Genetic Resources and Crop Evolution **49:** 511–520, 2002.

Phaseolus genetic diversity maintained on-farm in central Italy

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Received 29 June 2001; accepted in revised form 22 December 2001

Key words: AFLP, Landrace diversity, On-farm conservation

Abstract

Thirty-one *Phaseolus vulgaris* L. and five *Phaseolus coccineus* L. landraces reproduced on-farm were found in central Italy. They were mostly grown by elderly farmers who usually select for a certain type of seed. Different varieties are often grown in each location and on each farm. They are maintained on-farm because of a local market request for high quality products or because of sticking to traditional family use in cooking (21.2 and 75.8% of recorded cases, respectively). Three AFLP primer combinations were used to assess genetic variation among collected materials, a wild accession of *P. vulgaris* and commercial varieties of both species. They revealed a quite high percentage of polymorphism (90.2% of polymorphic bands as an average). A wide genetic variation was observed among collected materials and each accession showed a unique pattern of polymorphism. Within *P. vulgaris*, landraces were discriminated in two main subgroups, the former including the accessions from the Mediterranean area around the Lake Trasimeno and the latter including accessions from the humid Mediterranean area within the Appennine Mountains. These findings demonstrate the peculiar genetic identity of the landraces studied also in relationship to human and environmental selection pressures. Possible on-farm conservation strategies are briefly discussed in relationship to the information collected.

Introduction

Phaseolus vulgaris, a recently introduced species in Europe, was one of the most important protein sources for people until a few decades ago and, after a period of decline, its use is now being reevaluated for dietary reasons.

The world crop production of dried seeds is estimated to be around 18.8 million tons, with the major producers being China, Brazil, Mexico, the USA, Ethiopia, Uganda, Burundi, Tanzania, Turkey, Argentina, Rwanda, Angola and Colombia (source FAO web site). Europe currently imports most of what it consumes. Nonetheless some countries (Spain, France and Italy) are presently gambling on products with guaranteed origin and with high added value (Schneider and Lacampagne 2001; Piergiovanni and Laghetti 1999) since consumers have progressively acquired specific preferences for various combinations of bean size and shape and the market reflects this trend giving preference to good quality types. Some types, typical and peculiar to certain areas (i.e. local varieties) are sold at very high prices (4–5 times higher than commercial types) in local Italian markets. Since there is no information about their seed sold on the seed market, they are probably landraces. Landraces, reflecting the cultural identity of the people of Europe and harbouring a diversity that is of interest for future breeding work, as well as for developing new farming systems and new products, deserve to be preserved for future generations.

Little is known about the current levels of crop diversity in Europe and the need for scientific work to catalogue and characterise landraces for prospective on-farm conservation was recently acknowledged (Negri et al. 2000b). Knowledge of the existing level

of diversity is fundamental in planning conservation activities because, without monitoring, it is not possible to verify the effectiveness of conservation. Why farmers continue to grow landraces and the level of variation existing among landraces are poorly documented scientific issues that need to be understood to achieve on-farm conservation (Brush 1999; Brown 1999).

An overall picture of crop germplasm conserved on-farm in central and northern Italy, which includes the results of several collection missions in the area, was recently published (Hammer et al. 1999). This paper presents the results of an investigation on the existence of bean landraces, the reason for maintaining them on-farm and the level of diversity existing among them in a large area of central Italy. AFLP (Amplified Fragment Length Polymorphism) molecular markers were used to assess diversity due to their repeatability and efficiency in this kind of study, as demonstrated in previous studies on *P. vulgaris* and *P. lunatus* (Tohme et al. 1996; Caceido et al. 1999). DNA markers can also distinguish among *P. vulgaris* accessions with similar morphology (Becerra Velasquez and Gepts 1994; Beebe et al. 2000).

Materials and methods

Germplasm and information collection

With the aim of identifying really typical material, explorations and collections of bean landraces have been undertaken in different areas of central Italy (Figure 1) since the beginning of the 90ies.

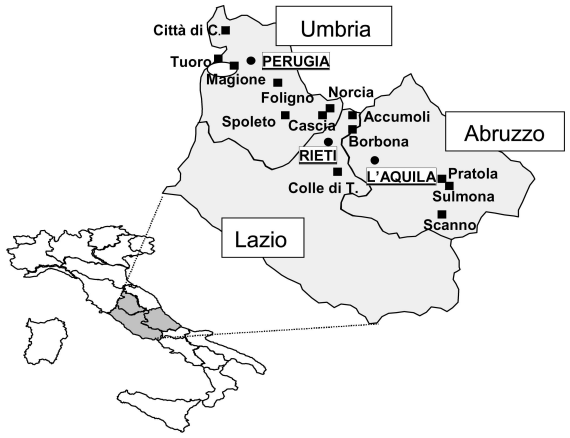

Figure 1. Collection sites of *Phaseolus* landraces.

In some cases, it was possible to collect with the help of the local extension services, in others visits were organised by chance. Farmers were approached in a friendly manner, explaining the reason for the visit; they were interviewed asking information about the presence of materials reproduced on their farm for generations. If present, this germplasm was collected. Information on adaptative, agronomic, qualitative and organoleptic traits of landraces found, as well as information related to seed exchange among farmers, use, local names, traditions and social context were collected. The seeds of landraces found were then stored in the DBVBA germplasm bank.

Both *P. vulgaris* and *P. coccineus* were found, assessed by visiting the fields where the crop were grown and, later, by the respective epigeous and hypogeous behaviour of the cotyledonary leaves.

Commercial varieties of both species and an accession of *P. vulgaris* var. *aborigenus* (Burkart) Baudet from Morelos in Mexico (collected at 1981 m asl, 19°00′ latitude North and 99°15′ longitude West) were used as controls in the following characterization and assessment of genetic variation work. Commercial varieties (hereafter identified as 'Q', 'R' and 'S') were of unknown origin and were found on the local grain legume market. In particular 'Q' and 'R' were chosen because they could represent other specimens of landrace 4198 which is known to be sold in the town market of Perugia.

Characterisation

No multiplication occurred before the characterization phase. Fifteen seeds for each original seed lot (accession) and control were sown (50 × 60 cm apart) according to a randomized block design with three replication (5 plants per each replicate) in the DBVBA experimental field in spring 2000. Ten plants were used for molecular (see below) and morphological characterisation. The following data were collected on each plant per accession:

Growth habit was recorded using a scale from 1 to 6 and 1 to 3 as reported in the *P. vulgaris* and *P. coccineus* descriptors, respectively (IBPGR 1982, 1983). The average of ten plants per accession is reported;

Seed weight (g) The average 100 seed weight of ten plants per accession is reported (20 randomly chosen seeds per plant were evaluated);

Seed shape was recorded on the sample used for seed weight determination using a scale from 1 to 5

and from 3 to 7 for *P. vulgaris* and *P. coccineus*, respectively (IBPGR 1982, 1983);

Seed colour was recorded on the sample used for seed weight determination following the already mentioned IBPGR descriptors.

Assessment of genetic diversity among collected materials

Molecular markers were used to assess the level of genetic diversity among the collected materials.

Genomic DNA was extracted from young leaves of a bulk of 10 plants per accession following a modified procedure of Doyle and Doyle (1990). AFLP analysis was carried out following the procedures described in Zabeau and Vos (1994), Vos et al. (1995) with modifications. Five U of EcoRI (NEB) and Mse I (NEB) were used for digesting DNA. Ligation of adapters was conducted with 5 pmol of Eco and 50 pmol of Mse adapter and 1 U of T4 DNA ligase (Pharmacia). Digestion and ligation cocktail was incubated for 4 hours at 37 °C in RL buffer (OPA 5X, DTT 25 mM, BSA 250 ng μL^{-1}) and then diluted 1 : 10 in TE (10 mM Tris HCl, 1 mM EDTA, pH 7.5). Primers complementary to adapter sequences, having one additional nucleotide on their 3′ end (Mse + A and Eco + C), were used to carry out a selective pre-amplification of the DNA template. Fifty nanograms of each primer, 0.2 mM dNTPs, 10X PCR buffer, 1 U Taq DNA polymerase (Pharmacia) and 5 μL of diluted digested-ligated DNA in 50 μL of total volume were amplified in the following conditions: 45 s and 94 °C for denaturation, 30 s and 65 °C for annealing and 60 s and 72 °C for extension in the first cycle, 30 s denaturation time and annealing temperature decrease of 0.7 °C per cycle in the following 11 cycles, 19 further cycles in the achieved conditions and a final cycle of 5 minutes at 72 °C. The quality of pre-amplified DNA was observed on an ethidium bromide stained agarose gel (1.5%). The rest of the amplification product was diluted 1 : 10 with TE buffer. For the final selective amplifications, two more selective bases were added to each primer combination so as the primer combinations used were: Eco-CAC Mse-ACT, Eco-CAC Mse-ACA, Eco-CAG Mse-ATA. Eco primers were flourescine labelled. Five microlitres of diluted pre-amplified products, 30 ng of Mse primer, 33 ng of Eco, 0.2 mM dNTPs, 10X PCR buffer and 0.4 U Taq DNA polymerase reacted in 20 μL under the same time and temperature conditions described above. Then, 8 μL of modified formamide dye (98% formamide, destran blue 2% and 0.25 mM EDTA) was added to the reaction products; 6 μL of each sample, denaturated for 5 min at 94 °C, were run on acrylammide gel (6% acrylamide/bis-acrylamide 19 : 1, 7 M urea, TBE buffer 1X). Electrophoresis was performed at constant power, 95 W, for approximately 2.15 h on a Genomix LR apparatus (Beckman). The same equipment was used for the acquisition of the fluorescent images of the gel. Only reproducible well-marked amplified fragments were scored; faint bands were ignored. For all markers and in each sample, the presence and absence of fragments were recorded as 1 or 0, respectively. Genetic similarity estimates were worked out using the Dice coefficient (Dice 1945). Cluster analysis was conducted on similarity estimates using the unweighted pair-group method with arithmetic average (UPMGA), from which the dendrogram representing the relationship between accessions was obtained. Analysis was performed using the NTSYS.PC package version 1.8 (Rohlf 1993).

Results and discussion

Bean germplasm maintained on-farm and related information

Table 1 shows species, accession number in the DBVBA germplasm bank, donor's name, collection site and its relevant characteristics (altitude, latitude, longitude, annual rainfall, average of minimum temperatures of the coldest month and average of maximum temperatures of the hottest month), farm size, farmer's age and reason for continuing to maintain landraces on-farm and characterisation data (growth habit, seed weight, colour and shape) relative to the collected materials.

Thirty-one *P. vulgaris* and five *P. coccineus* local varieties reproduced on-farm were found in hilly or mountainous areas, at altitudes ranging from 250 to 1100 m asl; they were grown under climatic characteristics from sub-Mediterranean to humid-Mediterranean, as defined by (Le Houérou 1977).

They are mostly grown by elderly farmers (average age 65.2 years) on small farms (average farm size 13.0 ha) and under traditional farming systems which nonetheless always include the use of mechanical tools for soil preparation and sometimes chemical fertilisers. Each farmer who donated germplasm insisted that the beans belonged to his/her heirloom, some also claimed to have applied deliberate selection

Table 1. Information on bean germplasm maintained on-farm in the area investigated: species, local name, accession N°, donor's name, geographic and climatic information of collection sites, farm size, farmer's age, prevalent purpose of cultivation (P.v. = *Phaseolus vulgaris*, P.c. = *Phaseolus coccineus*, FC = family consumption, LM = local market, TM = town market) and characterisation data (growth habit, seed weight, shape and colour), n.r. = not recorded.

Sp.	Local name	Acc.N°	Farmer's name	Site	Alt m asl	Lat (N)	Long (E)	Ann. Rain. mm	Min T°	Max T°	Ann. T°	Farm area ha	F.'s Age yrs	Prev. purp.	Growth habit 1–5	Seed Weight g	Seed Shape 2–5	Seed colour
P.v.	tipo borlotto	3502	Bucchi	Norcia	600	42,48	13,08	875	−3.0	29.0	11.5	n.r.	45	FC	1	38.8	2/5	Pale brown purple striped, purple
P.v.	quarantino	3504	Filippini	Trestina, Città di Castello	280	43,22	12,15	749	−2.4	25.9	12.6	n.r.	n.r.	FC	1	40.4	3	Pale brown eyed
P.v.	cannellino	3506	Filippini	Trestina, Città di Castello	280	43,22	12,15	749	−2.4	25.9	12.6	n.r.	n.r.	FC	5	21.6	3	White
P.v.	unnamed	3928	Sportoletti	Lombardo, Magione	300	43,10	12,12	725	1.6	29.1	14.1	n.r.	59	FC	1	28.0	4	Pale yellow
P.v.	cannellino	3931	Rinchi	Macerone, Tuoro	260	43,12	12,10	718	1.7	30.2	13.8	n.r.	69	FC	4	36.0	3	White
P.v.	cannellino	3935	Sportoletti	Lombardo, Magione	300	43,10	12,12	725	1.6	29.1	14.1	n.r.	59	FC	1	16.8	2	White
P.v.	zolfino	3963	Ragnini	Dirindello, Magione	300	43,10	12,12	725	1.6	29.1	14.1	3.0	65	FC	4	28.9	3	Yellow
P.v.	grigio	3964	Ragnini	Dirindello, Magione	300	43,10	12,12	725	1.6	29.1	14.1	3.0	65	FC	4	36.7	3	Maroon purple striped
P.v.	cannellino	3966	Sportoletti	Lombardo, Magione	300	43,10	12,12	725	1.6	29.1	14.1	n.r.	59	FC	1	16.7	2/3	White
P.v.	grigio	3967	Sportoletti	Lombardo, Magione	300	43,10	12,12	725	1.6	29.1	14.1	n.r.	59	FC	3	38.1	3	Maroon purple striped
P.v.	colorati	3991	Dormalfuoco	Tuoro	300	43,12	12,10	718	1.7	30.2	13.8	7.0	70	FC	5	28.8	3	Maroon purple striped
P.v.	della vigna verdino	4083	D'Ambrosio	Cave, Foligno	250	42,55	12,42	688	1.7	30.3	14.5	7.0	76	FC	1	45.7	3	
P.v.	dall'occhio	4084	D'Ambrosio	Cave, Foligno	250	42,55	12,42	688	1.7	30.3	14.5	7.0	76	LM	1	43.0	2	Pale buff, eyed
P.v.	borlotto nano	4087	Lolli	Palude di Colfiorito	870	43,04	12,57	995	1.0	26.4	12.3	5.0	77	LM	4	47.2	2/5	Pale brown purple striped, purple
P.v.	quarantino	4088	Armillei	Scopoli, Foligno,	540	42,58	12,48	1144	1.1	28.4	13.5	1.0	72	FC	n.r.	65.2	3/4	Buff eyed
P.v.	fagiolina dello zio	4089	Simonetti	Piandoli, Cascia	600	42,46	12,59	875	−3.0	29.0	11.5	10.0	69	FC	n.r.	57.8	2	Pale brown eyed
P.v.	fagiolina	4090	Ranucci	Poggio Primocaso, Cascia	800	42,44	12,59	1523	−2.6	26.6	10.3	0.2	44	FC	n.r.	49.2	2	Pale brown eyed
P.v.	bianco	4102	Giuliarini	Borghetto, Tuoro	259	43,13	12,10	718	1.7	30.2	13.8	2.0	77	FC	5	37.4	3	White
P.v.	a pisello	4198	Marino	Colle di Tora, Rieti	750	42,12	12,56	1261	−2.2	27.6	10.8	10	47	TM	6	40.9	2	White
P.v.	borlotto borbontino	4360	Giorgi	La Terra, Borbona, Rieti	750	42,30	13,08	1261	−2.2	27.6	10.8	15.0	70	LM	5	95.6	2	Maroon purple striped
P.v.	borlotto chiaro da m.	4361	Giorgi	La Terra Borbona, Rieti	750	42,30	13,08	1261	−2.2	27.6	10.8	15.0	70	LM	5	73.6	2	Pale brown eyed
P.v.	borlotto	4391	Coop. Zootecnica	Grisciano, Accumoli, Rieti	700	42,44	13,16	1261	−2.2	27.6	10.8	150.0	42	FC	6	78.6	2	Maroon purple striped
P.v.	aquilano bianco	4454	Di Censo Iole	Sulmona, L'Aquila	350	42,03	13,56	652	−0.1	31.4	13.7	0.2	75	LM	6	69.4	2/5	Pale brown
P.v.	a pane	4455	Di Censo Efelda	Sulmona, L'Aquila	350	42,03	13,56	652	−0.1	31.4	13.7	0.5	66	FC	5	56.8	2/5	Pale brown
P.v.	aquilano nero	4456	Anzini	Sulmona, l'Aquila	350	42,03	13,56	652	−0.1	31.4	13.7	n.r.	n.r.	n.r.	5	54.2	2	Black
P.v.	pisello	4460	Panepucci	Paganica, L'Aquila	700	43,03	13,59	678	−1.2	27.8	11.7	2.0	70	LM	5	49.8	2	White
P.v.	tondino bianco	4462	Di Censo Ettore	Sulmona, L'Aquila	350	42,03	13,56	652	−0.1	31.4	13.7	2.5	90	LM	4	60.4	2	White
P.v.	a pane	4464	Di Censo Efelda	Sulmona, L'Aquila	380	42,03	13,56	652	−0.1	31.4	13.7	0.5	66	FC	5	73.0	2/5	Pale brown purple striped
P.v.	tabacchino	4466	Battaglini	Pacentro, L' Aquila	700	42,05	13,55	773	−1.2	30.0	12.8	n.r.	70	FC	6	50.2	3/5	Pale maroon
P.v.	a pane	4467	Notarmuzi	Scanno, L'Aquila	1100	41,54	13,53	1143	−2.5	26.5	9.8	11.0	28	n.r.	5	91.2	3/5	Pale brown eyed
P.v.	cannellino	4468	Sigismundo	Pratola Peligna, L'Aquila	350	42,05	13,52	653	−0.1	31.4	13.7	4.0	91	FC	1	44.6	3	White
P.c.	borlotto bianco	3615	Moretti	Trivio, Monteleone, Spoleto	945	42,39	12,58	1523	−2.6	26.6	10.3	n.r.	n.r.	FC	n.r.	78.6	5	White
P.c.	ciabattone	3745	Amici	Casesparse, Norcia	600	42,48	13,08	875	−3.0	29.0	11.5	n.r.	n.r.	FC	3	189.4	5	White
P.c.	bianco tondo	4300	Taddei	Tuoro	260	43,12	12,10	718	1.7	30.2	13.8	5.0	46	FC	3	n.r.	5	White
P.c.	ciabattone	4392	Coop. Zootecnica	Grisciano, Accumoli, Rieti	700	42,44	13,16	1261	−2.2	27.6	10.8	150.0	42	FC	3	206.4	5	White
P.c.	fagiolone	4461	Notarmuzi	Scanno, L'Aquila	1100	41,54	13,53	1143	−2.5	26.5	9.8	16.0	28	n.r.	3	136.4	5	White

for a certain seed or plant type both in the field and/or while cleaning the seed for the next cropping season. Seed exchange was anyway reported to occur as a sporadic practice among local farmers.

Different varieties are often grown in each location and on each farm. Farmers maintain distinct types which differ in colour, size and shape of seed and growth habit or use (Table 1). Up to four different varieties were found per farm: two 'cannellino' types (one used for fresh seed during the summer and another one used during the winter), a yellow-seeded determinate type and an indeterminate maroon purple striped type are maintained on Sportoletti's farm. Local names mostly relate to seed colour and shape either directly (i.e. zolfino = yellow, tabacchino = tobacco brown, bianco = white seed coloured, respectively; con, dall'occhio = eye seeded, tondo = round shaped, a pisello = pea-shaped) or indirectly referring to well-known types (i.e. cannellino = white, cuboid seeded type) or to local folk language (ciabattone = old shoe shaped, fagiolone = large bean). In some cases the names relate to the growing period (i.e quarantino = which starts to be harvested forty days after sowing) or to the heirloom (i.e. dello zio = inherited from uncle) or to the importance the crop had in the past (i.e. pane = wheat, in the meaning of staple food). In addition, the same local name may refer to quite different types. For example, accessions n. 3935, 3966 and 3931, coming from a restricted area around Lake Trasimeno in Umbria and all locally named 'cannellino', show the growth habit of determinate bush (3935 and 3966) or indeterminate with moderate climbing ability (3931), low seed weigth (3935: $\bar{x}$ = 16.8 g and 3966: $\bar{x}$ = 16.7 g) or high seed weight (3931: $\bar{x}$ = 36.0 g). They also differ in other characteristics (not reported in Table 1) such as length of legume (3931: $\bar{x}$ = 121.2 mm, 3935: $\bar{x}$ = 88.5 mm, 3966: $\bar{x}$ = 89.4 mm) and size of central leaflet (3931: $\bar{x}$ = 70.8 and 67.0 mm, 3935: $\bar{x}$ = 92.2 and 61.0 mm and 3966: $\bar{x}$ = 84.2 and 57.0 mm length and width of central leaflet, respectively).

Some varieties are sold on local markets and are appreciated for their distinctness and peculiar taste (21.2% of recorded cases), but the main reason for conserving and managing local varieties on-farm is to stick to traditional family use in cooking (75.8% of recorded cases). There was only one case in which the farmer grew the crop mainly for the town market.

The commercial varieties 'Q' and 'R' used as control were very similar to landrace 4198 with respect to seed size (seed weight 42.1 and 41.4 g,

Table 2. Total number of bands, number of polymorphic bands and percentage of polymorphism detected overall and in each species for each primer combination used, and in total, and species-specific number of bands.

Primer combinations	Overall			*P. vulgaris*				*P. coccineus*			
	Total N° bands	N° polym. bands	Polym %	N° species specific bands	Total N° bands	N° polym. bands	Polym %	N° species specific bands	Total N° bands	N° polym. bands	Polym %
Eco-CAC Mse-ACT	121	119	98.3	38	111	107	96.4	10	83	64	77.1
Eco-CAC Mse-ACA	83	71	85.5	32	77	57	74.1	6	51	37	72.5
Eco-CAG Mse-ATA	122	104	85.5	36	114	84	73.7	8	86	62	72.1
Total	326	294		106	302	248		24	220	163	
Average per marker	108.6	98.0	90.2	35.3	100.7	82.7	81.4	8	73.3	54.3	73.9

respectively) and had the same shape, colour and growth habit. From the twining wild *P. vulgaris*, it was not possible to obtain seed under the photoperiodic conditions present during the characterization phase. *P. coccineus* 'S' was an indeterminate climber with white, relatively large seeds (100 seed weight = 181.3 g).

Genetic similarity among collected materials

Efficiency of the marker system used

The total number of bands, number of polymorphic bands and percentage of polymorphism detected overall and in each species for each primer combination used and in total, as well as the number of species-specific bands are reported in Table 2. A total of 294 polymorphic bands were detected out of a total of 326 bands. The primer combinations used differed in the number of bands detected (which overall ranged from 122 to 83), but all were highly efficient in detecting polymorphisms and species-specific bands in the materials studied. The percentage of polymorphism ranged overall from 73.7 to 96.4 in common bean and from 72.1 to 77.1 in runner bean, depending on the primer combination used. It is worth noting that the first primer combination yielded a percentage of polymorphism in common bean comparable to that obtained analyzing a wide collection of wild material (Tohme et al. 1996). In total, 106 and 24 species-specific bands were detected in *P. vulgaris* and *P. coccineus*, respectively. Also, average percentages of polymorphism were equal to 81.4 and 73.9% in *P. vulgaris* and *P. coccineus*, respectively.

Peculiarity of single local varieties

Even when collected at the same site and under the same local name, each accession showed a distinct pattern of polymorphism (i.e. a peculiar genetic identity). It has to be considered that each profile represents the sum of ten plants which makes it improbable to have sampled different genetic patterns by chance. This also suggests that differences in morpho-agronomic characteristics can be ascribed to different genetic contexts. Moreover, 29.4% of the *P.*

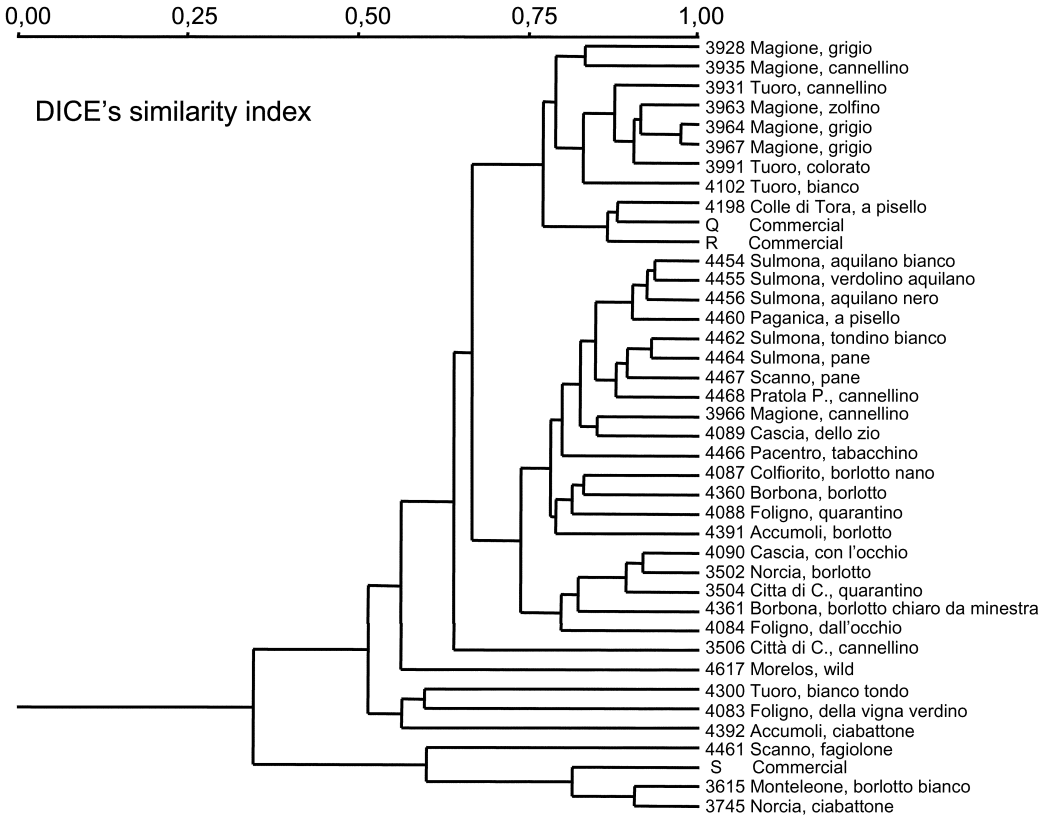

Figure 2. Dendrogram illustrating genetic similarities among examined accessions.

vulgaris and 83.3% of the *P. coccineus* accessions studied (10 and 5 populations per species, respectively) showed peculiar bands, i.e. bands not found in other accessions (*P. vulgaris*: 4083 n.9, 4617 wild n.7, 4361 n.4, 3935 and 3991 n.3, 3506 n.2, 4084,4087, 4089 and 4102 n.1; *P. coccineus*: 4392 n.4, 4300 n.3, 3745, 4461 and 'S' n.2). The lower number of private bands generally found in the cultivated material, compared with the wild accession, is feasible considering the reduction of diversity which characterises the domestication process (Sonnante et al. 1994). An exception was the cultivated accession of *P. vulgaris* 4083, which showed 9 peculiar bands. The genetic differences among landraces could be ascribed to individual farmer's preference and selection for a certain type as well as to different introductions that occurred in a relatively distant past in each location.

Relationships among examined varieties

A wide genetic variation was observed among collected materials. Genetic similarity estimates and similarity relationships among accessions studied are reported in Figure 2. Two main groups were discriminated at a Dice similarity index equal to 0.35: one group included all the *P. vulgaris* accessions along with two *P. coccineus* accessions (4300 and 4392) and the second group included only *P. coccineus* accessions.

Within the former group, a first subclustering included all the *P. vulgaris* accessions (landraces, cultivars and wild) except one (4083) and a second subclustering included the common bean and the two already mentioned runner bean landraces. These subclustering were separated at D = 0.51.

Focusing on the first subclustering, landraces were clearly distinguished from the wild accession 4617 (D = 0.56). Other subgroups were then identified: one including accession 3506 and another including all the rest of the landraces (D = 0.64). The latter included two groups (D = 0.67), the accessions from the area with a Mediterranean climate (as defined by Le Houérou (1977)) around the Lake Trasimeno and the accessions from the area within the Appennine Mountains with a humid Mediterranean climate (Le Houérou 1977). Both these groups included accessions characterized by different seed types. Accessions from the same collection site were often grouped together (see for example the group from Sulmona, Paganica, Scanno and Pratola Peligna which are in the Abruzzi Region). Groupings related to geographic origin were also recognised in a study carried out on a large sample of common bean landraces from Middle America (Beebe et al. 2000). There were few exceptions to the clustering trend according to geographic origin (i.e. accession 4198 from the mountainous area included in the Mediterranean subcluster and accessions 3504 and 3506 and 3935 and 3966, in couples from the same farmer, each included in a different cluster). This may relate to a relatively recent introduction, even though the farmers always stated that their family had been growing that landrace for ages. Only for accession 4198 it was possible to ascertain from verbal records of local farmers a relatively recent introduction by an emigrant who returned to Colle di Tora from South America at the beginning the twentieth century.

Considering the second subclustering, it was surprising to find a common bean clustered with a runner bean accessions, though it is worth noting that all three accessions constituting this grouping were quite distinguishable from each other. Accession 4083 may have derived from an occasional cross between *P. vulgaris* and *P. coccineus*, both cultivated in the examined area, as a relatively short hypocotyl seems to suggest. *P. vulgaris* and *P. coccineus*, are related species and produce fertile hybrids when *P. vulgaris* is the female parent (Delgado Salinas 1988; Delgado Salinas et al. 1999). This hypothesis is presently being verified with appropriate markers.

Within the second main cluster, a landrace from the Abruzzi Region (4461) was separated from the commercial cultivar and two landraces from relatively close sites in the Umbrian Appennine Mountains (3615 and 3745) at D = 0.60. The lower genetic similarity observed among runner bean accessions, as compared with the similarity levels found among the majority of common bean accessions, could be due to the prevalent outcrossing mating system of this species (Nevo 1978; Hamrick and Godt 1989).

Relationships between genetic similarities and origin of landraces

Accessions from neighboring sites differing in several morphological traits were often grouped together in our study (see discussion above). These findings suggest that environmental constraints played an important role in differentiating landraces, though we are not able to exclude other evolutive factors such as a more frequent intermating among varieties belonging to the same introduction gene pool. Contrary to what happens for natural populations, for landraces it

is impossible to ascribe genetic variation only to environmental selective pressures and similarity may simply reflect the same introduction, which is very difficult to ascertain.

Mesoamerican and Andean gene pools are present in common bean (see review in Gepts (1996)). Numerous introductions from different parts of the world have taken place in Europe since the first introduction of *Phaseolus* around A.D. 1500 (Zeven 1997) and previous studies have shown that both gene pools are present in Europe, and in Italy in particular (Gepts and Bliss 1988; Lioi 1989; Limongelli et al. 1996; Masi 2001; Piergiovanni et al. 2000a). In the area under investigation, variation for Mesoamerican and Andean phaseolin types was found among four landraces as well as within one of them (Piergiovanni et al. 2000b).

Morphological characterisation data suggest that both gene pools are also present in our sample (Table 1). Morphological traits (number of nodes to the first flower, leaflet dimensions, seed shape and seed weight, in particular) are generally correlated with the origin of the accessions and, in more than 96% of the cases, the classification based on them correspond with the classification based on phaseolin type for landraces in the area of domestication (Singh et al. 1991a,b). The subgroupings observed could then be evidence of specimens belonging to either one of the two gene pools, but the wild accession from Mexico (i.e. belonging to the Mesoamerican gene pool) did not match any of the *P. vulgaris* subgroupings found.

Cultivated accessions from the centers of domestication generally cluster with wild material from the same area (Becerra Velasquez and Gepts 1994; Sonnante et al. 1994). Since in our experiment the sample from the Mesoamerican gene pool was not clearly associated with any of the subgroupings found, the hypothesis of an important role played by local selective pressures in differentiating landraces appears to be more consistent. On the other hand, even for cultivated populations in the area of domestication it is sometimes impossible to trace back to introduction the history of a single accession or group of accessions, introgression from non-native gene pools is reported and a lack of a clear association between isoenzyme variants and geographic distribution exists (Paredes and Gepts 1995).

Our study seems to indicate that the landraces examined, due to environmental and human selective pressures different from those present in the area of origin, frequency of new introductions and, probably, gene exchange between Andean and Middle American germplasm, have evolved into a gene pool peculiar to the area examined. It is worth noting that another study, recently carried out on a collection from the Netherlands, led to the same conclusions (Zeven et al. 1999). Further studies are certainly needed to clarify the identity, peculiarities and extent of the European gene pool.

Conclusions

This study shows that, although generally considered extinct in post-modern societies, landraces continue to be maintained on-farm as in the area investigated. The marker system used in this study efficiently detected genetic variation in cultivated material characterised by a restricted genetic variation in comparison to wild material (Sonnante et al. 1994; Gepts 1996). Similarities among landraces were relatively low showing that there is a well-differentiated gene pool in Italy. The wide variation and peculiarities observed and the strong link with local cultural heritage, strongly recommend their continued on-farm conservation and management since it can safeguard genetic resources by maintaining their ability to evolve in the face of biotic and abiotic pressures, social and cultural changes and to meet the needs of unpredictable future demands (Frankel et al. 1995). Until now on-farm conservation has been proposed more as a theoretical model rather than a real possibility. In the few examples of on-farm conservation present in the EU, farmers have been paid as ''guardian farmers'' but this does not necessarily address the future needs or create lasting incentives for conservation (Orlowe and Brush 1996; Zeven 1996). Nevertheless the pressing need to sustain *in situ* (on-farm) conservation and to use plant genetic resources with an integrated approach was recently acknowledged by the Global Plan of Action (FAO 1996). The reasons why bean landraces are conserved on-farm in central Italy may be of help in suggesting different conservation strategies.

We found that 22% of the collected accessions were maintained because of local market request for typical, profitable products. Since the demand for typical products is increasing in Italy, as elsewhere, if the relationships between the agrifood system and plant genetic resources were strengthened it could lead to an effective on-farm conservation. If landraces exist and consumers are willing to pay a good price for them, a self-sustainable system could be triggered. In

this way the cultivation of landraces would become advantageous for local farmers and effective on-farm conservation could become a reality. This approach would also fit well with the diversification of productions policy currently in action in some countries. Some specific EU/National/Regional laws may help make on-farm conservation of landraces economically feasible for farmers. Also the possibility of attributing marks of origin and quality can be an important support to on-farm conservation of élite landraces (Negri et al. 2000a; Piergiovanni and Laghetti 1999). However, the most of the collected material was only grown for private consumption and appears to be in danger of extinction due to the advanced age of the farmers. To reinforce the links between rural communities and their plant genetic resources and the pride of their heirloom, it is important to encourage young generations to continue growing landraces in the future. Further anthropological and sociological studies are needed to understand how to motivate on-farm conservation.

Acknowledgements

We wish to thank Prof. P. Gepts (University of California) for kindly providing the wild accession of *P. vulgaris*. Part of this research was funded by the Italian Ministry of University and Scientific Research 'Conservazione *in situ* di una specie coltivata autogama. Valutazione del sistema: popolazioni locali di *Phaseolus vulgaris* ambiente montano del Lazio' (coord. Prof. P.L. Spagnoletti Zeuli, scientist responsible for research unit Prof. V. Negri).

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