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Amplified fragment length polymorphism for genetic diversity assessment in globe artichoke

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Abstract Globe artichoke (*Cynara cardunculus* L. var. *scolymus* L.) is a diploid (2*n*=2*x*=34), predominantly cross-pollinated plant native to the Mediterranean basin, and Italy contains the richest primary cultivated 'gene pool'. Commercial production is mainly based on perennial cultivation of vegetatively propagated clones that are highly heterozygous and segregate widely when progeny-tested. Analysis of the artichoke genome by means of molecular markers has been limited to a few studies; here we report on the genetic relatedness among 118 artichoke accessions, including clones belonging to the same varietal type, two accessions of cultivated cardoon (*C. cardunculus* L. var. *altilis* DC.) and four accessions of wild cardoon [*C. cardunculus* L. var. *sylvestris* (Lamk) Fiori] as measured by amplified fragment length polymorphism (AFLP). Eight primer combinations yielded a total of 667 bands, of which 519 were polymorphic. Genetic similarities among accessions were calculated according to Jaccard's Similarity Index and used to construct a dendrogram based on the unweighted pair group method using arithmetic averages. Our results demonstrate that AFLP markers can be useful in evaluating *Cynara cardunculus* genetic diversity and in classifying accessions to phylogenetic groups based on their genetic similarity values. Genetic variation among artichoke clones belonging to the same varietal type was in some cases higher than that found among accessions differently named and coming from different areas. The lowest Jaccard's Similarity Index found within a varietal type can be considered as a threshold for the identification of accessions which share an analogous genetic background. This will enable the selection of representatives in order to develop and manage a germplasm 'core collection' as well as the identification of suitable material for future artichoke breeding efforts.

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Introduction

Globe artichoke (*Cynara cardunculus* L. var. *scolymus* L.) (2*n*=2*x*=34) is a perennial and cross-pollinated vegetable (De Vos 1992; Pécaut 1993) native to the Mediterranean basin, a region where the cultivated cardoon (*C. cardunculus* L. var. *altilis* DC.) and at least seven other wild *Cynara* taxa are present: the wild cardoon [*C. cardunculus* L. var. *sylvestris* (Lamk) Fiori], *C. syriaca* Boiss, *C. cornigera* (Lindely) (syn. *C. sibthorpiana* Boiss.), *C. algarbiensis* Cosson, *C. baetica* (Sprengel) Pau (syn. *C. alba* Boiss.), *C. humilis* L. and *C. cyrenaica* Maire & Weiller (Rottenberg and Zohary 1995). Globe artichoke represents an important component of the European agricultural economy, with more than one million tons of crop (FAO data 2001) and more than 100,000 ha in cultivation (120,837 ha; FAO data 2001). Nearly 85% of world production comes from Europe. Italy is the leading producer (FAO data 2001) followed by Spain, France and Turkey.

Artichoke heads or capitula, which are immature composite inflorescences, are the edible part of the plant and are used as a fresh, frozen or canned delicacy all over the world. Globe artichoke leaves have also been widely used in herbal medicine as hepatoprotectors and choleretics since ancient times (Bruneton 1995; Gebhardt 1997, 1998, 2002). The chemical components of artichoke leaves have been found to be rich in compounds originating from the metabolism of phenylpropanoids, with mono- and dicaffeoylquinic acids and flavonoids as the major components (Adzet and Puigmacia 1985;

Debenedetti et al. 1993; Slanina et al. 1993; Wagenbreth 1996; Sevcikova et al. 2002; Wang et al. 2003).

Italy has the richest globe artichoke primary cultivated "gene pool" and harbours many distinct clonal varietal groups best adapted to different local environments. The germplasm in cultivation has been classified into four main groups mainly on the basis of capitulum characters (Porceddu et al. 1976; Dellacecca et al. 1976; Vannella et al. 1981): (1) the Spinosi group, containing types with long sharp spines on bracts and leaves; (2) the Violetti group, with medium-sized, violet-coloured and less spiny heads; (3) the Romaneschi group, with spherical or subspherical non-spiny heads; (4) the Catanesi group, with relatively small, elongated and non spiny heads. More recently, cluster analysis based on the discrimination of eight quantitative characters among 104 artichoke accessions (Elia and Miccolis 1996) identified five large groups having similar characters and presumably similar genomes.

In globe artichoke cross-fertilizations are promoted by protandry, but selfing is not precluded (Mauromicale and Ierna 2000). At present, commercial production is mainly based on the cultivation of clones, which are obtained through vegetative propagation by means of "carducci" (basal shoots) or "ovoli" (semi-dormant shoots with a limited root system). The clones are highly heterozygous as, when progeny tested, they show a wide segregation of morphological and production traits (Basnizki and Zohary 1987). Seed-propagated cultivars are becoming popular in some parts of the world, but at present no concrete results have been obtained with Italian germplasm.

Artichoke breeding to date has been limited to a few studies on the inheritance of a few main characters (Pècaut 1993; Lòpez Anido et al. 1998; Mauromicale et al. 2000). Breeding programmes have been based on intra-clonal selection (Deidda 1967; Abbate and Noto 1981; Pècaut 1983; Mauromicale and Copani 1989) or hybridization among varietal groups followed by selection (Miller 1975; Scarascia Mugnozza and Pacucci 1976; Tesi 1976; Baznisky and Zohary 1987, 1994). Molecular marker studies of the artichoke genome have also been extremely limited and primarily aimed at estimating the variation among American varieties (Tivang et al. 1996) and variation among a limited number of artichoke and cardoon accessions (Sonnante et al. 2002) using randomly amplified polymorphic DNAs (RAPDs). Acquadro et al. (2003) reported on the development of the first set of microsatellite (simple sequence repeats; SSRs) markers obtained by an enriched library approach and search in sequence database. Due to the high range of genetic variation we previously found in cultivated populations (Lanteri et al. 2001), we consider the term 'varietal type' more appropriate than 'variety' for defining the accessions of germplasm at present in cultivation.

Scientific approaches to the conservation and utilization of plant genetic resources require accurate assessment of the amount and distribution of genetic variation within a gene pool. In recent years DNA markers have provided the tools needed to do this (Tomkins et al. 2001). The multilocus amplified fragment length polymorphism (AFLP) DNA fingerprinting technique (Vos et al. 1995) has been widely used to study genetic relationships within and among many different plant species (Hill et al. 1996; Maughan et al. 1996; Sharma et al. 1996; Hongtrakul et al. 1997; Paul et al. 1997; Cervera et al. 1998; Angiolillo et al. 1999; Barcaccia et al. 1999; Leus et al. 2000; Tomkins et al. 2001; Van Huylenbroeck et al. 2001; Carr et al. 2003).

The objectives of the investigation reported here were: (1) to evaluate the usefulness of AFLP in differentiating between artichoke varietal types; (2) to determine genetic relationships in a sample of 118 accessions which also include clones within the same varietal type.

Materials and methods

Plant material

A total of 118 artichoke accessions, including 89 varietal types, were analysed. Ninety-one of these came from a living artichoke collection maintained at the CRAS (Centro Regionale Agrario Sperimentale, Sardinia, Italy) and are listed in Table 1. The C3 clone of Romanesco (collected in the field in Tarquinia, Italy), the F_1 hybrid Orlando from Nunhems Seed Company (The Netherlands) and three accessions of Tudela (collected from the field in Tudela, Spain) were also surveyed. In order to estimate the genetic variation within varietal type, we also analysed nine clones of Spinoso sardo (accessions II–X, selected at the CRAS on the basis of morphological and production traits) as well as Turkish accessions. The latter included six provenances of Bayrampasa—accessions II, III and IV (from the Yalova Research Station at Marmara, Turkey) and accessions V, VI and VII (collected in the field at Izmir, Turkey)—and seven Sakiz selections—accessions II, III and IV (from Ege University, Izmir, Turkey), accession V (from Yalova Research Station) and accessions VI, VII and VIII (collected in the field at Karaburun Peninsula, Turkey).

Two accessions of cultivated cardoon [*C. cardunculus* var. *altilis*, accessions I and II, sampled from the field in Scalenghe (Turin, Italy)] and four accessions of wild cardoon [*C. cardunculus* var. *sylvestris*, accessions I–IV, sampled from the wild in Bronte and Piano Tavola (Catania, Italy)] were also included in the analysis.

DNA extraction and AFLP analysis

Samples were collected from young leaves, and DNA was extracted following the procedure of Lanteri et al. (2001). The AFLP protocol was essentially that of Vos et al. (1995) with minor modifications (Lanteri et al. 2003). Briefly, 5 μl extracted DNA (400–500 ng) was concurrently digested with *Eco*RI and *Mse*I and ligated to adapters. Digested and ligated DNA fragments were first pre-amplified with primers complementary to the adapters plus an additional selective 3′ nucleotide (*Eco*RI+A and *Mse*I+C primers). Selective amplification was subsequently carried out using primers with two or three selective nucleotides. Initially, 64 primer pairs (combinations of eight *Eco*RI primers and eight *Mse*I primers) were tested in four artichoke genotypes. On the basis of the results obtained from this pilot study, the following eight primer combinations were selected and applied to all samples: E35/M17 (ACA/CG), E35/M50 (ACA/CAT), E35/M60 (ACA/CTC), E36/M62 (ACC/CTT), E37/M47 (ACG/CAA), E38/M47 (ACT/CAA), E38/M50 (ACT/CAT), E38/M62 (ACT/CTT). Reproducibility for each primer pair was checked by running the AFLP protocol at different DNA concentrations; 20 ng DNA/μl before digestion was found to be the lowest concentration that avoided appearance of artifacts or disappearance of some bands.

Table 1 List of the 91 artichoke accessions from the artichoke living collection maintained at the CRAS (Centro Regionale Agrario Sperimentale, Sardinia, Italy) included in this study

Varietal type	Origin	Varietal type	Origin
A. Pigno	Italy	Moretto	Italy
AVM 7	France	Niscemese	Italy
Baladi	Egypt	Nostrano violetto di Pesaro	Italy
Bamafsigi	Egypt	Ogni mese	Italy
Bayrampasa I	Turkey	Pasquaiolo	Italy
Bianco tarantino	Italy	Pietralcina	Italy
Blanc oranais	Tunisia	Precoce violetto di Chioggia	Italy
Blanco	Argentina	R 35	France
Cacique	France	Romana	Italy
Camard	France	Romanesco	Italy
Campagnano	Italy	Romano	Italy
Camus de Bretagne	France	Sakiz I	Turkey
Caribou	France	Selezione 67	Italy
Castellamare I	Italy	Selezione romanesco	Italy
Castellamare II	Italy	Siracusano	Italy
Catanese I	Italy	Spinoso di Licata (Sp. di Gela)	Italy
Catanese II	Italy	Spinoso di Palermo	Italy
CB 641	France	Spinoso di Sciacca	Italy
CB 642	France	Spinoso sardo I	Italy
Centofoglie	Italy	Spinoso violetto di Liguria	Italy
CP 11	France	Terom	Italy
Di Catania	Italy	Testa di ferro	Italy
Di Niscemi	Italy	Tonda di Paestum	Italy
Di Palermo	Italy	VP 45	France
Di Teramo	Italy	VP 49	France
Di Vasto	Italy	VP 558	France
Di Viterbo	Italy	Verde di Pesaro	Italy
EB 9	Spain	Verde di Putignano	Italy
Empolese	Italy	Violet d'Algerie	Egypt
Gagliardo Sgrò	Italy	Violet de Campagne	France
Green globe I	USA	Violet du Gapeau	France
Green globe II	USA	Violet French	France
Gross Camus	France	Violet Margot	France
Hyerois	Egypt	Violetto	Italy
Locale di Fano	Italy	Violetto di Maremma	Italy
Locale di Cuneo	Italy	Violet de Provence	France
Locale di Mola	Italy	V. de Provence sel. INRA	France
Locale di Montelupone	Italy	Violetto di Putignano I	Italy
Locale di Ostuni	Italy	Violetto di Putignano II	Italy
Locale di Sibari	Italy	Violetto di S.Luca	Italy
Locale di Strancona	Italy	Violetto di Toscana I	Italy
Blanc Hyerois (Macau)	France	Violetto di Toscana II	Italy
Masedu	Italy	Violetto precoce	Italy
Mazzaferrata	Italy	928	France
Mazzaferrata di Termoli	Italy	932	France
		968	France

Amplified fragments were resolved on 5% denaturing polyacrylamide gels and silver stained as described by Bassam et al. (1991).

Data scoring and analysis

AFLP amplifications were repeated at least once in order to test their consistency. Electrophoretic patterns were documented using the Gel Documentation System (Quantity One Programme; Bio-Rad, Hercules, Calif.). Each PCR product was assumed to represent a single locus, and only reproducible polymorphic bands were scored as present (1) or absent (0).

AFLP data were evaluated by means of Shannon's index, marker index and polymorphic information content. Shannon's index (H'_j) (Shannon and Weaver 1949) was calculated over all loci as follows: $H'_j=-\Sigma p_i \log p_i$, where p_i is the frequency of the i^{th} fragment in the sample. In order to compare levels of diversity detected by different primer combinations, H'_j was calculated for each primer combination separately.

The marker index (MI) was calculated according to Powell et al. (1996) as the product of expected heterozygosity (H_n) and effective multiplex ratio (EMR). H_n of a locus is defined as: $1-\Sigma p_i^2$, where p_i is the frequency of the i^{th} allele (band). EMR of a primer is defined as: ßn, were ß is the percentage of polymorphic loci and n is the number of polymorphic loci detected per primer combination (Milbourne et al. 1997).

The polymorphic information content (PIC) was calculated by applying the simplified formula of the expected heterozygosity (Anderson et al. 1993): PIC=2f (1-f), where f is the percentage of plants in which the fragment is present.

A binary matrix was imported into NTSYS-pc (numerical taxonomy and multivariate analysis system) version 1.80 package (Rohlf 1993) for cluster analysis. Genetic similarity among all accessions was calculated according to Jaccard's Similarity Index (JSI) (Jaccard 1908), using the SIMQUAL (similarity of qualitative data) routine. The similarity coefficients were used to construct a dendrogram using the UPGMA (unweighted pair-group method, arithmetic average) and a thousand bootstraps were performed over AFLP loci using the PHYLIP package (Felsenstein 1993; http://evolution.genetics.washington.edu/phylip.html). A co-phenetic matrix was produced using the hierarchical cluster system by means of the COPH routine and correlated with the original distance matrices in order to test for association between the cluster in the dendrogram and the JSI matrix. Principal co-ordinate analysis (PCO) was also carried out to show differentiation of accessions in a multi-dimensional space.

In addition, the clustering abilities of eight selected AFLP primer combinations were tested to determine the optimal number of primer pairs needed to discriminate the maximum number of artichoke varietal types and accessions. The primer combination (PC) with the highest cluster ability (evaluated on the basis of the number of varietal types and accessions identified) was first analysed alone (PC1.J=primer combination 1, based on JSI) and then in combinations with the other PCs with progressively lower discrimination power. The last combination (PC1-8.J) comprised all eight PCs used in the study.

Results

Primer selection and AFLP analysis

A total of 433 polymorphic bands (64.9% of the total amplified bands) between artichoke accessions were scored (Table 2). The number of polymorphic bands per primer combination ranged from 43 to 68, with an average of 54.1 (Table 2).

Shannon index (H'_j), PIC and MI values for each primer pair are also reported in Table 2. Primer combination E37/M47 showed the highest values for H'_j and PIC, while primer combination E38/M50 showed the highest value for MI and distinguished 70 of the 89 varietal types and 94 of the 118 accessions in this study. The lowest values for H'j, PIC and MI were obtained with E35/M60, which distinguished 35 varietal types and 49 accessions (Table 2).

Table 2 Summary of AFLP primer combination characteristics[a]

Primer combination	TNB	NPB-1	P%	H'_j	PIC	MI	NVT	NA	UDB	NPB-2
E35/M17	82	53	64.6	0.592	0.262	8.975	52	73	1	10
E35/M50	73	47	64.4	0.545	0.235	7.100	38	55	1	8
E35/M60	69	47	68.1	0.438	0.183	5.856	35	49	1	6
E36/M62	74	43	58.1	0.664	0.300	7.490	45	59	1	9
E37/M47	87	56	64.4	0.693	0.323	11.627	66	90	2	10
E38/M47	88	51	58.0	0.535	0.235	6.948	44	57	3	14
E38/M50	99	68	68.7	0.680	0.315	14.713	70	94	3	19
E38/M62	95	68	71.6	0.501	0.217	10.562	63	81	1	10
total	667	433							13	86
Average	83.4	54.1	64.7	0.581	0.259	9.159	51.6	69.8	1.6	10.8

[a] TNB, total number of bands; NPB-1, number of polymorphic bands between artichoke accessions; P%, percentage of polymorphic bands; H'_j, Shannon index; PIC, polymorphic information content; MI, marker index; NVT, number of varietal types identified; NA, number of accessions identified; UDB, number of unique/distinctive bands;NPB-2, number of polymorphic bands among artichoke and cardoon accessions

Each primer combination detected unique/distinctive bands (i.e. fragments present in only one accession), for a total number of 13 distinctive bands and a range of one to three distinctive bands per primer combination (Table 2). Twelve artichoke accessions were characterized by distinctive bands. It may be possible to convert these into STS (sequence tagged site) markers of great value for varietal fingerprinting (Table 3).

Eighty-six bands that were polymorphic between the accessions of artichoke and those of cardoon were also scored, with an average number per primer pair of 10.8 and a range of 6 to 19 (Table 2). Overall, 519 polymorphic bands (77.8% of the total number of amplified bands) were scored between the 124 *Cynara cardunculus* accessions in this study. An example of an AFLP profile is shown in Fig. 1.

The number of varietal type and accession subsets recovered by the successive clustering analyses with the eight primer combinations are shown in Table 4. The number of artichoke varietal types clustered by using different primer pairs was found to increase from 70 in PC1.J to 89 in PC1-3.J. Clustering power reached a maximum of 114 subsets (accessions) after the AFLP data of the fifth primer combination were added. The addition of the three remaining primer combinations (PC1-6.J, PC1-7.J, PC1-8.J; data not shown) only resulted in a minor modification of the dendrogram.

Table 3 List of the 12 varietal types in which unique/distinctive bands were detected. (UDB number of unique/distinctive bands, PCSs primer combinations)

Varietal type	UDB	PCs
Blanco	1	E38/M50
Cacique	1	E37/M47
Di Teramo	1	E38/M50
Di Vasto	1	E38/M47
Locale di Cuneo	1	E35/M60
Mazzaferrata di Termoli	1	E38/M62
Precoce violetto di Chioggia	1	E38/M47
Romanesco	1	E38/M47
Spinoso sardo	1	E37/M47
Terom	2	E35/M17–E36/M62
Testa di ferro	1	E35/M50
Verde di Pesaro	1	E38/M50
Total	13	

Genetic relatedness

The lowest JSI value of 0.238 was detected between an accession of wild cardoon (accession III) and one of artichoke (Terom). Wild and cultivated cardoon accessions were also highly genetically differentiated, and the lowest JSI value of 0.327 was found between *C. cardunculus* var. *altilis* I and *C. cardunculus* var. *sylvestris* II. Within artichoke accessions, the smallest JSI value (0.373) was found between Terom and Spinoso di Palermo, while values of 1.00 (identity) were measured between accessions of the same varietal type (Sakiz IV and II; Spinoso sardo II, VIII and IX; Spinoso sardo III and IV).

Table 4 Primer combinations and the number of polymorphic bands (NPB), number of different artichoke varietal types (NVT) and number of different accessions (NA) they were able to distinguish

Dendrogram	Primer combinations	NPB	NVT	NA
PC1.J	E38/M50	68	70	94
PC1-2.J	E38/M50, E37/M47	124	85	102
PC1-3.J	E38/M50, E37/M47, E38/M62	192	89	109
PC1-4.J	E38/M50, E37/M47, E38/M62, E35/M17	245	89	111
PC1-5.J	E38/M50, E37/M47, E38/M62, E35/M17, E36/M62	288	89	114
PC1-6.J	E38/M50, E37/M47, E38/M62, E35/M17, E36/M62, E38/M47	339	89	114
PC1-7.J	E38/M50, E37/M47, E38/M62, E35/M17, E36/M62, E38/M47, E35/M50	386	89	114
PC1-8.J	E38/M50, E37/M47, E38/M62, E35/M17, E36/M62, E38/M47, E35/M50, E35/M60	433	89	114

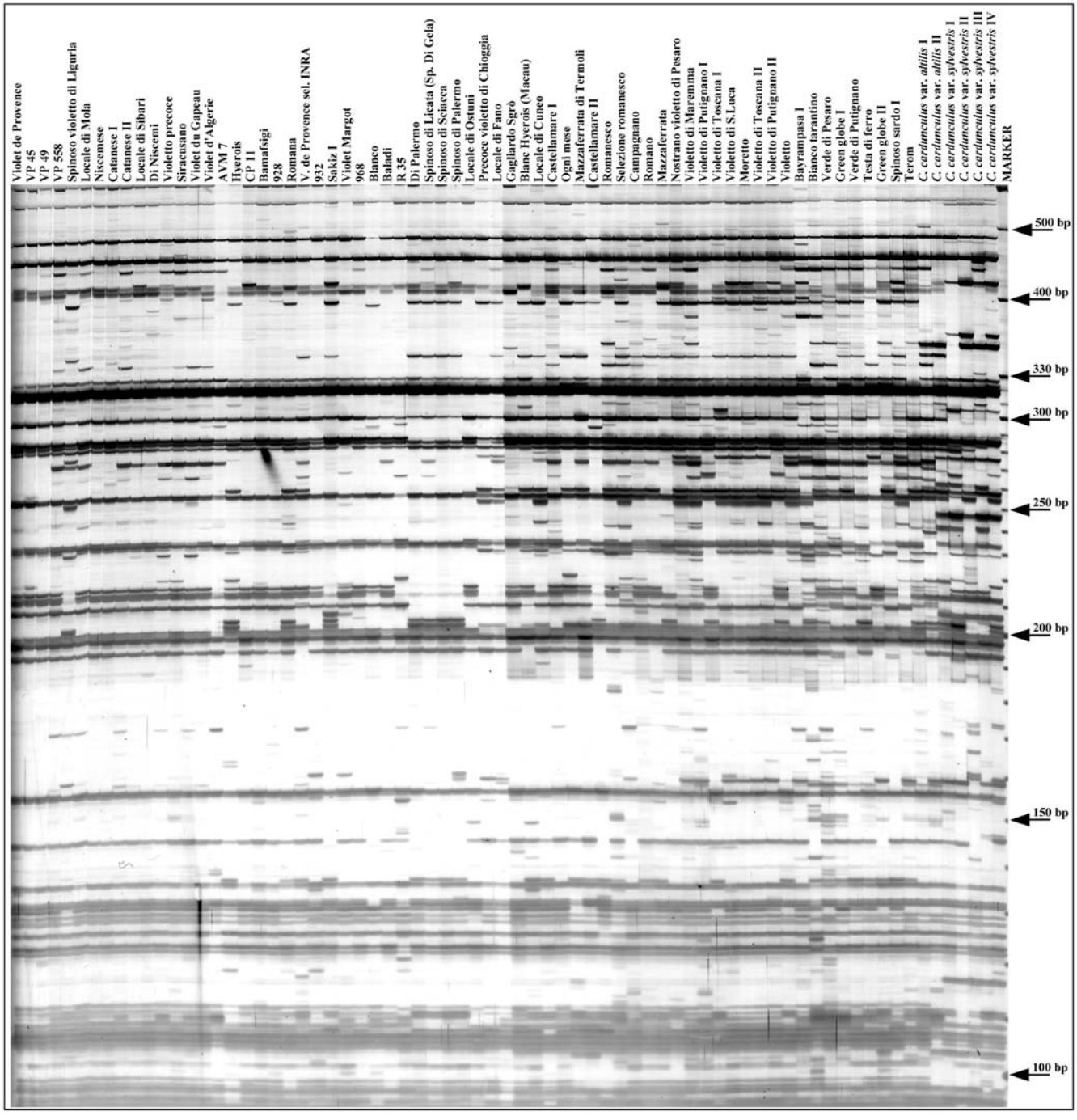

Fig. 1 Example of AFLP band profiles produced by primer combination E38/M47 in 72 of the accessions tested

The dendrogram, based on the similarity values generated using UPGMA clustering analysis, is shown in Fig. 2. The co-phenetic correlation coefficient (r-value) between the data matrix and the co-phenetic matrix for AFLP data was 0.92, suggesting a very good fit between the dendrogram clusters and the similarity matrices from which they were derived. The wild cardoon (var. *sylvestris*) accessions analysed clustered together and showed an average genetic differentiation of 64% from the *Cynara cardunculus* cultivated forms (var. *altilis* and var. *scolymus*). The two accessions of cultivated cardoon (var. *altilis*) also formed a separate cluster, but showed a lower genetic differentiation (54%) from artichoke accessions.

Apart from Testa di ferro, Caribou and Terom, the dendrogram separated (with 95.4% bootstrap probability) the artichoke accessions into two main branches: A, which included the Catanesi (1) and Romaneschi (2) types

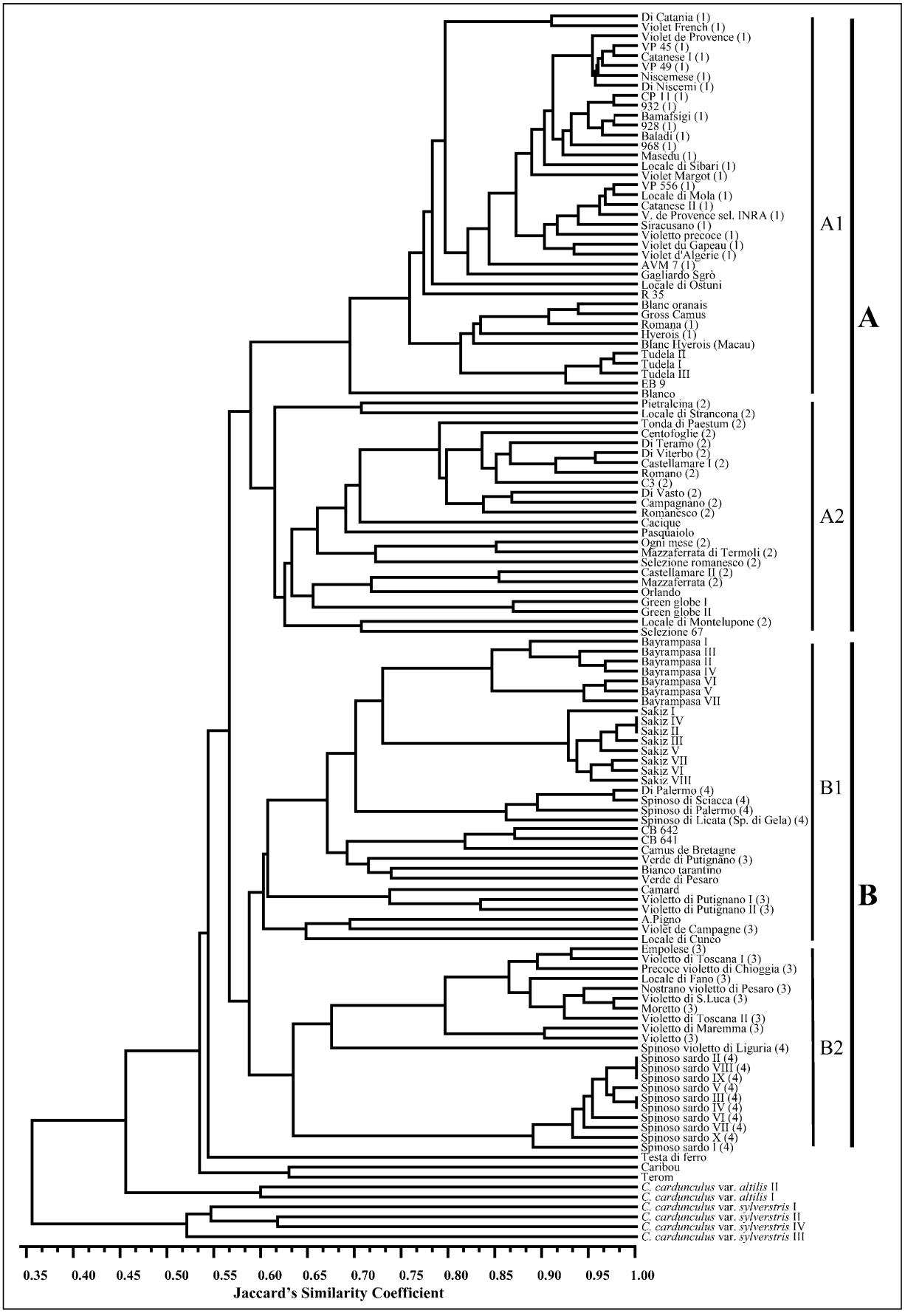

Fig. 2 Dendrogram obtained from UPGMA cluster analysis of AFLP data generated by the eight primer combinations tested. *1* Catanesi type, *2* Romaneschi type, *3* Violetti type, *4* Spinosi type. Co-phenetic correlation coefficient = 0.92

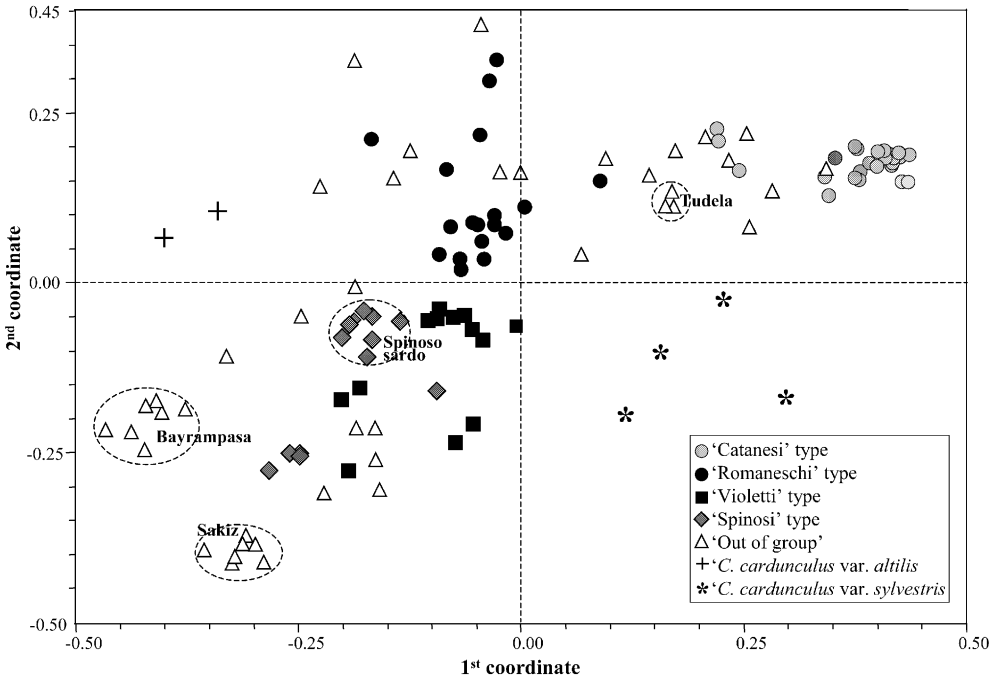

Fig. 3 Principal coordinates analysis, based on AFLP data, depicting the genetic relationship among the 124 *Cynara cardunculus* accessions in this study

together with some varietal types not attributable to any group (subsequently defined as 'out of groups'); B, which included the Violetti (3) and Spinosi (4) types together with a few 'out of groups' (Fig. 2). Each branch was further separated into two main clusters—A1, A2 (with 96.7% bootstrap probability) and B1, B2 (with 82.7% bootstrap probability), respectively.

Cluster A1 contained 39 accessions, 28 of which were Catanesi or Violet de Provence types, all quite similar to each other. Of these 28, 23 showed an average genetic similarity of 88%, while the other five had JSIs of 0.85 (AVM7), 0.80 (Di Catania and Violet French) and 0.76 (Romana and Hyerois). Within cluster A1 the following 11 accessions of different origin were not attributable to any of the four main typologies: Gagliardo Sgrò and Locale di Ostuni from Italy; R35, Gross Camus and Blanc Hyerois from France; Blanc oranais from Tunisia; Blanco from Argentina; three accessions of Tudela from Spain and its selection EB9.

Cluster A2 included 24 accessions, among which were all those of the Romaneschi type (18). Of the Romaneschi type Pietralcina and Locale di Strancona were the most genetically differentiated (JSI=0.6) from the others. JSIs within the remaining Romaneschi ranged from 0.941 (Di Viterbo vs. Castellamare I) to 0.475 (Locale di Montelupone vs. Ogni mese). Six 'out of groups' were also included within cluster A2 (two Green globe from the USA, Pasquaiolo and Selezione 67 from Italy, the French Cacique and the Nunhems F_1 hybrid Orlando).

Cluster B1 contained 31 accessions, including four Violetti and four Sicilian Spinosi types. It also included four French (CB 641, CB 642, Camus de Bretagne and Camard) and four Italian (Bianco tarantino, Verde di Pesaro, A. Pigno and Locale di Cuneo) 'out of groups', together with all of the Bayrampasa and Sakiz accessions from Turkey. Selections I to IV of Bayrampasa clustered together showing an average JSI of 0.89. Among the Sakiz accessions, the one from the CRAS collection (accession I) was genetically more different than the others.

The 21accessions in cluster B2 included ten Violetti and 11 Spinosi types. Eight Violetti accessions were more genetically similar (average JSI of 0.87), while Violetto di Maremma and Violetto differentiated at a JSI of 0.80 from the others. The ten Spinoso sardo accessions clustered together and showed an average JSI of 0.89. The Spinoso Violetto di Liguria occupied an intermediate position between the Violetti types and the Spinoso sardo accessions.

The scatter-plot obtained from PCO analysis (Fig. 3) showed similar genetic clustering of the accessions, with cultivated and wild cardoon accessions well separated. Principal co-ordinates 1, 2 and 3 accounted for about 52% of the total variation with each coordinate contributing 25.9%, 15.4% and 10.5%, respectively. The first co-ordinate distinguished the Catanesi types from Violetti, Spinosi and most of the Romaneschi types; the second co-ordinate separated Catanesi and Romaneschi from Violetti and Spinosi.

Discussion

Many artichoke varietal types are currently grown, but little is known about domestication and subsequent diversification (Elia and Miccolis 1996). Germplasm variability has been described and classified on the basis of head (capitulum) morphology (presence/absence of

spines, size and shape, pigmentation) or relative size of the plant. On the basis of harvest time, varietal types can be classified as early—producing heads from autumn to spring (e.g. Violetto di Sicilia, Spinoso sardo, Spinoso di Palermo, Violet de Provence)—and late—producing heads from March to June (e.g. Romanesco and Violetto di Toscana) (Mauromicale and Ierna 2000).

Most varietal types are cultivated in limited geographic areas and are identified with vernacular names, so presumably very similar genotypes can be differently named in different areas. Furthermore, varietal types differing by only a few characters, due to mutation in a limited number of genes, might share a similar genetic background. On the whole, artichoke genomes are rather poorly known at present and have not been adequately investigated.

Our report is apparently the first on the use of AFLP markers to identify genetic differentiation among a wide sample of artichoke varietal types that represent the genetic variation of material at present in cultivation. All of the primer pairs applied in this study revealed high levels of polymorphism, and similar levels were observed among all of the primer combinations tested, confirming that high genetic diversity exists within the artichoke genome. Pejic et al. (1998) reported that 150 polymorphic bands make it possible for a researcher to reliably estimate genetic similarities among genotypes within the same species. In confirmation of this, we found that with three primer pairs, generating 192 polymorphic bands, it was possible to fingerprint all of the 89 varietal types included in this study (Table 4). The maximum resolution of our 114 distinguishable subsets, which included accessions within the same varietal type, was obtained using five primer combinations that amplified 288 polymorphic bands. Nevertheless, our study was based on the detection of 433 polymorphic bands, which enabled us to make more accurate estimates of the genetic relationships among genotypes.

Co-phenetic correlation values showed that the genetic clusters accurately represented the estimates of genetic similarity. Wild cardoon accessions clustered together and were highly differentiated from the cultivated forms. Similarly, the two cultivated cardoon accessions were highly differentiated from artichoke accessions but with a lower JSI value. Cytogenetic and isozyme studies (Rottenberg and Zohary 1995) support the hypothesis that wild cardoon was the progenitor of both cultivated forms, with which it is cross-compatible with the fully fertile F_1 hybrids. Our data support the hypothesis that both artichoke and cultivated cardoon evolved separately from wild cardoon as a result of different agricultural selection criteria. The cultivated cardoon was selected for the production of inner stalks, which are succulent and used in traditional dishes in Southern Europe, while artichoke was selected mainly for the production of heads.

Artichoke varietal types Terom and Spinoso di Palermo had the lowest genetic similarity value (JSI=0.373). Using RAPD markers, Sonnante et al. (2002) obtained 0.817 as the lowest JSI value when 32 cultivated artichoke accessions belonging to different varietal types were compared. This discrepancy might be explained by the limited number of genotypes included in their analysis as well as by the smaller number of polymorphic bands they used for discriminating genotypes. In fact, their study was based on the application of 18 RAPD primers generating only 69 polymorphic bands.

The UPGMA dendrogram (Fig. 2) shows that artichoke accessions fall into two main groups: A, containing only non-spiny types (Catanesi and Romaneschi), and B, including all of the spiny Spinosi and Violetti types together with a few non-spiny types. The presence/absence of spines seems to be controlled by a single gene with two alternative alleles (Pochard et al. 1969; Basnizki and Zohary 1994): the dominant non-spiny (Sp) and the wild type spiny (sp). This trait appears to be a key to understanding the origin of the material at present in cultivation. Barbieri (1959) hypothesized that the spiny types (Spinosi) were selected first, then the violet types (Violetti), which possess less spiny heads, and finally the non-spiny Romaneschi and Catanesi. The older origin of the Spinosi and Violetti types is supported by their higher within-genetic variation. However, the high genetic differentiation between spiny and non-spiny types seems to support the hypothesis of their evolution side by side.

Within branch A two main clusters were found: A1 and A2. Cluster A1 mainly contained Catanesi types having small elongated heads weighing about 110–120 g and included accessions cultivated in Sicily (Di Catania, Catanese, Niscemese, Siracusano, etc.) as well as in Southern France (Violet de Provence, Violet Margot, Violet du Gapeau, Violet French, etc.) or areas where the influence of French civilization has been quite great over time (Bamafsigi, Violet d'Algerie, Baladi). Also within the cluster, although more genetically differentiated, were the three accessions of Tudela and its selection EB9, whose cultivation is concentrated in the Navarra region of Spain.

Cluster A2 included the Romaneschi types, which are mainly cultivated in central Italy and whose major peculiarity is the large size of the principal and secondary heads (up to 200 g). The cluster also included two accessions of Green globe, a varietal type accounting for more than 85% of the artichoke production in the United States. This varietal type has very big green heads and originates from germplasm introduced from Southern Europe in the 19th century (Tivang et al. 1996).

Branch B contained two additional clusters: B1 and B2, both of which included Spinosi and Violetti types with medium-small heads (about 120–140 g)—although the former has more elongated capitula—suggesting their common origin. Cluster B1 also included all the accessions of Bayrampasa and Sakiz from Turkey, which differentiated at a JSI of 0.70 with the Spinosi types from Sicily (Di Palermo, Spinoso di Sciacca, Spinoso di Palermo and Spinoso di Licata). Analogous results were previously obtained by Sonnante et al. (2002).

On the basis of our data it appears that no clear relationship can be found between capitulum pigmenta-

tion and clustering based on molecular data. According to Pochard et al. (1969) and Foury (1969) pigmentation is controlled by a dominant anthocyanin-producing gene and an additional dominant colour inhibitor. However, Basnizki and Zohary (1994), after crossing pigmented and non-pigmented types, hypothesized that the character involves a series of modifiers in addition to one or two major genes. Further studies confirmed that pigmentation intensity is strongly influenced by environmental conditions, mainly temperature.

Based on the discrimination of eight quantitative characters, Elia and Miccolis (1996) found that harvest time was one of the main parameters for discriminating among germplasm, and they grouped the 104 accessions in their study as: (1) early, (2) medium-early, (3) late with small heads, (4) late-violet and (5) late with large heads. Based on our data the early and medium-early types correspond to the accessions included in cluster A1, while the late types with large heads cluster in A2. However, the accessions they included in the groups named 'late with small heads' and 'late violet' were quite randomly distributed in cluster B1 and B2. Furthermore, we disagree with the classification of varietal types like Spinoso sardo form Sardinia, Spinoso di Palermo, Spinoso di Licata, Spinoso di Sciacca, etc. from Sicily and Spinoso violetto di Liguria from the Liguria region as being late with small heads since these varietal types, as well as Catanesi, start their production in late autumn-early winter when resumption of plant growth in mid-July is carried on through irrigation.

The highest value of genetic differentiation within the same varietal type was found among the seven accessions of Bayrampasa (15%) and the ten of Spinoso sardo (11%). These data are consistent with the high molecular genetic variation we previously found in five populations of Spinoso sardo (Lanteri et al. 2001) and which was attributed to the multiclonal composition of the populations (De Vos 1992) due mainly to the limited selection adopted by farmers, to mutations occurring over time (chromosomal aberrations, aneuploidy, polyploidy etc) which have been selected and vegetatively propagated together with non-variant plants and to adaptation to different environmental conditions. Moreover, analogous results were obtained by Tivang et al. (1996) by analysing RAPD heterogeneity in two breeding populations of Green globe.

The genetic variation detected within the same varietal type was in some cases higher than that found between varietal types. This can be presumably attributed to the multiclonal composition of the populations in cultivation, but might also be due to mutations occurring over time which have been selected and vegetatively propagated together with non-variant plants (Lanteri et al. 2001). The JSI among clones of the same varietal type may be considered to be a threshold value that identifies material sharing the same genetic background. This threshold will enable the selection of representative plants to develop and manage a 'core collection' of *Cynara cardunculus* germplasm and to identify suitable material for future breeding efforts with this species.

The present results show that AFLP markers are useful in evaluating *Cynara cardunculus* genetic diversity and in classifying accessions to phylogenetic groups based on their genetic similarity values. Despite the inability of AFLPs, as dominant markers, to distinguish between the homozygous and heterozygous stage of an individual at a given locus, the good consistency of our AFLP-based dendrogram with the classification based on morphological characters proves that AFLPs can be successfully applied to study genetic relationships even in the highly heterozygous artichoke. Traits selected in cultivated plants may result in confusion for taxonomists and lead to a false sense of genetic distinctness (Kirkbride 1993), however our data suggest that traits selected by man also play an important role in understanding variation and differentiation within cultivated artichoke germplasm.

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