Genet Resour Crop Evol (2009) 56:445–453
DOI 10.1007/s10722-008-9377-z

RESEARCH ARTICLE

Assessing diversity in common bean (*Phaseolus vulgaris* L.) accessions at phenotype and molecular level: a preliminary approach

A. Foschiani · F. Miceli · M. Vischi

Received: 6 September 2007 / Accepted: 4 September 2008 / Published online: 7 November 2008

Abstract Assessing diversity across and within crop varieties is relevant to improve the description of collections in genebanks and in on-farm conservation. The differences among and within several common bean (*Phaseolus vulgaris* L.) landraces, collected from and maintained *ex situ* in NE Italy, were initially studied using a set of 15 phenotypic descriptors. From a subgroup of five bean accessions, microsatellite genotyping was performed after the extraction of DNA from each of 38–40 individuals. One of the 15 phenotypical descriptors (plant architecture) was of no use. Only ten out of the 23 molecular markers did work, however sufficient to discriminate the accessions. The phenotypic descriptors identified a portion of the within-population variability. A few discrepancies were obtained when observations of phenotype descriptors were run independently in two alpine locations in NE Italy. The genetic approach carried out separately on DNA of about 40 individuals clarified the structure of the five accessions.

Keywords Genebanks · Landraces · Molecular markers · *Phaseolus vulgaris* · Phenotypical descriptors

A. Foschiani · F. Miceli · M. Vischi (✉)
Dipartimento Scienze Agrarie e Ambientali, Università di Udine, Via delle scienze 208, 33100 Udine, Italy
e-mail: massimo.vischi@uniud.it

Introduction

Common bean (*Phaseolus vulgaris* L.) is by far the most widely consumed grain legume in the world (Singh 2001). A major food in Latin America and eastern Africa, common bean is gaining momentum in developed countries, where the population is concerned with healthier diets (Acosta-Gallegos et al. 2008). In the last few decades, a reversed trend has been observed in Italy, where the crop occupied only 11,000 ha by the end of the century. The stagnation of yields, the labour required to hand-harvesting climbing varieties and the changed food preferences were all mentioned to explain this negative trend (Ranalli et al. 2001). Nevertheless, a number of landraces are presently grown in Italy, mostly because of their nutritive, culinary and organoleptic values (Bove 1994; Piergiovanni et al. 2000).

In alpine valleys, climbing bean is grown in small patches and kitchen gardens, as in Friuli Venezia Giulia region (NE Italy), where a large diversity is informally maintained on "farms". Those beans have little, if any, market share and the season' product is used at home for traditional soups, or donated to friends and relatives. Indeed, Friuli Venezia Giulia is a land of differences, at the crossroad of Latin, Slavic and Germanic cultural influences. Three out of five biogeographical regions of Europe (Alpine, Continental and Mediterranean) can be found, and a number of languages, dialects and idioms are spoken there. Therefore, cultural or "non-geographic"

limitations have been invoked to tentatively explain the enduring presence of crop diversity in the region (Miceli et al. 2007; Hammer et al. 2007).

Collecting crop germplasm from Friuli area started after the quake of May–September 1976. On recent years, several bean populations and landraces, mostly belonging to *P. vulgaris* were collected, characterised and evaluated (Pozzi et al. 2004). Regional Law no. 11/2002 set up a germplasm repository (the *Autochtonous Plant Genebank*—BaGAV). Presently, bean landraces are being considered for registration as "Conservation Varieties". Therefore, available protocols to assess and monitor diversity at reasonable costs are a key issue.

A broad approach, using phenotypic and molecular markers, is required for analysing diversity and support plant genetic resources management (Brown et al. 1997). Among phenotypical descriptors for common bean, IBPGR (1982) and UPOV (1994) are of standard use. The former set is better suited for characterising ancestral (i.e. *wild* and *weedy*) materials, the latter for commercial varieties; in both cases a large number of descriptors is involved. We adopted a compact set of 15 descriptors, identified by the *Phaselieu* Concerted Action (Schachl and De la Rosa 2001). The system is intended for inspecting and describing large bean collections in the field at reasonable costs.

Different approaches for assessing diversity at molecular level are presently available. Microsatellite markers have been developed that for many characteristics have been considered the reference markers for variety fingerprinting in bean (Yu et al. 1999), being co-dominant, widely distributed in the genome, highly polymorphic and permitting a high level of repeatability of the analyses (Powell et al. 1996; Rafalsky and Tingey 1993). Several microsatellite markers are now available in the common bean (see Sicard et al. 2005). In case of large germplasm collections, DNA analysis techniques are however subjected to limitations of a practical order (Gilbert et al. 1999). A rapid, simple and reliable molecular protocol would be essential for managing a vast number of samples. Pooling the DNA of several individuals is a common strategy for monitoring the diversity within a large number of accessions. Indeed, high-capacity automated sequencers have become available, making it possible to carry out separate analyses on the DNA of dozens of individuals per population.

Here we present a molecular procedure based on the separate extraction of DNA from 38 to 40 individuals and the use of microsatellites. The abjective is to ascertain genetic diversity in a few bean accessions, as a preliminary step towards a routine application of the procedure to the entire bean collection maintained *ex situ* by BaGAV. Previously run phenotyping did detect some diversity within and across bean landraces. A molecular protocol to resolve the structure of, and establish relations among, bean landraces would complement our genebank management procedures on *Phaseolus* and hopefully add momentum to agro-biodiversity studies in the area.

Materials and methods

Plant material

Five common bean accessions were considered (Table 1). Two are commercial varieties: 'Pegasus', a large-seeded borlotto type, usually marketed as shelled beans (Cattivello and Del Zan 2004) and 'Kondor', a small-seeded cannellino type intended for greenhouse cultivation, but also suitable in the field (Parisi et al. 2000). Both cultivars have been also used as checks in local field evaluation tests. Three putative landraces ('Borlotti 4', 'Militons 3' and 'Cesarins') were included, as top scorers in field evaluations for agronomic and qualitative traits (Miceli et al. 2003). Due to the very limitate diffusion of landraces in the area, only one population per landrace was available, i.e. the seeds originated from multiplication of the originally collected stock. No statistics about the cultivation area for landraces are available. However, taking into account a crop surface of about 11 ha in Friuli Venezia Giulia mostly from commercial varieties, it is safe to say that 'Borlotti 4' and 'Militons 3' occupy 0.1–0.3 ha each, and 'Cesarins' a slightly larger area. Indeed, a small-seeded agro-ecotype grown in the contiguous Belluno province under the name "Gialet" or "Fasol Bisio" (Piergiovanni et al. 2004), is phenotypically very close to 'Cesarins'. Those materials may belong to the same landrace.

From our observations (Tables 2, 3) each of the first two landraces ('Borlotti 4', 'Militons 3') consisted of at least two lines, while the structure of the third one ('Cesarins') at phenotype level appeared as single line. Seed from the three

Table 1 General description of the five climbing bean (*P. vulgaris*) accessions

Accession number	Name	Sample status	Collecting source	Location	Elevation (m a.s.l.)	100-seed weight (g)	Colour	Coat pattern	Use
3680001	'Pegasus'	Improved cultivar	Seed company	Cesena (FC)	–	80.9	Bicolour	Pinto type	gs
3680024	'Kondor'	Improved cultivar	Seed company	Cesena (FC)	–	43.5	White	Absent	gs-ds
3680009	'Borlotti 4'	Landrace	Field	Lauco (UD)	719	72.2	Bicolour	Pinto type	gs-ds
3680018	'Militons 3'	Landrace	Field	Forni di Sopra (UD)	942	74.6	Bicolour	Pinto type	gs-ds
3680019	'Cesarins'	Landrace	Field	Prato Carnico (UD)	736	34.9	Pale green	Absent	ds

Shelled green seeds (gs) and/or dry seeds (ds) as typical utilisations in bean accessions are indicated

Table 2 Names and GenBank accession numbers of the 23 microsatellites used

Locus	GenBank no.	Core motifs	Allele size (bp)	T_a
PH10B11	M75856	(CT)11	157	47
PH1B4	J04555	(CCT)3(T)3(CTT)6	152	48
PH3B4	X60000	(AT)4(T)2(AT)6	139	49
PH4B6	X61293	(AT)18	163	47
PH9B2	X79722	(CCT)7	149	49
PH6B9	X80051	(ATCC)3(AG)2(TAC)3T(CTA)3	192	49
PH5B5	X74919	(AT)5	132	50
PH7B3	X96999	(AT)9	161	49
PH8B1	X04001	(AG)8	164	49
PH2B2	U77935	(GCCACC)5	95	48
PV1	X04660	(AG)8	201	48
PV2	X57022	(AATG)6	163	49
PV3	X63525	(AT)7	305	48
PVBR5	DQ185875	(GA)22	195	56
PVBR10	DQ185878	(AG)23	88	54
PVBR11	DQ185879	(TC)8(GT)4	142	56
PVBR14	DQ185881	(AG)23	196	56
PVBR16	DQ185883	(GA)28	198	56
PVBR18	DQ185884	(CT)29	198	46
PVBR20	DQ185886	(AG)22	197	56
PVBR21	DQ185887	(AG)18	229	56
PVBR23	DQ185888	(AG)19	345	56
PVBR25	DQ185890	(CT)23	158	56

landraces, as well as other locally grown bean materials, was donated by farmers or kitchen-garden owners, either by visiting them or in occasion of an agrobiodiversity event in Arta Terme (Udine, NE Italy). 'Cesarins' and 'Militons' denominations were the originally recorded in the area, while for 'Borlotti 4', local name was unavailable so a temporary denomination was coined.

Phenotype characterisation

The set of 15 morpho-phenological descriptors, thoroughly described in the "Handbook on evaluation of Phaseolus Germplasm" (Schachl and De la Rosa 2001) was routinely used on 34 putative landraces, i.e. the materials collected during the first 2 years of the work programme (unpublished data); data from

Table 3 Phenotyping data of *P. vulgaris* landraces ('Borlotti 4', 'Militons 3', 'Cesarins') and cultivars ('Pegasus', 'Kondor') grown in the field at two alpine locations

	Plant			Flower			Pod			
	First flower JD	Plant type[a]	Leaf shape[b]	Colour of standard[c]	Colour of wings[d]	Veins in the standard[e]	Position in the plant[f]	Fibre hardness[g]	Colour fresh pod[h]	Colour mature pod[i]
Location 1—Pesariis (750 m a.s.l.)										
Pegasus	189	4	1	3	3	+	4	3	1	RM
Borlotti 4	191	4	2	5	1	+	4	4	1	VS
			4	3	3		3	3		RM
Militons 3	189	4	4	1	1	+	4	1	1	YB
			2	3	3			2		RM
Kondor	190	4	2	1	1	+	4	2	1	WH
Cesarins	200	4	2	1	1	+	4	2	1	YB
Location 2—Piano D'Arta (560 m a.s.l.)										
Pegasus	187	4	1	3	1	0	4	3	1	RM
Borlotti 4	187	4	1	5	1	+	4	4	1	VM
			2	9	3				4	RM
Militons 3	190	4	4	1	1	+	5	1	1	YB
			2	3	3			3		RM
Kondor	190	4	2	1	1	+	4	2	1	WH
Cesarins	195	4	2	1	1	+	4	2	1	YB

Plant, flower and pod descriptors, after Schachl and de la Rosa (2001)

[a] Plant type 4 = indeterminate climber; [b] leaf shape 1 = triangular, 2 = quadrangular, 4 = ovate; [c] col. standard 1 = white, 3 = lilac, 5 = white with lilac stripes, 9 = purple; [d] col. wings 1 = white, 3 = lilac; [e] veins in the standard + present, 0 = absent; [f] position 3 = top, 4 = combination base, centre, top, 5 = other; [g] fibre hardness 1 = absent to 5 = strongly present; [h] col. fresh pod 1 = green, 4 = yellow with purple stripes; [i] colour mature pod, see Schachl and de la Rosa (2001)

the five bean accessions were then extrapolated. To encompass possible environmental variation in phenotypical descriptors, the survey was carried out in unreplicated plots at two Alpine villages (Pesariis: 46°31′11″ N, 12°46′32″ E, 750 m elevation; Piano d'Arta: 46°28′41″ N, 13°01′18″ E, 560 m elevation) close to the provenance of the landraces.

For each of the 15 descriptors, variants were assigned from a concise evaluation made by two observers, by considering 5–10 plants per plot and 5–15 seeds for each accession after harvest. Additionally, in a few cases observations from potted plants were made, to resolve traits inconsistently characterised in the field.

Microsatellite genotyping

DNA was extracted from young leaves of 38–40 individuals using the Dneasy 96 Plant Mini Kit (Qiagen GmbH, Hilden, Germany) and was kept separate throughout the entire analysis. Twenty-three pairs of primers were used, thirteen of which were identified by Yu et al. (2000), analysing the sequences present in the database of GenBank. A further 10 were isolated by Buso et al. (2006) starting from a genome library (Table 2). The primers were chosen on the grounds of their Tm, length and degree of polymorphism. The forward primers of the 23 SSRs were 5′-labelled with 6-FAM™ (Applied Biosystems). The PCR reaction was carried out in 20 µl of a solution containing 10 ng genomic DNA, 1× Mg-free PCR buffer solution, 0.2 mM dNTP mix, 2.5 mM $MgCl_2$, 0.25 µM of each primer, 0.8 U AmpliTaq Gold DNA polymerase (Applied Biosystems). Amplification was performed in a 9600 Thermal Cycler (Applied Biosystems) as follows: 5 min at 95°C followed by 35 cycles of: 30 s at 94°C, 30 s at the Tm indicated in Table 1, 45 s at 72°C, and a final extension stage of 5 min at 72°C (Lioi et al. 2005).

PCR products were separated with an ABI 3730 DNA sequencer (Applied Biosystems) and the fragments were sized by means of a ladder labelled with a fluorochrome (ROX = 6-carboxy-X-rhodamine). Data were analysed with GeneMapper software (Version 3.0, Applied Biosystems).

Statistical analysis

GenAlEx6 software (Peakall and Smouse 2006) was used to estimate allele number (A_n), expected heterozygosity (H_e), number and frequency of genotypes for each accession. Nei's (1978) genetic distance was calculated using the TFPGA software programme (Miller 1994). A cluster diagram was constructed based on these distances by the UPGMA (average linkage) method. To quantitatively evaluate the ability of the SSR set to assign each individual plant to the correct accession, an assignment test (Paetkau et al. 1995) was carried out based on genotype frequencies. This involved calculating the expected frequency of each individual's genotype in each of the five accession and subsequent assignment of each individual to the accession where its expected genotype frequency was highest.

Results and discussion

Phenotypical approach

The observations relative to plant, flower, pod and seed characteristics are reported in Tables 2 and 3. 'Plant Type' descriptor was of no use in discriminating materials: all the accessions felt into one class (indeterminate plant type). Useful variation was however observed for the other fourteen descriptors.

Intra-population diversity was detected within 'Borlotti 4' and 'Militons 3' landraces, while this did not hold true in 'Cesarins' (Table 2). Within each of the two previously mentioned landraces, after anthesis some differences in the colour and characteristics of the perianth were noted. The differences were confirmed by other descriptors, such as the colour of the ripe pod, primary and secondary colouration, and colour pattern and shape of the seeds.

Indeed, discrepancies by using descriptors appeared. In one of the genotypes pertaining to 'Borlotti 4', the colour of standard was recorded as 'lilac' (at Pesariis) but also as 'purple' (at Piano d'Arta). The colour of fresh and mature pods was also differently recorded at the two locations (Table 3). In any case, phenotypical approach enlighted a multilinear structure for 'Borlotti 4' and 'Militons 3'. This evidence was confirmed in 'Militons 3' by seed size and colour descriptors (Table 4). Darker, constant mottled seeds (BI-M5) were also thinner relative to the pale coloured, 'Pinto-type' (P) seeds. For the latter seeds, different variants (P2 vs. P6) appeared at Pesariis and Piano d'Arta.

In our small group of bean accessions, marked differences in size, weight and seed colour allows to separate borlotto-like, large-seeded materials ('Pegasus', 'Borlotti 4', 'Militons 3') from small-seeded materials, having cannellino-type ('Kondor') or roundish ('Cesarins') seeds (Table 4).

Indeed, within the borlotto-type subgroup, 'Pegasus' can be distinguished either from 'Borlotti 4' or from 'Militons 3' by the absence of veins in the standard (Table 3), and by the marked coating pattern of the seeds (Table 4). The hardness of pod fibre, the shape and coating pattern of seeds underscore distinctness of 'Borlotti 4' and 'Militons 3' discrepancies and/or variable trait expression in different locations seem hardly evitable for a phenotypical approach. On the whole, discrimination substantially holds, provided at least two descriptors are used to mark a difference in the phenotypes. As for the two small-seeded materials, 'Kondor' and 'Cesarins', they can be easily distinguished by mature pod colour, shape and length of the seed, and colour pattern of seeds.

Molecular approach

Microsatellite analysis

The data obtained were classified according to a qualitative scale, with scores ranging from 1 to 5, describing the complexity of the amplification profile for each primer (Stephenson et al. 1998). Of the 23 loci considered, 12 generated an electroferogram of a single locus, of easy interpretation as stuttering was absent or very slight. Loci PVBR21 and PVBR25 generated irregular stutter bands, which hindered easy interpretation. PH7B3, PV1, PVBR10, PVBR23, PH1B4, PVBR18 and PV3 produced multiple loci with various amplification products, making it

Table 4 Phenotyping data of *P. vulgaris* landraces ('Borlotti 4', 'Militons 3', 'Cesarins') and cultivars ('Pegasus', 'Kondor') grown in the field at two alpine locations

	Seed						
	Size (mm)			Shape[a]	100 seed weight g	Colour primary and secondary[b]	Coat pattern[c]
	Length	Width	Height				
Location 1—Pesariis							
Pegasus	17.6 (0.2)	6.18 (0.15)	9.08 (0.10)	4	81.2	BI	P8
Borlotti 4	16.2 (0.3)	6.31 (0.17)	8.15 (0.13)	4	70.1	BI	P6
Militons 3	16.5 (0.3)	6.8 (0.20)	9.02 (0.28)	2	73.7	BI	P2
	16.4 (0.4)	6.22 (0.08)	7.96 (0.27)	3			M5
Kondor	13.5 (0.2)	5.88 (0.10)	6.74 (0.10)	3	45.3	WH	21
Cesarins	10.4 (0.2)	5.63 (0.12)	6.71 (0.16)	1	31.7	GR	11
Location 2—Piano D'Arta							
Pegasus	16.9 (0.5)	6.83 (0.11)	9.61 (0.14)	4	80.6	BI	P8
Borlotti 4	16.8 (0.3)	6.65 (0.14)	8.59 (0.22)	4	73.8	BI	P2
Militons 3	17.1 (0.3)	7.20 (0.07)	9.40 (0.27)	2	75.6	BI	P6
	16.8 (0.9)	6.40 (0.10)	7.70 (0.14)	3		BI	M5
Kondor	13.1 (0.3)	5.56 (0.07)	6.33 (0.11)	3	41.7	WH	21
Cesarins	10.5 (0.3)	6.33 (0.09)	7.30 (0.12)	1	38.1	GR	11

Seed descriptors, after Schachl and de la Rosa (2001). Standard errors of seed size means are indicated on parenthesis

[a] Shape 1 = round/circular, 2 = oval/circular to elliptic, 3 = cuboid/elliptic, 4 = kidney shaped; [b] colour and [c] coat pattern: see Schachl and de la Rosa (2001)

difficult to relate the peaks between the different genotypes. Finally, the loci PH6B9 and PH4B6 failed to give rise to amplification products.

Loci PH9B2 and PH8B1 were monomorphic for all 5 accessions and could provide no useful information for this work. In regard to the other loci, the number of alleles of the single loci identified per each accession ranged from 2 (PH2B2, PVBR16, PVBR20) to 5 (PVBR11) with an average number of 3.4, giving a total of 34 alleles present in the 10 loci considered. Of the 10 useful sequences, the five SSRs identified by Yu et al. (2000) in GenBank (PH10B11, PH3B4, PH5B5, PH2B2, PV2) have a PIC between 0.1611 and 0.5193; the remaining five SSRs obtained from genome libraries have values ranging from 0.3535 to 0.6321, in agreement with findings in other molecular studies (Gaitan-Solis et al. 2002). As expected on the basis of *P. vulgaris* reproduction, the loci are completely or highly homozygotic and consequently the alleles are fixed. In regard to the percentage of polymorphic loci within the accessions, values ranging between 20% and 50% were observed, with an average value of 38%.

Genetic relationships between the five accessions

The number of alleles that are unique for a population is a simple measure of genetic diversity. In this regard it can be seen that the 'Cesarins' landrace has the greatest number of population-specific alleles. In 'Cesarins' among the 11 loci having unique alleles, there are four (PH10B11, PVBR14, PVBR20 and PVBR5) that being monomorphic or almost so, could be diagnostic for the identification of the variety. However, unique alleles are present in all the accessions examined, though normally they are rare alleles with a percentage below 1%. 'Pegasus' has an allele of locus PH3B4, 'Kondor' an allele of locus PH3B4 and PH10B11, 'Borlotti 4' an allele of locus PH5B5 and PV2, 'Militons 3' an allele of locus PH5B5 and two alleles of locus PVBR14.

The genetic distance between the accessions examined was calculated using Nei's index. Cluster analysis applied to this matrix produced the cluster diagram shown in Fig. 1, whose nodes are supported by high bootstrap values, well above 60%. The values obtain amply back up the tree where the close distance between 'Pegasus' and 'Borlotti 4' can be

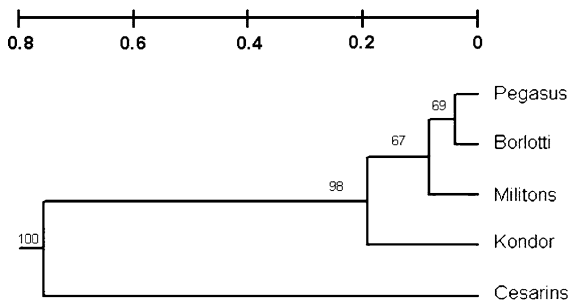

Fig. 1 Cluster diagram built according to the genetic distances between the accessions and relative bootstrap values

seen, while the landrace 'Cesarins' forms a separate branch.

The assignment value, based on the allele frequency of each accession allows all the individuals of the 'Cesarins' landrace to be correctly assigned, and those of the 'Pegasus', 'Kondor' and 'Militons 3' accessions at a percentage above 90%. The 'Borlotti 4' landrace has the highest number of individuals assigned to other accessions (17 out of 40, of which 12 assigned to the 'Pegasus' cultivar and 5 to 'Militons 3'). It should be noted that incorrect assignment is only limited to the borlotto-like accession.

Genotype structure of the accessions

The number of genotypes found within each accession was established, in order to verify whether the five accessions could be considered inbred lines or a mixture of lines in relation to phenotype observations. Four genotypes were found in 'Pegasus' and 'Kondor', one of which was clearly predominant, at a percentage of 92% and 86%, respectively. We conclude that they are inbred lines whose multiplication was not performed under secluded conditions. Seven different genotypes were found within 'Borlotti 4', two of which at 35% and 22.5% respectively, and the others at percentages ranging between 12% and 7%, with the exception of one that was below 2%. Similar results were found in 'Militons 3', where five genotypes were identified, one at 65%, another at over 20% and the remaining varying between 7.5% and 2.5%. The results therefore confirm phenotype observations, with individuals belonging to several lines.

Of the seven genotypes found in 'Cesarins', only one is predominant (47.5%), whereas the others are present at a percentage ranging between 15% and 2.5%. Phenotype observations failed to reveal any variability within this landrace. Comparing this with the information reported above, it can be said that the phenotype approach relies on a survey on limited numbers (5–10 individuals). Pinpointing genotypes within the accessions depends on their proportion, which should be approximately over 20%.

Conclusions

The overall number of accessions to be managed *ex situ* can increase considerably over time. Consequently the chances of monitoring their diversity may be at risk. Genetic diversity of populations *in situ* can be altered, due to management practices they are subject to (Gomez et al. 2005) and in general as a consequence of adaptation and evolution. Our attempt focuses on parallel phenotype and DNA characterisation on five bean accessions. Of the compact set of phenotypical descriptors used, one resulted inefficient: the small group shared the same plant architecture. Indeed, of the 23 molecular markers tried only 10 did work. This was enough, however, to distinguish the accessions analysed. All the loci of the accessions examined had a high degree of homozygosis, with the exception of one that perhaps is not neutral (PV2).

In accordance with phenotype observations, they appear to be inbred lines or a mixture of lines. Molecular investigations revealed that the two commercial varieties, 'Pegasus' and 'Kondor', appear to be formed by a genotype of long-standing predominance, as expected. Pegasus in addition is genetically very similar to the 'Borlotti 4' landrace. This is probably due to the fact that in the genealogy of 'Pegasus' commercial variety, the breeder used germplasm of borlotto type, collected from the same area (Cattivello and Del Zan 2004). Even by a phenotype viewpoint 'Borlotti 4' and 'Militons 3' appear to be a mixture of at least two lines, which has indeed been confirmed by molecular analysis. Moreover, in 'Militons 3' a substantial intra-population diversity in seed shape and colour facilitates a distinction into two lines. On the other hand, while phenotypical data failed to expose variability for 'Cesarins', microsatellite analysis did detect the presence of seven genotypes. From the cluster tree,

as well as at phenotype level, 'CESARINS' appeared to be a well separated accession. This landrace had also the largest number of population-specific alleles, that could be diagnostic for variety identification.

Traditional practices call for some maintenance selection on bean landraces, usually performed in winter times. After threshing dried pods by hand, farmers consider characteristics of individual seeds and segregate off-types, i.e. beans with different appearance, or showing symptoms of pests and *Anthrachnose* (Peresson, pers. comm.) Selection against susceptible materials is probably more severe, compared with the responses to small differences in bean mass, colour and shape. The diffuse habit of growing landraces close to each other may indeed facilitate physical mixture at harvesting. On the other hand, natural hybridation at distances as little as 0.5 m between beans, was measured to be around 0.14%, i.e. almost irrelevant (Ferreira et al. 2007). Such factors are however in place at each multiplication step, so they might explain a certain amount of diversity within landraces, and the presence of distinct genotypes of very similar morphology at seed level.

On the whole, the phenotype approach seems adequate to detect the general occurrence of within-population diversity. Taking into account the above mentioned discrepancies to consistently assess characterisation variants, whether this phenotyping approach may consistently discriminate across landraces can be seen as a matter of setting an appropriate threshold. In other words, do we need to have one, two or more different phenotype descriptors, to safely discriminate between two landraces? Of course, this in turn depends upon the objective(s) and the resources assigned to the project. The simplified phenotype approach was helpful for a preliminary characterisation, and the discrimination across the small group of accessions was largely in agreement with the molecular analyses. On the other hand, SSR fingerprinting carried out separately on 38–40 individuals resolved in detail the diversity across the accessions and indicated genetic relationships among them.

From a practical standpoint, the question of which approach can be adequately and efficiently implemented for *ex situ* collections seems to have multiple answers, according to the availability of resources and the condivision of goals among scientific and technical institutions. Integrated strategies (*ex situ* and *on farm*) proposed for genetic resources conservation can rely on distinct molecular approaches on specific collection's segments or core collections. The use of appropriate molecular tools in monitoring the diversity of crop landraces, particularly when they are conserved on farms, do not exclude a field characterisation at phenotype level. Should time be available, this traditional approach may offer basic information on the plant materials and complement the more expensive, demanding and powerful molecular procedure in assessing crops diversity.

Acknowledgements The research was supported in part by the Regione Autonoma Friuli Venezia Giulia-Regional Law No. 11/2002. We gratefully acknowledge the technical assistance of Riccardo De Infanti.

References

Acosta-Gallegos AJ, Kelly JD, Gepts P (2008) Prebreeding in common bean and use of genetic diversity from wild germplasm. Crop Sci 48(S1):1

Bove E (1994) Considerazioni economiche sulla coltivazione del fagiolo in Basilicata. Atti Congresso Nazionale Il fagiolo fresco in Italia, stato attuale e prospettive, 16–17 Settembre 1994, Potenza, 53–71

Brown AHD, Brubaker CL, Grace JP (1997) Regeneration of germplant samples: wild versus cultivated plant species. Crop Sci 37:7–13

Buso GSC, Amaral ZPS, Brondani RPV, Ferreira ME (2006) Microsatellite markers for the common bean *Phaseolus vulgaris*. Mol Ecol Notes 6:252–254. doi:10.1111/j.1471-8286.2006.01210.x

Cattivello C, Del Zan F (2004) Il miglioramento del fagiolo friulano: un impegno di 25 anni fra tradizione e innovazione. In: Devetag A (a cura di): Fagioli della Carnia. AIAT Carnia e CCIAA di Udine, Tolmezzo, pp 11–32

Ferreira JL, de Souza Carneiro JE, Teixeria AL, de Lanes FF, Cecon PR (2007) Gene flow in common bean (*Phaseolus vulgaris* L.). Euphytica 153:165–170. doi:10.1007/s10681-006-9251-z

Gaitan-Solis E, Duque MC, Edwards KJ, Tohme J (2002) Microsatellite repeats in common bean (*Phaseolus vulgaris* L.): isolation, characterization, and cross-species amplification in *Phaseolus* spp. Crop Sci 42:2128–2136

Gilbert JE, Lewis RV, Wilkinson MJ, Caligari PDS (1999) Developing an appropriate strategy to assess genetic variability in plant germplasm collections. Theor Appl Genet 98:1125–1131. doi:10.1007/s001220051176

Gomez OJ, Blair MW, Frankow-Lindberg BE, Gullberg U (2005) Comparative study of common bean (*Phaseolus vulgaris* L.) landraces conserved *ex situ* in genebanks and *in situ* by farmers. Genet Resour Crop Evol 52:371–380. doi:10.1007/s10722-005-2249-x

Hammer K, Miceli F, Cifarelli S, Laghetti G (2007) Sauris (Zahre), a cultural and linguistic island in Carnia (Italy).

In: Bullitta S (ed) Proceeding XVII Eucarpia genetic resources section meeting—plant genetic resources of geographical and "other" islands, 30 March–2 April 2005, Castelsardo, Italy, pp 185–192

IBPGR (1982) Country abbreviations for use with IBPGR descriptors. Plant Genet Resour Newsl (FAO: Rome) 49:45–48

Lioi L, Piergiovanni AR, Pignone D, Puglisi S, Santantonio M, Sonnante G (2005) Genetic diversity of some surving *on-farm* Italian common bean (*Phaseolus vulgaris* L.) landraces. Plant Breed 124:576–581. doi:10.1111/j.1439-0523.2005.01153.x

Miceli F, De Infanti R, Dalla Costa L (2003) Valutazione agronomica e qualità culinaria in varietà locali di fagiolo rampicante (*Phaseolus vulgaris* L.). *Atti XXXV Convegno SIA*, Napoli-Portici, 16–18 settembre 2003

Miceli F, Dalla Costa L, Laghetti G, Hammer K (2007) Resia: a deep-rooted Slavic community in the Northeastern Italian Alps and its garlic landrace. In: Bullitta S (ed) Proceeding of the XVII Eucarpia genetic resources section meeting—plant genetic resources of geographical and "other" islands, 30 March–2 April 2005, Castelsardo, Italy, pp 257–259

Miller MP (1994) Tools for population genetic analyses (TFPGA) v. 1.3: a windows program for the analysis of allozyme and molecular population genetic data

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small numbers of individuals. Genetics 89:583–590

Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. Mol Ecol 4:347–354. doi:10.1111/j.1365-294X.1995.tb00227.x

Parisi B, Ranalli P, Del Re P, Parisi M (2000) Kondor: nuova cultivar di cannellino rampicante adatta alle produzioni in coltura protetta. *V Giornate Scientifiche della Società Orticola Italiana*, Sirmione 28–30 Marzo

Peakall R, Smouse PE (2006) GenAlEx6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295. doi:10.1111/j.1471-8286.2005.01155.x

Piergiovanni AR, Cerbino D, Della Gatta C (2000) Diversity in seed quality traits of common bean (*Phaseolus vulgaris* L.) populations from Basilicata (Southern Italy). Plant Breed 119:513–516. doi:10.1046/j.1439-0523.2000.00531.x

Piergiovanni AR, Taranto G, Sanson S (2004) Il fagiolo 'Gialet'. Una popolazione autoctona della Val Belluna. In: Russo G (ed) Atti del VI Convegno Nazionale 'Biodiversità. Opportunità di Sviluppo Sostenibile', vol III. Istituto Agronomico Mediterraneo, Valenzano, pp 973–976

Powell W, Gordon Marchay C, Provan J (1996) Polymorphism revealed by simple sequence repeats (reviews). Trends Plant Sci 1:215–222

Pozzi E, De Infanti R, Peresson N, Miceli F (2004) Recenti percorsi di recupero e valorizzazione di varietà locali di fagiolo rampicante. In: Devetag A (ed) Fagioli della Carnia. AIAT della Carnia e CCIAA di Udine, Tolmezzo, pp 33–79

Rafalsky JA, Tingey SV (1993) Genetic diagnostics in plant breeding: RAPDs, microsatellites and machines. Trends Genet 9:275–279. doi:10.1016/0168-9525(93)90013-8

Ranalli P, Parisi P, Soressi GP, Habashy AA, Santangelo E (2001) Fagiolo (*Phaseolus vulgaris* L.). In: Ranalli P (ed) Leguminose e agricoltura sostenibile. Specie da granella e cover crops. Calderini Editore, Bologna, pp 447–502

Schachl R, De la Rosa L (2001) Characterisation of *Phaseolus* accessions. In: De la Cuadra C, De Ron AM, Schachl R (eds) Handbook on evaluation of *Phaseolus* germplasm, PHASELIEU-FAIR-PL97-3463. http://www.cesga.es/phaselieu/handbook_germplasm.pdf

Sicard D, Nanni L, Porfiri O, Bulfon D, Papa R (2005) Genetic diversity of *Phaseolus vulgaris* L. and *P. coccineus* L. landraces in Central Italy. Plant Breed 124:464–472. doi:10.1111/j.1439-0523.2005.01137.x

Singh SP (2001) Broadening the genetic base of common bean cultivars. A review. Crop Sci 41:1659–1675

Stephenson P, Bryan G, Kirby J, Collins A, Devos K, Busso C et al (1998) Fifty new micro-satellite loci for the wheat genetic map. Theor Appl Genet 97:946–949. doi:10.1007/s001220050975

UPOV (1994) Guidelines for the conduct of tests for distinctness, uniformity and stability. Internat Union Protect New Var Plants. TG/3/11

Yu K, Soon SJ, Poysa V (1999) Abundance and variation of microsatellite DNA sequences in beans *Phaseolus* and *Vigna*. Genome 42:27–34. doi:10.1139/gen-42-1-27

Yu K, Park SJ, Poysa V, Gepts P (2000) Integration of simple sequence repeat (SSR) markers into a molecular linkage map of common bean (*Phaseolus vulgaris* L.). J Hered 91:429–434. doi:10.1093/jhered/91.6.429