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Characterization of some Italian common bean (*Phaseolus vulgaris* L.) landraces by RAPD, semi-random and ISSR molecular markers

Ilaria Marotti*, Alessandra Bonetti, Maurizio Minelli, Pietro Catizone and Giovenni Dinelli

*Department of Agroenvironmental Science and Technology, University of Bologna, Viale Fanin 44, I-40127 Bologna, Italy; *Author for correspondence (e-mail: imarotti@agrsci.unibo.it; phone: +390512096669; fax: +390512096241)*

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Abstract

Randomly amplified polymorphic DNA (RAPD), inter-simple sequence repeat (ISSR) and a semi-random PCR system were used to analyze the genetic diversity of 16 Italian common bean landraces and their relationship to four commercial cultivars. Of the primers tested, 8 ISSR, 6 RAPD and 7 semi-random primers produced polymorphic and reproducible DNA fragments. A higher proportion of polymorphic bands were observed using ISSR (85%) and semi-random (90%) primers than RAPD (69%) method. The combination of any two semi-random markers allowed the identification of all 20 bean genotypes. In contrast ISSR (except for primer $(CAC)_3GC$) and RAPD markers appeared to be less informative as more than two markers were necessary to achieve the same diagnostic level. Moreover, 7 ISSR, 2 RAPD and 8 semi-random exclusive bands were identified as putative population-specific markers. Semi-random and ISSR derived dendrograms showed similar tendencies in terms of genetic relatedness, whereas clustering of genotypes within groups was not similar when compared with the RAPD technique. Despite the different ability to resolve genetic variation among the investigated landraces, two major clusters with less than 60% (ISSR) and 40% (RAPD and semi-random) genetic similarity were formed with all three marker systems. The two groups were correlated with the phaseolin patterns and seed size of the landraces. The analysis showed that the cultivar 'Lingua di Fuoco' and most of the landraces (13 out of 16) collected in Italy belong to the Andean gene pool, whereas only the three populations from Pratomagno belong to the Middle American gene pool.

Introduction

The common bean (*Phaseolus vulgaris* L.) is the most important grain legume for direct human consumption in the world (CIAT 1993). Based on archaeological observations from Peru and South-western United States in the late 19th century, it was concluded that the common bean originated from the New World. Separate domestications in the Andes and in Mesoamerica led to two distinct cultivated gene pools (Gepts et al. 1986).

In Italy common bean is widely grown (Toniolo 1989; Miccolis 1995) along all the Italian peninsula. Although commercial bean cultivars are gradually

displacing landraces, several old bean populations exist in various Italian regions where traditional agriculture is still practiced. These landraces have the best adaptation to the pedoclimatic conditions of restricted geographical areas and represent local specialties very appreciated for their high nutritional value, short cooking time, thin coat and good yield (Piergiovanni et al. 2000). They generally have a local name identifying the well established geographical area of cultivation and some of them have obtained the PGI mark (Protected Geographical Indication), one of the quality recognition standards introduced by the European Community (CEE regulations n. 2081/92 passed by the European Council on 14/7/92). Some examples are the common beans grown at Lamon (three *Phaseolus vulgaris* L. populations named *Spagnolon, Calonega, Canalin* and *Spagnolet*), [and *Phaseolus coccineus* from Vallepietra (named *Ciavattone*)], Sarconi (Limongelli et al. 1996), and Sorana. To rescue and exploit the available genetic resources, useful to obtain new cultivars, studies focused on the knowledge and understanding of the genetic structure of these landraces are important for the implementation of measures addressing their management and conservation.

The evaluation of phenotypic differences is a traditional method for evolutionary and pedigree relationship determination. In the case of bean, considerable effort has been directed at analysis of variation in electrophoretic patterns of seed storage proteins, especially phaseolin (PHAS) (Gepts 1993). Four PHAS patterns named 'Tendergreen' (T), 'Sanilac' (S) 'Contender' (C) and 'Boyaca 22' (B) were identified among cultivars of common bean in the Mediterranean area (Lioi 1989; Rodino et al. 2001). Advances in molecular biology have allowed the development of rapid, sensitive and specific screening methods to study genetic diversity and relatedness between individuals. Over the last 10 years, polymerase chain reaction (PCR) technology has led to the development among others of two simple and quick techniques called RAPD and ISSR. The former detects PCR fragment polymorphisms, using a single primer of arbitrary nucleotide sequence (Williams et al. 1990), and the latter permits detection of polymorphisms in inter-microsatellite loci, using a primer designed from di-, tri- or tetranucleotide simple sequence repeats (Zietkiewicz et al. 1994). RAPD markers have been used in several studies concerning the genetic variations among cultivars and landraces of common bean from Southern Brazil and North-Western Argentina (Galvan et al. 2001; Maciel et al. 2001), the distribution of common bean landraces from Chile and Tanzania into the major gene pools (Johns et al. 1997; Briand et al. 1998), while cpDNA amplification was employed to investigate the phylogenetic relationship among taxa belonging to *Phaseolus vulgaris* complex (Llaca et al. 1994). More recently, a modified RAPD technique with a system containing additional DNA sequences partly complementary to the semi-conservative sequences of intron–exon junctions proved to be very useful in a variety of plant species (Rafalski et al. 1998). These primers, also known as semi-random primers, were successfully used by Nowosielski et al. (2002) to target diverse regions of the genome in Polish common and runner beans varieties. ISSR markers have been widely used for cultivar identification in oilseed rape and cauliflowers (Charters et al. 1996; Bornet et al. 2002), for mapping of plant chromosome (Kojima and Ogihara 1998) and for the assessment of genetic diversity in corn (Kantety et al. 1995) and common bean (Galvan et al. 2001).

Our investigation focused on two objectives: (1) to compare RAPD, semi-random and ISSR systems in order to evaluate the suitability and congruence of these different markers for estimating genetic similarity and (2) to develop landrace-specific fingerprints able to discriminate among sixteen Italian common bean populations and four commercial cultivars. Moreover, we attempted to group the tested bean landraces into their specific gene pool of origin by including PHAS analysis in the present study.

Material and methods

Plant material

A collection of sixteen common bean populations from Sarconi, Lamon and Pratomagno areas was evaluated in this study (Table 1). The map of Figure 1 shows the geographical origin of these populations. The material consists of landraces maintained by farmers for generations and representative of the bean populations growing in the North, in the Centre and in the South of Italy. Four commercial bean cultivars ('Tendergreen',

Table 1. List of *Phaseolus vulgaris* L. Italian landraces and cultivars used for the ISSR, RAPD and semi-random analysis.

WorkCode	Origin	Name	Producer	PHAS type	(100 seeds weight (g))
S13	Sarconi	Riso bianco	'Al Fagiolo d'Oro', Belisario, Dalessandri, De Rosa, Di Spagna, Florio, Giovinnazzo.	C	49.6
S14a	Sarconi	Tuvagliedda bianca.	Belisario	C	87.7
S14b	Sarconi	Tuvagliedda rossa.	Belisario, Lauria	C	54.8
S15	Sarconi	Nasieddu	Belisario, Lauria	T	55.8
S17	Sarconi	Munachedda	Belisario	C	45.5
S18	Sarconi	Ciuoto	Al Fagiolo d'Oro', Belisario, Dalessandri, De Rosa, Di Biase, Di Spagna, Florio	T	44.7
S19	Sarconi	Cannellino bianco	Al Fagiolo d'Oro', Belisario, Di Biase, Giovinnazzo	C	53.4
S20	Sarconi	Verdolino	Al Fagiolo d'Oro', Belisario, De Rosa, Di Biase, Di Spagna, Lauria	T	43.6
S21	Sarconi	Tabacchino nano	Belisario, Lauria, Florio, Di Biase	C	41.2
S22	Grumento Nova	San Michele o' Ciuoto	Belisario	T	65.9
LB1	Lamon	Spagnolon	Consorzio per la tutela del fagiolo di Lamon	C	78.2
LB2	Lamon	Spagnolet	Consorzio per la tutela del fagiolo di Lamon	T	69.3
LB3	Lamon	Calonega	Consorzio per la tutela del fagiolo di Lamon	C	103.9
Zolfino bean					
ZB1	Reggello		Ciabatti, Pasquale, Turini, Franci	S	27.5
ZB2	Loro Ciuffenna		Miniati, Neri, Bacci, Mealli, Scarselli	S	22.0
ZB3	Terranuova Bracciolini		Morandi	S	31.4
Reference cultivars					
CON		Contender	SAIS- Bologna	C	33.8
SAN		Sanilac	Michigan State University	S	17.7
TNG		Tendergreen	Olter SRL	T	28.6
LDF		Lingua di Fuoco	LaRAS- Università di Bologna	T	78.3

Figure 1. Geographical origin of the studied bean landraces. (1) Lamon area; (2) Pratomagno area; (3) Sarconi area.

'Contender', 'Sanilac' and 'Lingua di Fuoco'), supplied by private seed trade companies were also included in the analysis and used as a reference. 'Tendergreen', 'Contender' and 'Sanilac' were chosen according to Gepts and Bliss (1988) as their phaseolin electrophoretic patterns are used as standards. 'Lingua di Fuoco' was chosen as it is one of the most widely grown bean cultivars in Italy.

For DNA analysis bean seeds were germinated in a growth chamber on wet sand under controlled conditions of temperature (20 °C) and photoperiod (12 h). After 15 days, the primary leaves were harvested and DNA was extracted.

PHAS extraction and electrophoresis

Phaseolin fraction was extracted following the procedure previously described by Limongelli et al. (1996). Briefly, five single seeds of each landrace/cultivar were ground to a fine powder and flour suspended for 30 min in 0.5 M NaCl. The suspension was centrifuged and the supernatant mixed with equal volume of cracking buffer (Tris–HCl 0.625 M) containing 2 mM EDTA, 2% (w/v) SDS, 10% (w/v) glycerol, 0.02% (w/v) DTT and 0.01% of bromophenol blue marker dye, and denatured. Sample amounts of 15 μl were used for SDS-PAGE and loaded on an Excel SDS Homogeneous 12.5 gel (Amersham Biosciences). The gel was run on a Multiphor II electrophoresis unit (Amersham Biosciences) at 50 mA for 80 min. Proteins were fixed and stained. The destaining and preserving procedures were according to the manufacturer instructions (Amersham Biosciences). Stained and dried gels were digitalized and evaluated using a BioRad GS700 densitometer.

DNA extraction

Total DNA was extracted from fresh leaves from individual plants according to the method of Saghai-Maroof et al. (1984). DNA concentration was measured by fluorometric analysis, and all samples were diluted in TE 1X (Tris/EDTA, pH 8) to a uniform DNA concentration of 10 ng/μl for RAPD, semi-random and ISSR analysis.

ISSR, RAPD and semi-random amplifications

The amplification analyses were carried out with a mixture of DNA from five individuals per sample. Thus, the band patterns were representative of landraces and cultivars and only differences among

them, not within genotypes, were observed. Bands identified as putative landrace/cultivar discriminating markers were further screened for their presence in all individuals composing the pool.

Twenty ISSR primers (Table 2) based on core repeats non-anchored or anchored either at the 5′ or 3′ end were screened for polymorphic loci in the twenty examined samples. Each amplification reaction contained 20 ng template DNA, 100 mM Tris/HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.25 mM each of dATP, dCTP, dGTP, dTTP, 4 μM primer (Genset SA, Paris, France) and 0.4 U of Taq DNA Polymerase (MBI Fermentas, Finland) in a total volume of 25 μl. ISSR amplification consisted of an initial denaturation step at 94 °C for 1.5 min, 35 cycles of 40 s at 94 °C, 45 s at 45 °C, 1.5 min at 72 °C and a cycle of 45 s at 94 °C, 45 s at 44 °C, followed by one last extension step of 5 min at 72 °C.

The 27 random primers (Table 2) tested in the current study were obtained from Genenco (Florence, Italy); these included fifteen decamer primers (from FAGR1 to FAGR15) and twelve semi-random primers (from FAGR16 to FAGR27). RAPD analyses were performed according to the protocol of Williams et al. (1990). Briefly, PCR amplifications were carried out in a reaction volume of 20 μl containing 20mM Tris–HCl (pH 8.4), 50 mM KCl, 2 mM $MgCl_2$, 0.2 μM of primer, 0.1 mM each of dATP, dTTP, dCTP and dGTP, 0.5 U of Taq DNA polymerase (MBI Fermentas, Finland) and 20 ng of genomic DNA. RAPD reactions were carried out using the following cycle profile: initial denaturation at 94 °C for 5 min followed by 45 cycles at 94 °C for 1 min, 35 °C for 1 min, 72 °C for 2 min and a final 7 min extension at 72 °C. Semi-random amplification protocol and PCR conditions were as described for ISSR analysis.

Amplifications were performed in a Whatman Biometra (Germany) T gradient Thermal Cycler for all three techniques. At least two PCR amplifications were performed for each sample with ISSR, semi-random and RAPD primers to evaluate the reproducibility of the obtained bands. Amplified products were separated in a 1.8% agarose gel using 0.5× TBE (Tris/Borate/EDTA pH 8.0) buffer. After ethidium bromide staining,

Table 2. Nucleotide sequences of tested primers. The underlined fragment of each semi-random primer was complementary to the semi-conservative sequences of intron-exon junctions.

Primer code	Nucleotide sequence (5′ → 3′)	Primer code	Nucleotide sequence (5′ → 3′)
ISSR			
LOL1	CTCTCTCTCTCTCTCTAC	LOL11	CTCCTCCTCGC
LOL2	CTCTCTCTCTCTCTCTGC	LOL12	GTGGTGGTGGC
LOL3	CACACACACACAAC	PHV1	ACTGACTGACTGACTG
LOL4	CACACACACACAGT	PHV2	GACAGACAGACAGACA
LOL5	GAGAGAGAGAGAGG	PHV3	GCCCTCTCTCTCTCTCTCT
LOL6	GTGTGTGTGTGTGG	PHV4	GGCGTGTGTGTGTGTGTGT
LOL7	GAGAGAGAGAGACC	PHV5	ACGCACACACACACACACA
LOL8	GTGTGTGTGTGTCC	PHV6	CCACTCTCTCTCTCTCTCT
LOL9	CACCACCACGC	PHV7	GTGGTGTGTGTGTGTGTGT
LOL10	GAGGAGGAGGC	PHV8	GAGCACACACACACACACA
RAPD/Semi-random			
FAGR1	CACCGCTTGT	FAGR15	AGCCGTGGAA
FAGR2	AGGTGACCGT	FAGR16	ACTTACCTGAGGCGCGAC
FAGR3	CAAACGTCGG	FAGR17	ACTTACCTGCTGGCCGGA
FAGR4	GTTGCCATCC	FAGR18	ACTTACCTGCCTGCCGAG
FAGR5	GGCTGCGACA	FAGR19	ACTTACCTGCCTACGCGG
FAGR6	AGGCAGAGCA	FAGR20	CCGGCAGGTCAGGTAAGT
FAGR7	AGCAGCGCAC	FAGR21	GCAGAGGGCCAGGTAAGT
FAGR8	CAAACGTGGG	FAGR22	ACTTACCTGCACCGTATG
FAGR9	GGGTCTCGGT	FAGR23	ACTTACCTGAGGTCACCA
FAGR10	GGTCGATCTG	FAGR24	ACTTACCTGGACGTGCCG
FAGR11	AGTCGCCCTT	FAGR25	ACTTACCTGGTCGATCAA
FAGR12	GGGCCAATGT	FAGR26	AGCCTATGTCAGGTAAGT
FAGR13	GACGTGGTGA	FAGR27	AGTGATTGTCAGGTAAGT
FAGR14	GTGGAGTCAG		

gels were visualized on a transilluminator 302 nm UV light and were recorded digitally with a Kodak Digital Science DC120 Zoom Digital Camera. The digital imaging files were analyzed with Kodak Digital Science 1D software. Fragment sizes were estimated based on 3000 MW ladder size standards (MBI Fermentas, Finland) according to the algorithm provided in the Kodak Digital Science 1D software.

Data analysis

Only distinct, reproducible, well-resolved fragments, in the size range from 300 to 3000 bp, were scored as present (1) or absent (0) for each of the RAPD, semi-random and ISSR markers with the 20 analyzed samples. The absence of an amplified product in at least one landrace was used as a criterion for considering a marker as polymorphic. The matrices were read by NTSYS-PC v.2.02 h (Rohlf, 1993) and analyzed using SIMQUAL (Similarity for Qualitative Data) with Jaccard's similarity coefficients (Jaccard 1928). Jaccard's similarity measure does not take into account double absence. This is closer to the biological reality, considering that the absence of a DNA fragment in two samples is an absence of information rather than an element of similarity.

Dendrograms were constructed independently for each type of molecular marker, ISSR, semi-random or RAPD, using Unweighted Pair-Group Method with arithmetic Averages (UPGMA) with Sequential, Agglomerative, Hierarchical, and Nested clustering (SAHN) routine. Dendrograms were also compared and the congruence of clustering produced with the three types of markers was assessed by the Pearson correlation coefficient. The robustness of nodes in the trees was tested by bootstrap re-sampling as proposed by Felsenstein (1985). To ensure an accuracy of the bootstrap higher than 95%, 600 repetitions of the analyses were done. The bootstrapping was performed by using the WinBoot program (Yap and Nelson 1996). Principal coordinates analysis (PCO-ORDA) (Gower 1966) was performed in addition to cluster analysis, and the ordinations displayed in three dimensions. PCOORDA allowed visualization of clusters of relatedness amongst reference cultivars and Italian bean populations, since the distance between the points is indicative of the genetic distance between the genotypes they represent.

The ability of the most informative primers to differentiate between landraces was assessed by calculating their Resolving power (Rp). According to Prevost and Wilkinson (1999) the Resolving power (Rp) of a primer is: $Rp = \sum Ib$ where Ib (Band informativeness) takes the value of: $1- [2\times| 0.5- p|]$, p being the proportion of the 20 genotypes containing the band.

Results

PHAS patterns

The Sarconi and Lamon landraces showed two PHAS patterns that were identified, by comparison with standards, as C and T types (Table 1). In particular the majority of Sarconi populations (S13, S14a, S14b, S17, S19, S21) and 'Spagnol' and 'Calonega' landraces from Lamon shared a C-type phaseolin pattern, whereas 'Nasieddu', 'Ciuoto', 'Verdolino nano' and 'San Michele o' Ciuoto' from Sarconi and 'Spagnolet' from Lamon showed a T-type pattern. The electrophoretic profile of the three landraces of Zolfino bean highlighted the occurrence of S-type phaseolin, as the 'Sanilac' reference cultivar. The analysis of five seeds for each sample showed the absence of individuals with different PHAS-type within each landrace. The commercial bean variety 'Lingua di Fuoco' was characterized by a T-type PHAS banding pattern.

ISSR/RAPD/semi-random analysis

A total of twenty ISSR, fifteen RAPD and twelve semi-random primers was used to investigate sixteen Italian common bean populations and four commercial cultivars. The total number of bands (TNB), number of polymorphic bands (NPB), percentage of polymorphic bands (P%), number of different genotypes (NG), Resolving power (Rp) and number of exclusive bands (NEB) obtained per each primer are shown in Table 3.

Eight ISSR primers produced a total of 130 clearly detectable amplified products, 110 of them polymorphic (84.6%) at an average of 13.7 markers per primer. The Rp of the eight ISSR

Table 3. Total number of bands (TNB), number of polymorphic bands (NPB), percentage of polymorphic bands (%P), number of different landraces or cultivars identified (NG), Resolving power (Rp) and number of exclusive bands (NEB) obtained per ISSR, RAPD and semi-random primer.

Primer 5′ → 3′	TNB	NPB	P%	NG	Rp	NEB
ISSRs						
LOL2 $(CT)_8GC$	16	14	87.5	16	8.5	0
LOL7 $(GA)_6CC$	18	15	55.5	6	2.7	2
LOL8 $(GT)_6CC$	17	12	70.6	7	2.7	1
LOL9 $(CAC)_3GC$	19	19	100.0	20	11.4	2
LOL10 $(GAG)_3GC$	13	10	69.2	14	6.9	0
LOL12 $(GTG)_3GC$	14	11	78.6	9	4.2	0
PHV6 $CCA(CT)_8$	15	10	66.7	5	1.6	1
PHV7 GTG $(GT)_8$	18	17	94.4	3	2.6	1
Total	130	110	84.6	80	40.6	7
RAPDs						
FAGR2 AGGTGACCGT	12	5	41.6	11	5.0	0
FAGR3 CAAACGTCGG	9	8	88.8	12	3.5	1
FAGR4 GTTGCCATCC	10	4	40.0	5	1.5	1
FAGR5 GGCTGCGACA	14	12	85.7	15	6.4	0
FAGR6 AGGCAGAGCA	13	10	76.9	16	4.5	0
FAGR10 GGTCGATCTG	9	7	77.7	4	2.8	0
Total	67	46	68.6	63	23.7	2
Semi-random						
FAGR16 ACTTACCTGAGGCGCGAC	19	19	100.0	16	7.3	2
FAGR17 ACTTACCTGCTGGCCGGA	24	22	91.6	20	13.4	0
FAGR18 ACTTACCTGCCTGCCGAG	17	17	100.0	17	7.7	3
FAGR21 GCAGAGGGCCAGGTAAGT	18	16	88.8	12	5.4	3
FAGR22 ACTTACCTGCACCGTATG	13	10	76.9	12	6.4	0
FAGR23 ACTTACCTGAGGTCACCA	18	15	83.3	16	8.9	0
FAGR24 ACTTACCTGGACGTGCCG	11	9	81.8	16	4.6	0
Total	120	108	90.0	106	53.7	8

primers ranged from 1.6 for primer PHV6 to 11.4 for primer LOL9. Besides its high Rp value, ISSR primer LOL9 was able to distinguish all 20 bean accessions. The 3′-GC/CC anchored primers based on GC/CC motifs were the most informative since the fingerprints had the highest number of amplified bands which ranged between 13 and 19.

Out of 15 RAPD primers screened, six generated reproducible DNA fragments with an average of 11.2 bands per primer. The number of polymorphic bands ranged from 4 to 12 with a mean of 7.7 bands, for a total of 46 polymorphic bands scored. The Rp of the six selected RAPD primers ranged from 1.5 for primer FAGR4 to 6.4 for primer FAGR5. The number of populations that could be distinguished by each primer varied from 4/20 (FAGR10) to 16/20 (FAGR6).

Seven semi-random primers produced a total of 120 bands with 90% of polymorphic fragments. FAGR17 primer had the highest Rp value (13.4) and was able to distinguish all 20 bean genotypes. An example of semi-random DNA patterns is shown in Figure 2.

The banding patterns obtained with all three techniques were clustered using UPGMA method. ISSR (Figure 3a), RAPD (Figure 3b) and semi-random (Figure 3c) techniques separated out one cluster in which the Zolfino beans (ZB1, ZB2, ZB3) were grouped with 'Sanilac'. For ISSR and semi-random techniques, the remaining samples were grouped into two main clusters (groups I and II for ISSR; A and B for semi-random), while for RAPDs in addition to these two clusters (G3 and G4) the cultivar 'Contender' appeared as separate group (G2). 'Tendergreen' (TNG) and 'Lingua di Fuoco' (LDF) samples were grouped together by RAPDs and the semi-random method in one tight subcluster but although ISSR placed them in the same larger cluster they were not as tightly grouped.

The three-dimensional ordinations confirmed the cluster analysis results, showing that 'Sanilac'

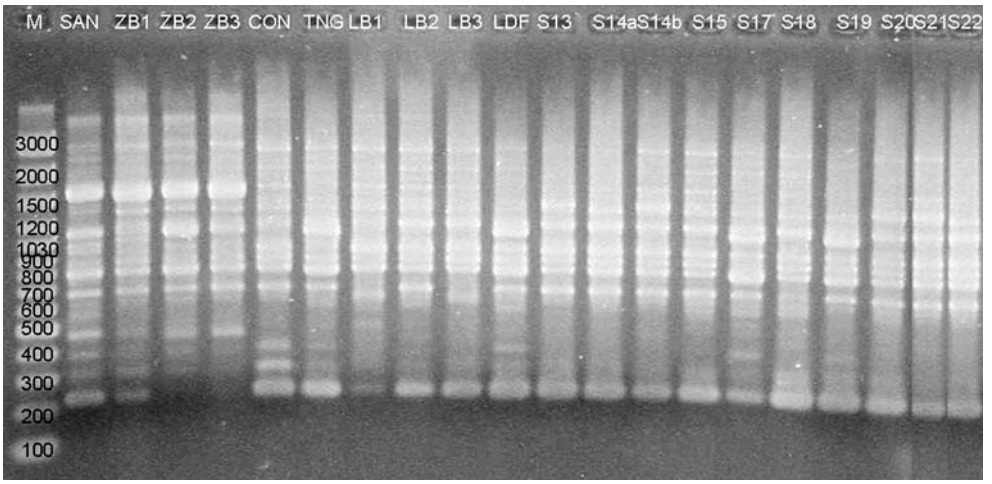


Figure 2. Amplification products of the studied common bean genotypes using FAGR22 semi-random primer. Lane codes as in Table 1. Lane marked as 'M' contain a 100 bp marker.

and Zolfino landraces were sharply separated from all other bean populations (Figure 4a–c).

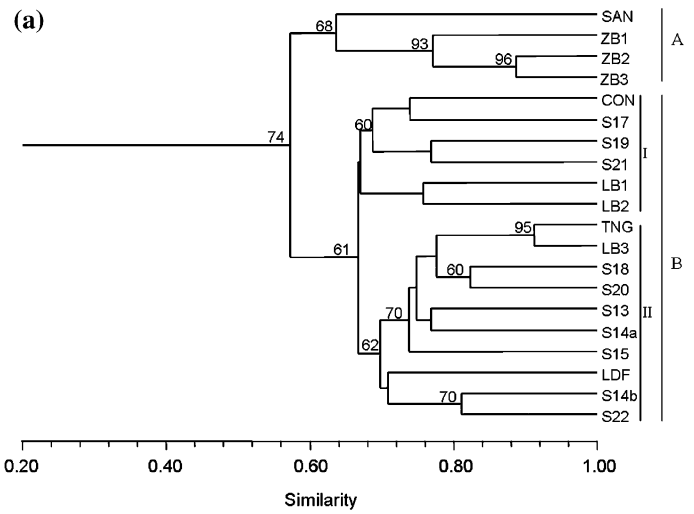
ISSR, RAPD and semi-random analysis also revealed putative landrace/cultivar-specific products. Such bands were observed in the Zolfino landrace 'Reggello' (primer FAGR16–1600 bp) and 'Loro Ciuffenna' (primer LOL9–650 bp) in the Sarconi S13 (primer FAGR16–900 bp) and S14b (primer LOL9–1350 bp) and in the Lamon bean 'Spagnolet' (primer FAGR3–690 bp). Primer FAGR21 was able to produce three unique specific fragments (2100, 1500, 1300 bp) in the S21 landrace. As far as the reference cultivars are concerned 'Sanilac' (primers FAGR4–850 bp, PHV6–570 bp, PHV7–900 bp, LOL8–1340 bp), 'Contender' (primer FAGR18–2050 bp), 'Tendergreen' (primer FAGR18–1100 bp and 680 bp) and 'Lingua di Fuoco' (primer LOL7–930 bp and 380 bp) showed a total of 9 exclusive bands.

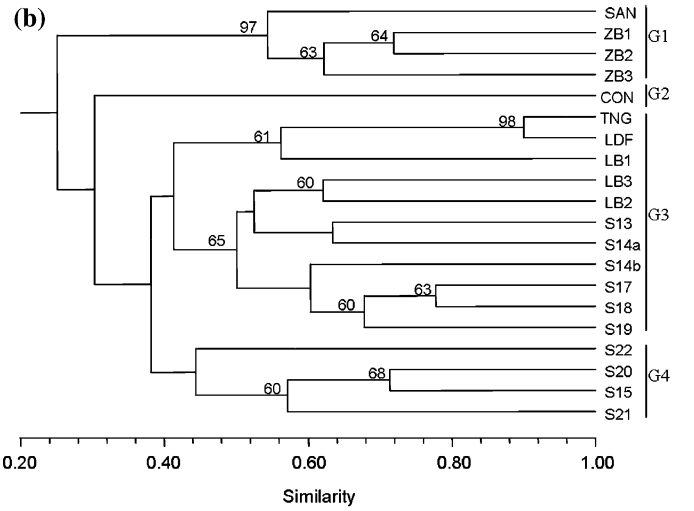
Discussion

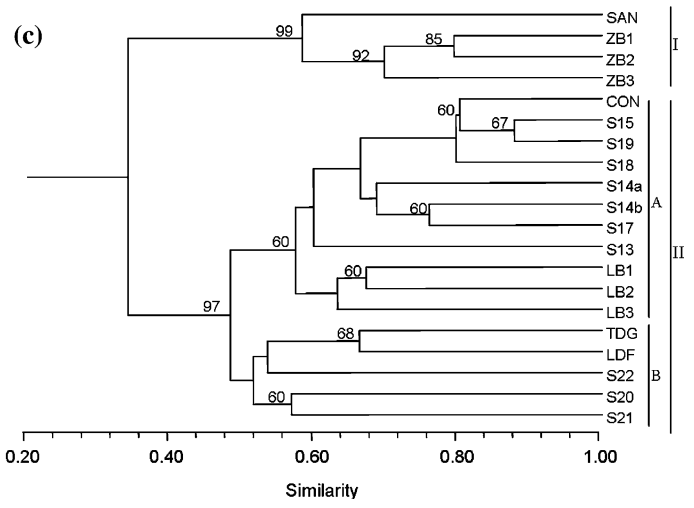
PHAS patterns

The distribution of the electrophoretic banding types of PHAS (Table 1) showed that all the analyzed bean landraces possess the three patterns typical of cultivated materials: i.e. C, T and S (Gepts and Bliss 1988). The observed frequency of the C type (50%) was in agreement with the reported predominance of this pattern in the Mediterranean basin (Gepts and Bliss 1988; Lioi 1989). The T banding pattern occurred in 31.2% of examined landraces and in the cultivar 'Lingua di Fuoco' and the S-type pattern, the least diffused one in Italy (Lioi 1989), was shared by the three Zolfino populations. In agreement with previous reports, the S phaseolin pattern was associated with the sole small seeded populations (Gepts and Bliss 1988). The geographic distribution of the different PHAS types among wild and cultivated beans in Middle America and the Andes strongly suggest the existence of two main areas of domestication: Middle America for small-seeded, S-phaseolin cultivars and the southern Andes for large-seeded, T- and C- phaseolin cultivars (Gepts and Bliss 1986; Gepts 1988; Gepts et al. 1988). Our results indicated that most of the landraces collected in Italy (13 out of 16) and the cultivar 'Lingua di Fuoco' belong to the Andean gene pool, whereas the Zolfino populations belong to the Middle American gene pool.

Figure 3. UPGMA dendrogram showing the genetic relationships among 16 Italian bean landraces and 4 commercial cultivars, using (a) ISSR, (b) RAPD and (c) semi-random data. The numbers at the forks indicate the bootstrap value, using the Winboot program. The code plotted corresponds to landraces and cultivars as listed in Table 1.


(a)
SAN
ZB1
ZB2
ZB3
CON
S17
S19
S21
LB1
LB2
TNG
LB3
S18
S20
S13
S14a
S15
LDF
S14b
S22
A
I
B
II
68
93
96
74
60
61
95
60
70
62
70
0.20
0.40
0.60
0.80
1.00
Similarity


(b)
SAN
ZB1
ZB2
ZB3
CON
TNG
LDF
LB1
LB3
LB2
S13
S14a
S14b
S17
S18
S19
S22
S20
S15
S21
G1
G2
G3
G4
97
64
63
98
61
60
65
63
60
68
60
0.20
0.40
0.60
0.80
1.00
Similarity


(c)
SAN
ZB1
ZB2
ZB3
CON
S15
S19
S18
S14a
S14b
S17
S13
LB1
LB2
LB3
TDG
LDF
S22
S20
S21
I
A
II
B
99
85
92
60
67
60
60
60
97
68
60
0.20
0.40
0.60
0.80
1.00
Similarity

Molecular marker band patterns

The PCR amplification performed with ISSR markers revealed a high percentage of polymorphic fragments (84.6%). This was not unexpected since the ISSR technique amplifies microsatellite regions that are potentially polymorphic (Morgante and Olivieri 1993). Some SSR motifs were better suited for genotyping than others. Although a higher number of primers need to be analyzed our results suggested that dinucleotide and trinucleotide SSR occur at high frequencies along common bean genomes. On the contrary, primers based on tetranucleotide repeats did not produce any polymorphic pattern. In previous surveys about rice, maize, soybean, the occurrence of di- and trinucleotide SSR at higher frequencies along the genomes than tetranucleotide repeats was also highlighted (Rongwen et al. 1995; Akagi et al. 1996; Chin et al. 1996). Yu et al. (1999) reported that dinucleotide motif primers were more important sources for developing polymorphic SSR markers in common bean. Our experiment agrees with above mentioned findings as five out of the eight primers that produced polymorphisms were based on dinucleotide motifs.

The six selected RAPD primers produced 68.6 % of polymorphic bands with an average of 7.6 polymorphic fragments per primer. This value resulted to be relatively high when compared to the reports of other RAPD studies, e.g. in *Brassica* spp. (Demeke et al. 1992), *Allium* spp. (Wilkie et al. 1993), sorghum (Tao et al. 1993), alfalfa (Yu and Pauls 1993) and sweet potato (Connolly et al. 1994). A possible reason could be the utilization of primers with 60 to 70% GC content in our experiments. In a previous report Fukuoka et al. (1992) observed an increase in the number of bands with increasing of primer GC content, and this correlation was explained with the higher stability of G-C complementation with respect to A-T pairing.

The application of a semi-random approach, using primers in which the 9 base long consensus sequences of the intron-exon junction were supplemented with some random sequences, proved to be convenient for the evaluation of genetic diversity among all twenty bean genotypes. This system was as cheap and fast as RAPD, but unlike the latter, the semi-random primers generated more complex band patterns with a high degree of polymorphism (90%). The sensitivity of the semi-random system and the ISSR method was almost comparable whereas the RAPD data were much more dissimilar (Table 3). The RAPD profile comprised up to 14 DNA fragments, while the average number of fragments amplified by the semi-random primers was 17.1. This was not unexpected since semi-random primers were known to provide considerably higher polymorphism than RAPD and were successfully used to assess genetic diversity among common bean landraces (Nowosielski et al. 2002) and cultivars of wheat and *Triticale* (Gawel et al. 2002).

From the comparison of dendrograms obtained with either ISSR and semi-random fragments, a value of 0.77 was obtained for Pearson correlation coefficient (r value), indicating a good fit of both type of markers. Although semi-random and ISSR dendrograms showed similar trends in terms of genetic relatedness, the similarity values based on ISSR (Jaccard's coefficient range of 0.57–0.86) data were higher than those based on semi-random (Jaccard's coefficient range of 0.27–0.84), meaning that closely related genetic groups could be more easily detected. On the contrary clustering of genotypes within groups was not similar when RAPD and ISSR/semi-random derived dendrograms were compared. The different ability to resolve genetic variation among the genotypes may be partially related to the number of PCR products analyzed with each marker system (130 for ISSR, 67 for RAPDs and 120 for semi-random primers). This is in agreement with a previous report suggesting that the number of loci and their coverage of the overall genome is of great importance in obtaining reliable estimates of genetic relationships among cultivars (Loarce et al. 1996). Another reason could be the differences in reproducibility between ISSR/semi-random PCR and RAPDs. Many authors reported the higher reproducibility of ISSR and semi-random PCR, due to the longer primers used, as compared to RAPD (Salimath et al.1995; Yang et al. 1996; Goulao et al. 2001; Gawel et al. 2002). The putatively similar bands originating from RAPDs in different individuals are not necessarily homologous although they may share the same size in base pair. This situation may lead to wrong results when calculating genetic relationships (Fernandez et al. 2002).

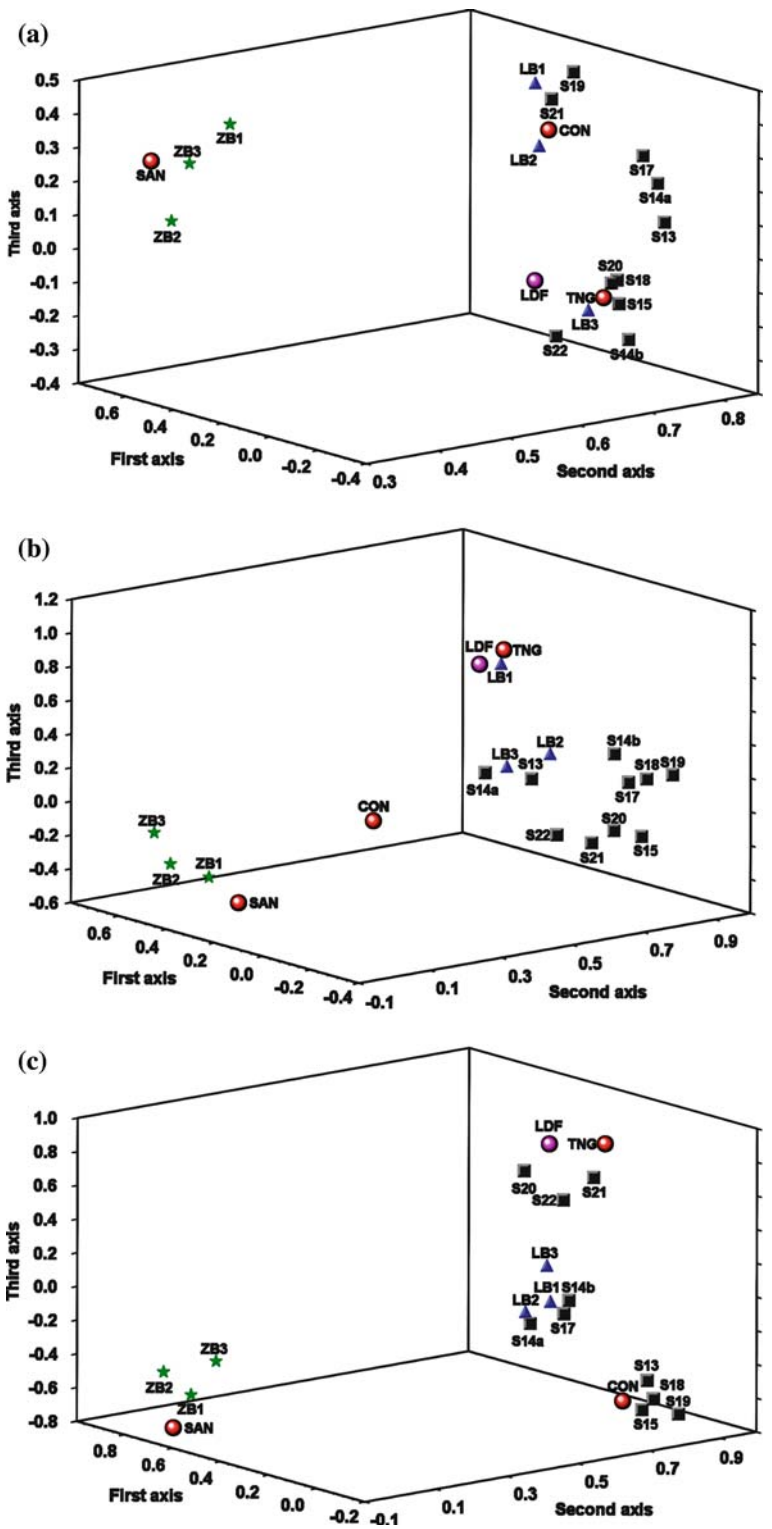


Figure 4. Three-dimensional plot of principal component analysis using (a) ISSR, (b) RAPD and (c) semi-random markers. The code plotted as listed in Table 1.

Despite the different discriminating power, RAPD, ISSR and semi-random based cluster analyses showed to be successful in grouping Italian bean landraces by their gene pool of origin. All three dendrograms highlighted a clear separation of the 20 tested samples onto two main branches with genetic similarity of 57.2% for ISSR and less than 40% for RAPD and semi-random approaches: Lamon and Sarconi populations on one group and Zolfino landraces on the other group. This separation was correlated with the phaseolin type and seed size (Table 1). The Lamon and Sarconi landraces had T or C phaseolin patterns and large/medium seed sizes. On the other hand the small-seeded Zolfino populations had the characteristic 'S' phaseolin pattern. A correlation among the formed clusters and seed weight was also observed in RAPD studies on cultivars and landraces of common bean of South Brazil (Freyre et al. 1996; Maciel et al. 2001). In the same work they also assigned the bean accessions to specific gene pools. In a previous report (Metais et al. 2000) it was suggested that the Andean and Mesoamerican gene pools seem to have specific SSR regions and because of this they could be considered useful markers to discriminate between different gene pool of origin. Similarly, Galvan et al., (2001) reported the usefulness of ISSR markers to provide reliable evidences of the earlier divergence of the Mesoamerican and Andean gene pools in North-Western Argentinean varieties of common bean. More recently, the semi-random PCR resulted to be a valuable system in studying the genetic diversity among Polish genotypes of *Phaseolus*, but no relation between the formed clusters and their belonging to particular gene pools was observed (Nowosielski et al. 2002).

The practical utility of any molecular approach for germplasm management is partly determined by the ability to differentiate between large numbers of populations (Gilbert et al. 1999). The ISSR, semi-random and RAPDs primers used in the present study varied in their ability to diagnose bean landraces. There was a seemingly linear relationship between the Rp of each primer and the number of genotypes identified, and primers with the highest Rp values were generally the most effective in distinguishing among landraces. This relationship was stronger for ISSR ($r^2 = 0.95$) than for semi-random ($r^2 = 0.57$) and RAPD markers ($r^2 = 0.63$). Similar results were observed by Prevost and Wilkinson (1999) on potato cultivars. The combined Rp values of two primers also provided a measure of their collective performance for identification purpose (Gilbert et al. 1999). In the present work the combination of any two semi-random primers allowed the diagnosis of all 20 bean genotypes. On the contrary at least four and three ISSR and RAPDs primers, respectively, were necessary to diagnose all tested populations. One exception is ISSR-LOL9 with a discrimination ability comparable to that of FAGR17 semi-random primer.

The present study provided evidence that semi-random and ISSR techniques are more suitable approaches for estimating the extent of genetic diversity as well as to determine the pattern of genetic relationship among different landraces of *P. vulgaris*, with polymorphism levels sufficient to generate potential diagnostic fingerprints with relatively fewer primers sets. The informative primers identified in our studies will be useful in genetic analysis of bean accessions in germplasm holdings. In addition, the putative landrace-specific bands may be used for genotype characterization and grouping germplasm accessions. Studies are in progress to confirm and convert secure landrace-specific random/ISSR fragments into sequence characterized amplification regions (SCARs) in order to develop robust landrace-specific marker. Identified landrace-discriminating sequences, together with exclusive fingerprints, would allow an accurate description of each Italian bean populations permitting thus to safeguard this genetic resource from fraudulently commercialized lines.

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