa di montagna" (sub-cluster *C3*) and "Sangue di porco" (sub-cluster *C4*) (Fig. 4b). Finally, analysis using the STRUCTURE software also inferred a potential natural hybridization between *P. coccineus* and *P. vulgaris* from the Andean gene pool found in group VI, in particular the landrace "Ciota Serpiata" (cluster B).

Discussion

Genetic population structure and the domestication events of *P. vulgaris* were broadly analyzed based on different types of markers such as morphological traits (Singh et al. 1991a, b; Gepts and Debouck 1991); seed proteins (Gepts et al. 1986; Gepts and Bliss 1986; Logozzo et al. 2007); allozymes (Koenig and Gepts 1989; Singh et al. 1991c); RFLPs (Freyre et al. 1998); RAPDs (Freyre et al. 1996); AFLPs (Tohme et al. 1996; Papa and Gepts 2003; Pallottini et al. 2004); and SSRs (Yu et al. 2000; Blair et al. 2003; Masi et al. 2003; Blair et al. 2006; Kwak and Gepts 2009, Burle et al. 2010). In particular, the last technique, first applied to the common bean by Yu et al. (2000), has been successfully used in recent years to characterize large bean germplasm collections from both America and Europe (Blair et al. 2009; Angioi et al. 2010). Furthermore, the analysis of genetic diversity based on SSRs could allow inferences on relationships among different groups of germplasm and the rate of intra- and interspecific hybridization in the bean worldwide collections.

In the present study, different markers, such as SSRs, morphological seed traits, phaseolin and 100-seed weight, have been combined with different statistical data analyzes in order to characterize the Calabrian collection of 87 *P. vulgaris* landraces. The aim was to increase understanding of the patterns of bean genetic diversity in this Italian region and to confirm the evolutionary origins of bean germplasm in Southern Europe. In general, this approach is of crucial importance in estimating the levels of germplasm diversity, to avoid its loss, and to allow its potential utilization.

Twelve SSRs, chosen among the most informative in bean and considered to characterize different worldwide core collections (Kwak and Gepts 2009; Blair et al. 2009), were used. However, with four of them it was not possible to distinguish among Calabrian landraces, although they were reported as informative SSR in previous studies (Blair et al. 2007; Zhang et al. 2008). The SSR ability to distinguish among landraces (PIC) was highly variable as reported in previous studies (Kwak and Gepts 2009; Blair et al. 2009). Conversely, the overall loci probability of identity (PI) was very low, confirming the informativeness of the SSR panel in the Calabrian germplasm. According to Kwak and Gepts (2009), BM160 appeared to be the best SSR for discriminating among Calabrian landraces, due to its higher PIC and lower PI values. The percentage of homozygosity (Hom) varied among loci, but was rather high on average, as would be expected in an autogamous species.

According to Nei's index (average $H_e = 0.595$, Nei 1987), a high level of genetic diversity was found in the Calabrian germplasm collection, which may reflect the large number of private alleles (19) and the allele richness (AR = 2.5). H_e was higher than that reported in previous studies on Sardinian (Italy) (Angioi et al. 2009) and worldwide bean (Blair et al. 2009) collections. The observed heterozygosity (H_o) was low as expected in an autogamous species (Wells et al. 1988; Ibarra-Perez et al. 1997), in which cross hybridizations, due to the close together cultivation of different landraces, have been frequently observed.

According to a number of Italian reports (reviewed in Piergiovanni and Lioi 2010), cluster analysis on the Calabrian common bean revealed the presence of both gene pools but with a large prevalence of Andean origin landraces (79.3 %). This result was also confirmed by STRUCTURE analysis which revealed that 39 landraces (44.8 %) were present in groups II and III and 30 (34.5 %) in groups IV and V when the Andean genotype testers were included. In contrast, the Mesoamerican gene pool contribution to Calabrian germplasm appeared to be consistent (12.6 %) as shown in group I; smaller than that reported in the Marche region collection (Sicard et al. 2005) but greater than was observed in Sardinia (Angioi et al. 2009). However, the high level of genetic diversity of the Calabrian landraces, in contrast with their prevalent Andean origin (>80 %), could be due to: (1) the high diversity of Andean germplasm; and (2) the cross hybridization phenomena between the Andean and Mesoamerican germplasm, prominent in

several areas of Calabria over the years, according to STRUCTURE analysis.

Thus, a three-step process could have occurred in Calabria: (1) a substantial introduction of germplasm from the Andean gene pool; (2) high diversity within this Andean germplasm, in contrast with that recently reported in Sardinia (Angioi et al. 2009); and (3) rare but significant cross hybridization events between gene pools or bean species.

According to STRUCTURE analysis, the cross hybridization between the 2 gene pools was identified in four landraces found in sub-clusters *C1*, *C3* and *C4*. In addition, the same suspect of cross hybridization event was observed between common and runner bean, confirmed by the genetic structure in "Ciota Serpiata", and by the 100-seed weight and germination results. In particular, the average 100-seed weight of the landraces putatively derived from runner bean was higher (85 g 100-seed weight^{-1}) than Mesoamerican gene pool small-medium seed (<25–40 g 100-seed weight^{-1}) or the large seeded (40–60 g 100-seed weight^{-1}) landraces of Andean origin (Gepts et al. 1986; Gepts 1988; Singh et al. 1991a). In addition, "Ciota Serpiata", "Favarula nera", "A fava", "Quarantino" and "Piani corona" landraces showed clear hypogeal germination, which is typical of runner bean, such as red color flowers observed in "Ciota Serpiata" and "Favarula nera". These events of interspecific hybridization could be expected since runner and common beans are often found in sympatry (Papa et al. 2006; Spataro et al. 2011).

The contribution of Andean and Mesoamerican gene pools in Calabria was also clarified by seed storage analysis. Indeed, phaseolin pattern, very useful for gene pool identification, confirmed that the Calabrian germplasm was of Andean origin, although the Mesoamerican gene pool contribution was still considerable. These results were in accordance with phaseolin data from other Italian regions such as Basilicata and Abruzzo (Piergiovanni et al. 2000a, b). Furthermore, the phaseolin analysis supported the hypothesis of a cross hybridization between the gene pool by a presence of C type in a landrace ("Bianco") of Mesoamerican origin. Hybridization events between the common (Andean gene pool) and runner beans are also suspected, since the "Ciota Serpiata" landrace, together with a genetic structure similar to runner bean, harbored a phaseolin type (T), typical of the Andean gene pool. Further analysis of the "Ciota Serpiata" landrace and the other members of cluster B-group VI is needed to confirm this rare natural cross hybridization event.

Finally, in Calabria, as in other regions, the local name could represent a 'label' of genetic divergence underlining a primary difference among landraces. Indeed, local names often refer to the seed traits (e.g., "Fagiolo a ughia" = nail bean; "Fagiolo ciuncu" = truncated bean; "Fagiolo bianco piccolo" = little white bean; "Russa janca" = red and white bean), possible geographical origins ("Paulitana" = bean from Paola; "Piani corona" = bean from an area near Reggio Calabria), cultivation system ("Azzicca" = "Azzicca grande" = sticking to a support because of a climbing growth habit, "Fasolu vasciu" = low bean with dwarf growth habit), or to the shape and origin simultaneously ("Poverello di Mormanno" = little bean from Mormanno). A similar approach to classification has been previously proposed (Jarvis et al. 2008; Angioi et al. 2009). Thus, the local name or 'label', together with the areas of origin, could allow a stratified sampling strategy able to recover rather all the genetic diversity, included landraces with the same name but from different areas, harboring different genetic profiles (homonymies).

In conclusion, the genetic resources of the common bean in a Mediterranean region have been described. More knowledge about bean evolution in areas far from their center of origin has been obtained. These results shed light on the genetic relationships between common beans and further demonstrated that the large genetic diversity of *Phaseolus vulgaris* L. is still unexploited in Calabria. Cross hybridizations between the 2 gene pools and 2 cultivated bean species are presumably present in the Calabrian germplasm, underlining that interspecific hybridization could represent an important source for common bean breeding (Beaver and Osorno 2009). Moreover, the present results will be useful in defining how bean genetic resources are managed in Calabria and for establishing a core collection.

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