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Summary

A number of horticultural crop landraces are still grown in Piedmont (NW Italy), despite the dominance of improved cultivars and hybrids. Conservation strategies, both in an *ex situ* and an *in situ* context are required to prevent their loss. Here we describe an AFLP-based assessment of the genetic structure of leek, garlic, celery, red beet, cultivated cardoon, sweet pepper and common bean autochthonous landraces. Each landrace was sampled by selecting 3-5 populations representative of the area of cultivation. The genotypic data showed that the crop's breeding system was less important for determining genetic structure than the selection criteria adopted by the producers, the extent of informal seed exchange among producers and natural selection imposed by the local environmental conditions. The genotypic data identified alleles which were common, some which were restricted to a particular locality and some which were either infrequent or rare. On this basis, the most representative population(s) for each landrace were recognized and targeted for conservation. The landraces in study differed markedly from one another with respect to their genetic structure, and so they may represent an appropriate reference model for the management of crop landraces grown in fragmented areas and at risk of genetic erosion or extinction.

Key words AFLP ⊙landraces ⊙*in situ* ⊙*ex situ* ⊙germplasm conservation

Introduction

The widespread adoption of a small number of elite crop varieties has led to a narrowing of the genetic base of some of our food crops and to the disappearance of many traditional crop varieties, referred to variously as 'landraces', 'farmer varieties' and 'local varieties' (Negri, 2003; Negri *et al.*, 2009). Loss of diversity is a problem for underpinning further varietal improvement, and also reduces the options available to deal with new production constraints. The extinction of landraces also has a negative impact on rural culture (Negri *et al.*, 2009).

The landraces of seed-propagated crops are genetically heterogeneous but identifiable by their phenotype and a local name. They have evolved by a combination of natural selection and selection imposed by the farmers to generate a product which fits the traditional uses and habits of the local consumers (www.ecpgr.cgiar.org/networks/iin_situi_and_on_farm.html). As classified by Zeven (1998), a landrace is said to be autochthonous when it undergone a continuous period of local cultivation over at least 100 years, while allochthonous landraces refer to those introduced from another region and locally adapted.

The Piedmont region of NW Italy, as in a number of other southern European localities, is home to numerous autochthonous landraces, which survive in a few fragmented areas. The region has made extensive efforts to catalogue the local autochthonous horticultural crop landraces (http://www.regione.piemonte.it/agri/biodiversita/orticolo/schede.htm), and supports research projects attempting to identify appropriate strategies for their *ex situ* and in *situ* conservation. Fundamental differences exist between these two strategies, however they should not be viewed as alternatives or in opposition to one another, but rather should be practised as complementary approaches to conservation, each providing a safety back-up for the other (Maxted *et al.*, 1997).

DNA fingerprinting provides an effective means for assessing the level and distribution of genetic diversity in plant populations. Its use to characterize landraces provides an

objective method of identifying representative populations within a landrace, allowing these then to be prioritized for conservation purposes. Here we describe an AFLP-based assessment of the genetic diversity present in autochthonous landrace populations of seven horticultural crop species, namely leek (*Allium porrum* L.), garlic (*A. sativum* L.), celery (*Apium graveolens* L. var. *dulce*), red beet (*Beta vulgaris* L.), cultivated cardoon (*Cynara cardunculus* L. var. *altilis* DC), sweet pepper (*Capsicum annuum* L.) and common bean (*Phaseolus vulgaris* L.). The landraces in study are characterized by different reproductive systems, ranging from the predominantly self –pollinating sweet pepper and common bean, to the predominantly out-crossing leek, red beet and cardoon; as a consequence they differed markedly from one another with respect to their genetic structure, so may represent an appropriate reference model for the management of crop landraces grown in fragmented areas and at risk of genetic erosion or extinction.

Materials and Methods

Plant materials and DNA extraction

The landraces analysed were ‘Lungo di Cervere’ leek, ‘Molino dei Torti’ garlic, ‘Dorato d’Asti’ celery, ‘Rossa di Castellazzo Bormida’ red beet, ‘Gobbo di Nizza Monferrato’ cardoon, ‘Tomaticot’ sweet pepper and ‘Bianco di Bagnasco’ common bean (Figure 1).

The leek landrace ‘Lungo di Cervere’ is characterized by long, thin, tender and sweet stems. The garlic landrace ‘Molino dei Torti’ produces cream/pinkish bulbs of oval shape and flattened base, and it is typically consumed raw in preparing traditional dishes. The fleshy stems of the celery landrace ‘Dorato d’Asti’ are characterized by deep-yellow/golden ribs and an almost full section. The red beet landrace ‘Rossa di Castellazzo Bormida’ produces

globular roots with peculiar organoleptic properties, and it is traditionally consumed after boiling or after oven baking. The thick, sweet, almost spineless stalks of the cultivated cardoon landrace ‘Gobbo di Nizza Monferrato’ are often consumed uncooked in traditional dishes after blanching to accentuate their flavour. The sweet pepper landrace ‘Tomaticot’ produces small, round and flat berries with a very thick pericarp (up to 14 mm) and a very low content in capsaicinoids. The common bean landrace ‘Bianco di Bagnasco’ is characterized by pods which, when fully ripe, turn to yellow and contain white medium-sized seeds highly valued because their very thin coat. A more detailed morphological description of the landraces in study can be found at the website: http://www.regione.piemonte.it/agri/biodiversita/orticolo/schede.htm.

Between three and five populations per landrace were sampled to cover the cultivation localities (Table 1); the stands were chosen as being representative of each landrace cultivation area which covers, on average, about 2,500-3,000 square km. The choice was also based on production of the propagating material by farmers themselves and no exchange of material usually occurs among them All the material was established to have been raised from producers' own stocks, obtained by collecting seed (or bulbs in the case of garlic) for next year from selected plants which particularly express the peculiar characters of the landraces, with particular attention in removing off-type or undesirable plants, possibly derived from introgression from commercial varieties cultivated in the same area. The distance between each pair of populations ranged from 10 to 50 km. The number of individuals in each population ranged from 5,000 to 20,000 depending on the species in study.

Genomic DNA was extracted from ~0.15 g fresh young leaf harvested from 18-24 individuals per population, following Doyle and Doyle (1990), providing in all a set of 72-90 individuals per landrace for a total of 556 genotypes analysed (Table 1). The quality and concentration of the DNA was established by measuring its UV absorbance and by

comparison with a known quantity of molecular weight marker after electrophoresis through a 1% (w/v) agarose gel.

AFLP analysis

The AFLP profiling method was based on that described by Vos *et al.* (1995) as modified by Lanteri *et al.* (2004a). The template was digested with either *Eco*RI/*Mse*I or *Eco*RI/*Taq*I and the product ligated to standard adaptors to provide the template for the first amplification, which employed primers complementary to the adaptor plus one selective nucleotide (*Eco*RI+A, *Mse*I+C, *Taq*I+T). On the basis of prior experience (Lanteri *et al.*, 2003; 2004a; Portis *et al.*, 2005a; 2006), the second round of amplification was based on five primer combinations (PCs) for cardoon and six for sweet pepper (Table 2). For celery, red beet and common bean, a pilot study tested 24 PCs (four *Eco*RI primers x six *Mse*I primers) against two individuals per species. For the two large genome species leek and garlic, *Mse*I was replaced by *Taq*I and the pilot explored 16 PCs (four *Eco*RI x four T*aq*I primers). As a result of the pilot tests, four PCs were retained for each of these five crop species (Table 2). The reproducibility of the PCs selected was tested on DNAs extracted from six plants per landrace. The amplicons were mixed with a formamide-containing dye and electrophoresed on a DNA analyser Gene ReadIR 4200 (LI-COR) device through a 6.5% polyacrylamide gel, as described by Jackson and Matthews (2000).

Data analysis

Each variable AFLP fragment, which ranged in size from 60 to 650bp, was assumed to represent a single biallelic locus, allowing the data to be scored in binary form. A

polymorphic marker allele frequency (p) was calculated for each landrace population. For the five mainly allogamous species (leek, garlic, celery, red beet and cardoon), it was assumed that the populations were at Hardy-Weinberg equilibrium, so the frequency of each fragment (Fb) was used to predict the allele frequency at each dominant locus by applying the equation expression $1-(1-Fb)^{1/2}$ (where $(1-Fb) = q^2$, the proportion of individuals lacking the fragment). For the two mainly autogamous species (sweet pepper and common bean), the allele frequency at a given locus was considered to be equal to the frequency of the AFLP fragment in the sample. The estimated allelic frequencies were used for the analysis of genetic diversity within and between populations, and for the estimation of basic statistics, using Microsoft Excel software. The following measures were calculated for all populations and landraces: average number of alleles observed per locus (n_o), effective number of alleles per locus (n_e) and genetic diversity within a population (Nei's unbiased expected heterozygosity H'; (Nei, 1987). Pearson correlation coefficients between fragment sizes and fragment frequencies (together with the P-value associated with the correlation) were calculated using AFLP-Surv v1.0 (Vekemans *et al.*, 2002). Genetic similarities between pairs of individuals were estimated from the similarity index of Jaccard (1908). Principal coordinate (PCO) analyses were performed, based on the triangular matrices of genetic similarity estimates using appropriate routines within NTSYS v2.02 (Rohlf, 1998); for each landrace, the first two axes were plotted graphically, according to the extracted Eigen vectors. AFLP-Surv and the PHYLIP package (Felsenstein, 1993) were used to calculated pairwise Nei's genetic similarities between populations (Nei, 1978), and to construct an UPGMA consensus tree based on 1,000 bootstrap replicates. POPGENE v1.21 (Yeh and Boyle, 1997) was used to compute Nei's genetic diversity statistics (Nei, 1973), which quantify the proportion of the genetic diversity distributed among populations. The within population diversity (H_S) and the total genetic diversity (H_T) over all loci and individuals were also computed, and the expression $1-H_S/H_T$

was used to derive the proportion of diversity present between populations (G_{ST}). The significance of genetic differentiation between groups was tested via 10,000 random permutations by comparing the observed G_{ST} with a distribution of G_{ST}, assuming as a null hypothesis the absence of any genetic structure. Finally, all the polymorphic AFLP fragments were classified, for each population, on the basis of their information content and frequency into locally common (restricted to one or two populations with frequency ≥5%), private (restricted to a single population), low frequency (<5%) and rare (both low frequency and private).

Results

Genetic variability

The statistical parameters derived from the genotypic data are summarised in Table 1. The AFLP PCs amplified between 54 (common bean) and 123 (celery) polymorphic fragments, with the average number of polymorphic fragments per PC ranging from 10.2 (sweet pepper) to 30.8 (celery). The highest n_e values occurred in leek (1.73) and garlic (1.70), in the same populations in which H' values were highest (0.41 and 0.40, respectively). Notwithstanding differences between populations of both the landraces were found, the high values of both n_e and H', reflect a remarkable level of within population variability. The lowest n_e values occurred in celery (1.38) and common bean (1.46), coinciding with the populations showing the lowest H' values (0.25 and 0.29, respectively). The lowest variation among populations for the mean number of observed alleles (n_o) was present in sweet pepper, while for n_e in leek populations which, together with the ones of cultivated cardoon showed the lowest H' variation (Table 1).

The reproducibility of the AFLP assays was high, with ~97.5% of the scored fragments in agreement between replicates. Fragment size and fragment frequency were negatively correlated with one another in celery and cardoon (-0.095 and -0.083, respectively, $P<0.1$), indicating the presence of some homoplasy in these two data sets (Vekemans *et al.*, 2002; Portis *et al.*, 2005b). For both these species, a re-analysis was conducted excluding fragments smaller than 150bp, and this reduced the size of the correlation between fragment size and fragment frequency; however, the level of genetic differentiation between populations was analogous to that obtained using the complete data set (results not shown). Thus the extent of homoplasy present, if any, did not compromise the estimates of genetic divergence.

Cluster analyses

The first two PCO components accounted for between 49.8% (red beet) and 61.2% (common bean) of the total genotypic variance. In leek, celery and cardoon, one population appeared to be well differentiated from the others (leak: population A, celery: population C, cardoon: population B), while in garlic, population C showed a weaker extent of genetic differentiation; in these landraces a variable but limited genetic differentiation was observed among the other populations. The red beet, sweet pepper and common bean populations were more homogeneous, with a higher within population genotypic variance (Figure 2). The UPGMA tree separated the leek populations into three major clades, the first grouping populations D and E, the second B and C (bootstrap support values of ~90%), while population A was distinct. In red beet, two clusters were evident (bootstrap values >85%), one consisting of A, B and C, and the other of D and E populations. For garlic, celery and cardoon, both the PCO and the phylogenetic analysis highlighted the separation of one population from a pair of rather similar ones. There was less variation present between the sweet pepper and common bean populations (average NGS of 0.935 and 0.977 respectively), and as a result the clusters were less distinct and the bootstrap probabilities (<55%) failed to support any marked population structure.

Population differentiation and information content of AFLP fragments

The Nei statistics revealed that, for each of the landraces, most of the genotypic variance was concentrated within, rather than between populations. However, differentiation between populations was significant within most of the landraces, as the measures of population differentiation were significantly positive ($P < 0.0001$, Table 3). The estimated overall

population G_{ST} value was high for leek and cardoon (0.19 and 0.17, respectively), moderate for celery and garlic (0.14 and 0.13) and low for red beet and sweet pepper (0.11 and 0.10). No significant differentiation between populations was detectable for common bean (G_{ST} = 0.05), since only 5% of the variance was partitioned into between populations.

The PCs varied significantly in their ability to detect the two components of genetic diversity (Table 3) and a high range of variation was observed among AFLP PCs in cardoon, sweet pepper, leek and garlic. Some 46% of the celery and 31% of the red beet AFLP fragments were polymorphic (Table 3), while a higher level of uniformity was observed in sweet pepper and garlic since the frequency of polymorphic fragments was about 16%.

After classifying the fragments on the basis of their informativeness, the populations harbouring the most informative alleles were identified as leek A and C, garlic A, celery B, red beet A and D, cardoon A, sweet pepper B, and common bean A and B (Table 4).

Discussion

The Mediterranean area is a hotspot for a number of crop landraces; as elsewhere, in the Piedmont region, a number of horticultural crop landraces suffer from genetic erosion arising from pollen flow or admixture of seeds from commercial cultivars, and some are close to extinction. Conserving these populations will require a combination of *ex situ* and *in situ* measures to be implemented. Here, we have focussed on seven crops, and assessed the distribution of genetic variation present within the local landraces using AFLP fingerprinting.

The genetic architecture of crop landraces has been widely explored using DNA fingerprinting (Lanteri *et al.*, 2003; Negri, 2003; Sanchez *et al.*, 2007; Qi-Lun *et al.*, 2008; Muñoz-Falcón *et al.*, 2011). Implementing rational conservation strategies on a large scale needs both informative and time efficient methods to identify the populations present within a given landrace which should be prioritized. In this respect, AFLP genotyping, while it has certain disadvantages associated with the dominant nature of the markers, is well suited, as each assay captures a large number of genetic loci. Furthermore, on the basis of our experience, a higher level of diversity is detectable within closely related materials by means of AFLP than SSR markers. Our previous researches show that the volume of microsatellite data were not sufficient for the analysis of genetic relatedness within the same varietal type of sweet pepper and cultivated cardoon (Portis *et al.*, 2004; 2005a), while the level of variability detected in a celery landrace was very low (Acquadro *et al.*, 2006). Vice versa, we demonstrated that AFLP markers made it possible the fingerprinting of closely related selected clones in *Cynara cardunculus* var. *scolymus* (Lanteri *et al.*, 2004b; Acquadro *et al.*, 2010)

The genetic architecture of natural populations is strongly influenced by the species' breeding system, with inbreeders typically being the more genetically variable between

populations and outbreeders showing a greater degree of within population variation (Schoen and Brown, 1991; Gaudeul *et al.*, 2000). Leek and garlic are both outbreeders with little tolerance to inbreeding (Simon, 1993; Smilde *et al.*, 1995). Farmed garlic is predominantly propagated by asexual means (Konvicka, 1984; Etoh *et al.*, 1988), but still displays a high level of genetic variation and heterozygosity (Ipek *et al.*, 2005). Red beet and cultivated cardoon are also outcrossers (Bartsch *et al.*, 1999; Lanteri and Portis, 2008), while in celery outcrossing have been estimated from 47 to 87% (Orton and Arus, 1984). Both sweet pepper and common bean are largely autogamous (Allard, 1960; Singh *et al.*, 1991), although there is some evidence for a degree of outcrossing in the latter (Berke, 2000). The genotypic data assembled from the present set of landraces suggested, however, that genetic structure was independent of the breeding system of the species, with little difference being evident between the genetic architecture of the autogamous species sweet pepper and common bean populations, while a clear differentiation between the populations of the allogamous leek, cardoon and celery populations was found. It seems therefore that the genetic structure of crop landraces, where they are cultivated only to a limited extent, and in fragmented areas, is more heavily influenced by a combination of the selection criteria adopted by the producers, the degree of seed exchange which occurs and the outcome of natural selection imposed by distinct environmental niches.

The primary goal of germplasm conservation in the context of landraces is to maximize the capture of the genetic variability present. Thus, the initial step is to identify those populations which are most genetically variable. As suggested (Maguire *et al.*, 2002), a further step entails characterizing these populations with respect to their allelic richness (Marshall and Brown, 1975; Schoen and Brown, 1993; Petit *et al.*, 1998), the frequency of locally common alleles (Marshall and Brown, 1975), and establishing the existence of low frequency or unique alleles (Brown and Briggs, 1991; Falk, 1991). In ‘Lungo di Cervere’

(leek), three major clusters were identified (Figure 2). On the grounds of allele richness and the presence of private and low frequency alleles (Table 4), the singleton population A should be prioritized, while in the other two clusters, priority should be given to populations C and D. In 'Molino dei Torti' (garlic), priority should be given to the most variable of the populations (C), together with population A being the allelically richest and containing more private, low frequency and rare alleles. In 'Dorato d'Asti' (celery), population C was the most genetically heterogeneous while population B contained the most allelic diversity. In 'Rossa di Castellazzo Bormida' (red beet), two major clusters were recognized (Figure 2). On the basis of informative alleles, populations A and D (Table 4) are the most representative of the genetic variation within the landrace. In 'Gobbo di Nizza Monferrato' (cardoon), priority should be given to population B as this is the most genetically differentiated (Figure 2), while population A contained a higher frequency of informative alleles (Table 4). In 'Tomaticot' (sweet pepper), only a limited degree of genetic structure was apparent, suggesting that presumably seed exchange between producers occurred; population B should be chosen having the highest frequency of informative alleles. In 'Bianco di Bagnasco' (common bean), as in the sweet pepper landrace, little genetic differentiation was apparent. The A and B populations contained the highest number of locally common, private, low frequency and rare alleles and to them priority should be given in implementing conservation strategies.

On the basis of our molecular data the most representative population(s) for each landrace were recognized and targeted for conservation. With the goal to make feasible and optimize the *in situ* ('on farm') preservation of a local landraces, attention has to be focused on a limited number of populations which respond to its distinctive features and, at the same time, capture most of the genetic variability. The less demanding *'ex situ'* germplasm preservation may include samples collected from all the landrace identified populations,

however, the maintenance in a germplasm bank of seed samples just from the most representative ones may facilitate their long-term management.

Local landraces are typically highly esteemed for their quality, but are generally unknown outside their production area. An appreciation of their biological and cultural importance is rapidly being lost among the current generation of producers, so it is becoming increasingly urgent to catalogue and preserve the diversity which they contain. Unless action is taken immediately, losses of landraces will continue and extinction is the only possible conclusion.

We have presented here a model approach to establishing a rational strategy for the conservation of crop landraces. In conjunction, their recognition in the form of Protected Designation of Origin (PDO) status may contribute in saving local landraces and help consumers to identify them as cultural heritage.

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Tables

Table 1 Genetic diversity statistics: population (Pop.); number of individuals/population (N° ind.); number of polymorphic fragments (NPB); percentage of polymorphic fragments (%); mean number of observed alleles (n_o); mean number of effective alleles (n_e); Nei's unbiased genetic diversity (H').

Ecotype	Pop.	N° ind.	NPB	%	n_o	n_e	H'
Leek 'Lungo di Cervere'	A	18	57	82.6	1.83	1.56	0.32
	B	18	54	78.3	1.78	1.61	0.33
	C	18	54	78.3	1.78	1.60	0.31
	D	18	58	84.1	1.84	1.59	0.33
	E	18	58	84.1	1.84	1.61	0.34
	Overall	**90**	**69**	**100.0**	**2.00**	**1.73**	**0.41**
Garlic 'Molino dei Torti'	A	24	55	91.7	1.92	1.74	0.39
	B	24	45	75.0	1.75	1.61	0.33
	C	24	38	63.3	1.63	1.49	0.28
	Overall	**72**	**60**	**100.0**	**2.00**	**1.70**	**0.40**
Celery 'Dorato d'Asti'	A	24	103	83.7	1.84	1.45	0.27
	B	24	77	62.6	1.63	1.32	0.19
	C	24	78	63.4	1.63	1.29	0.18
	Overall	**72**	**123**	**100.0**	**2.00**	**1.38**	**0.25**
Red beet 'Rossa di Castellazzo B.da'	A	18	66	91.7	1.92	1.63	0.35
	B	18	65	90.3	1.90	1.56	0.33
	C	18	66	91.7	1.92	1.60	0.35
	D	18	63	87.5	1.87	1.53	0.31
	E	18	58	80.6	1.81	1.51	0.29
	Overall	**90**	**72**	**100.0**	**2.00**	**1.64**	**0.37**
Cultivated cardoon 'Gobbo di Nizza M.to'	A	24	64	85.8	1.85	1.52	0.30
	B	24	63	84.0	1.84	1.54	0.31
	C	24	57	76.1	1.76	1.48	0.28
	Overall	**72**	**75**	**100.0**	**2.00**	**1.60**	**0.36**
Sweet pepper 'Tomaticot'	A	24	57	93.4	1.93	1.53	0.31
	B	24	56	91.8	1.92	1.50	0.29
	C	24	57	93.4	1.93	1.59	0.33
	Overall	**72**	**61**	**100.0**	**2.00**	**1.59**	**0.35**
Common bean 'Bianco di Bagnasco'	A	22	43	79.6	1.82	1.44	0.25
	B	22	46	85.1	1.87	1.46	0.27
	C	22	46	85.1	1.87	1.48	0.29
	D	22	42	77.7	1.80	1.42	0.24
	Overall	**88**	**54**	**100.0**	**2.00**	**1.46**	**0.29**

Table 2 AFLP primer combinations used for fingerprinting. PC codes as published at wheat.pw.usda.gov/ggpages/keygeneAFLPs.html.

Leek	Garlic	Celery	Red beet	Cultivated cardoon	Sweet pepper	Common bean
E35/T81	E35/T81	E35/M50	E33/M49	E35/M60	E32/M48	E35/M49
E35/T82	E35/T82	E36/M50	E35/M47	E35/M62	E32/M59	E35/M50
E36/T82	E36/T81	E37/M49	E35/M48	E36/M48	E33/M59	E36/M48
E36/T90	E36/T82	E37/M50	E35/M49	E36/M50	E35/M59	E36/M50
				E36/M62	E35/M61	
					E36/M48	

Table 3 Population differentiation statistics: number of AFLP primer combinations (PC); total number of fragments amplified (NTB); number of polymorphic fragments (NPB) and percentage (%), genetic diversity (HT, HS) and differentiation (GST with PC range).

Ecotype	**PC**	**NTB**	**NPB**	**%**	**H_S**	**H_T**	**G_{ST} (range)**
Leek ‘Lungo di Cervere’	4	372	69	18.5	0.330	0.407	0.19 (0.14 - 0.27)
Garlic ‘Molino dei Torti’	4	365	60	16.4	0.333	0.384	0.13 (0.11 - 0.20)
Celery ‘Dorato d’Asti’	4	269	123	45.7	0.215	0.249	0.14 (0.12 - 0.15)
Red beet ‘Rossa di Castellazzo B.da’	4	235	72	30.6	0.329	0.376	0.11 (0.09 - 0.13)
Cultivated cardoon ‘Gobbo di Nizza M.to’	5	279	75	26.9	0.298	0.358	0.17 (0.10 - 0.25)
Sweet pepper ‘Tomaticot’	6	388	61	15.7	0.305	0.342	0.10 (0.06 - 0.14)
Common bean ‘Bianco di Bagnasco’	4	256	54	21.1	0.263	0.277	0.05 (0.04 - 0.07)

Table 4 Number of AFLP informative alleles within each population. Alleles classified as locally common (when restricted to one or two populations), private (restricted to a single population), low frequency (<5%), and rare (both low frequency and private).

Ecotype	Pop.	Informative alleles			
		Locally common	Private	Low frequent	Rare
Leek ‘Lungo di Cervere’	A	6	3	8	1
	B	1		3	
	C	5	1	4	
	D	3		3	
	E	1		4	
Garlic ‘Molino dei Torti’	A	4	2	2	1
	B	3	1	3	
	C		2	1	1
Celery ‘Dorato d’Asti’	A	11	1	6	
	B	21	4	14	2
	C	17	2	9	1
Red beet ‘Rossa di Castellazzo B.da’	A	9	4	6	2
	B	5	1	4	
	C	2		6	
	D	7	2	4	1
	E	1		4	
Cultivated cardoon ‘Gobbo di Nizza M.to’	A	6		2	
	B	1	2	2	1
	C	3		1	
Sweet pepper ‘Tomaticot’	A	6	1	5	2
	B	8	3	7	4
	C	2		2	
Common bean ‘Bianco di Bagnasco’	A	8	3	6	2
	B	6	4	7	2
	C	2		3	
	D	5	1	5	

Figure 1 A) leek ‘Lungo di Cervere’, B) garlic ‘Molino dei Torti’, C) celery ‘Dorato d’Asti’, D) red beet ‘Rossa di Castellazzo Bormida’, E) cultivated cardoon ‘Gobbo di Nizza Monferrato’, F) sweet pepper ‘Tomaticot’ G) common bean ‘Bianco di Bagnasco’.

Figure 2 PCO plots based on the first two principal coordinates, depicting genetic relationships between individuals from each population, along with the associated UPGMA tree (bootstrap support values shown at each branch point). The first two principal coordinates accounted for 57.7% (leek), 52.6% (garlic), 55.3% (celery), 49.8% (red beet), 58.9% (cultivated cardoon). 51.0% (sweet pepper) and 61.2% (common bean) of the genotypic variance.