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# Relationships among artichoke cultivars and some related wild taxa based on AFLP markers

G. Sonnante[1]*, A. De Paolis[2] and D. Pignone[1]

[1]*CNR—Istituto di Genetica Vegetale (Institute of Plant Genetics), Via Amendola 165/A, 70126 Bari, Italy and* [2]*CNR—Istituto di Scienze delle Produzioni Alimentari (Institute of Food Science Production), Sez. di Lecce, Str. Prov. Lecce-Monteroni, Lecce, Italy*

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## Abstract

Artichoke, *Cynara cardunculus* var. *scolymus* is a diploid outcrossing species, originated in the Mediterranean basin, which has been much appreciated both for its tasty heads and pharmaceutical properties since ancient times. The species includes two more botanical varieties: *C. cardunculus* var. *altilis*, the cultivated leafy cardoon and *C. cardunculus* var. *sylvestris*, the presumed wild progenitor of artichoke, which are completely interfertile with the cultivated globe artichoke and all together they form the primary gene pool of artichoke. The secondary gene pool includes at least seven wild *Cynara* species. A high level of morphological variation, essentially in head shape and size, is observed in artichoke varieties. Amplified fragment length polymorphism (AFLP) markers were used in order to assess genetic variation and relationships among artichoke varieties and between these and some of their wild relatives. A selected group of wild and cultivated artichoke accessions belonging to different clusters detected on a morphological basis and from various geographical origins was chosen for the analysis. Twenty-four primer combinations were initially tested to evaluate their ability to detect polymorphism between samples. Nine primer combinations were chosen for further analysis on 39 cultivated artichokes, two wild progenitors, one cultivated cardoon, one sample of *C. cornigera*, one of *C. humilis* and two samples of *C. syriaca*. A high level of polymorphism was observed for AFLP markers. The polymorphic bands obtained were scored and used to assess genetic similarity among wild and cultivated accessions and finally to construct a UPGMA dendrogram and principal co-ordinate (PCO) analysis based on Jaccard's similarity index. The artichoke wild progenitor was quite distantly related to the cultigen and occupied a separate branch in the dendrogram. However, wild *C. cardunculus* was more similar to the artichoke than were the other wild species, corroborating the idea that it is the wild progenitor of cultivated artichokes. Within the cultivated artichoke, the dendrogram derived from AFLP analysis produced branches which roughly corresponded to the groups obtained on the basis of morphological and physiological characteristics. The groups were homogeneous enough, except for the 'Romaneschi' types, which proved to be quite genetically variable, and did not cluster in a single branch. This is interpreted on the grounds of the possible selection pathway of this more modern morpho-group.

**Keywords:** AFLP; *Cynara cardunculus* var. *scolymus*; wild cardoon; molecular markers; genetic relationships

* Corresponding author. E-mail: gabriella.sonnante@igv.cnr.it

## Introduction

Globe artichoke (*Cynara cardunculus* L. var. *scolymus* (L.) Fiori) is a diploid ($2n = 2x = 34$) outcrossing species, traditionally cultivated in the Mediterranean Basin and generally propagated vegetatively. The cultivated leafy cardoon, *C. cardunculus* L. var. *altilis* DC, belongs to the same botanical species as the artichoke. The domestication of these crops from their presumed wild progenitor (*C. cardunculus* L. var. *sylvestris* Lam., later on referred to as wild cardoon or wild progenitor) is still unclear, but it is supposed to be relatively recent and to date back to about the 1st century (Foury, 1989). Wild and cultivated cardoons are cross compatible and fully interfertile with the cultivated artichoke and therefore form the primary gene pool of artichoke (Basnizki and Zohary, 1994; Rottenberg and Zohary, 1996). Besides crossability tests, this hypothesis concerning the wild progenitor of cultivated artichoke was also suggested by isozyme markers (Rottenberg *et al.*, 1996) which showed that the wild cardoon was more closely related to the cultivated artichoke than were the other *Cynara* species. Artichoke and its wild progenitor also possess ribosomal genes of the same length (Tucci and Maggini, 1986; Maggini *et al.*, 1988). The other wild *Cynara* species (*C. syriaca* Boiss., *C. cornigera* Lindley, *C. algarbiensis* Cosson, *C. baetica* (Spreng.) Pau and *C. humilis* L.) are reproductively isolated from the crop species and its wild progenitor by strong crossability barriers and therefore are considered to form the secondary gene pool of the artichoke (Rottenberg and Zohary, 1996).

The Institute of Plant Genetics (IGV) in Bari possesses a living collection of artichokes which had been partly analysed by means of multivariate analysis considering 27 traits (Porceddu *et al.*, 1976) based on previously determined descriptors (Dellacecca *et al.*, 1976); the cultivated artichokes were classified into four main groups mostly according to head morphology and production characters: 'Spinosi', 'Violetti', 'Catanesi' and 'Romaneschi'. Relationships among accessions belonging to the same collection were also evaluated on the basis of eight quantitative characters (Miccolis *et al.*, 1989; Elia and Miccolis, 1996); five large groups were identified: 'Early', 'Medium-early', 'Late with small head', 'Late-violet' and 'Late with big head'. Recently, a study on genetic variation in wild and cultivated artichoke based on randomly amplified polymorphic DNA (RAPD) markers evidenced that in a dendrogram, based on Jaccard's similarity, samples were clustered more reflecting their head morphological characters than geographic distribution (Sonnante *et al.*, 2002). However, although a certain level of variation is revealed by RAPDs also among clones (Tivang *et al.*, 1996; Lanteri *et al.*, 2001), more powerful molecular tools are needed to ascertain genetic variation and possibly detect specific markers able to distinguish varieties and ecotypes. This need becomes more substantial when considering aspects of management of the collections: synonymy, that is the case that the same entity takes a different name according to the place where it is cultivated, often engenders much confusion and complicates collection management and study. Finally, it is worth considering that this aspect is particularly important for Italian material, since the number of cultivars or ecotypes present especially in Italy is higher than in other Mediterranean countries (Bianco, 1990).

DNA fingerprinting techniques offer the opportunity to characterize diversity at the genetic level. In particular, the amplified fragment length polymorphism (AFLP) procedure (Zabeau and Vos, 1993; Vos *et al.*, 1995) allows detection of polymorphism at a great number of loci, generating highly reproducible markers without any prior sequence knowledge. The AFLP method is a robust and reproducible technique (Erschadi *et al.*, 2000), which has been successfully employed for fingerprinting varieties, cultivars and clones (e.g. Barrett and Kidwell, 1998). Moreover, AFLPs also proved effective in analysing inter- and intra-specific genetic diversity among species, as illustrated for a wide range of crop species and their wild relatives (e.g. Aggarwal *et al.*, 1999).

The objectives of the present study were to assess genetic variation in a selected set of varieties and/or ecotypes of the IGV artichoke collection, and to estimate genetic relationships between the artichoke and some taxa belonging to its primary and secondary gene pool, using AFLP markers.

## Materials and methods

### *Plant material and DNA extraction*

Thirty-nine cultivated artichoke accessions and one cultivated cardoon were obtained from the living collection of the Institute of Plant Genetics (IGV, CNR), Bari, Italy. Wild *taxa* were kindly provided by Prof. Mauromicale and Dr P. Medagli, Italy; Dr A. Susanna, Spain; and Dr Lev-Yadun, Israel. All the material analysed is listed in Table 1. Portions of young leaves were collected, frozen in liquid $N_2$ and stored at $-80$°C. DNA was extracted from frozen young leaves according to Sonnante *et al.* (2002).

### *AFLP analysis*

The AFLP procedure was carried out as reported by Vos *et al.* (1995) with some modifications. Approximately

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**Table 1.** List of the material analysed

| Variety | Origin | Group[a] |
|---|---|---|
| 1. Gagliardo Sgrò | Messina, Italy | CAT |
| 2. Catanese | Catania, Italy | CAT |
| 3. Niscemese | Catania, Italy | CAT |
| 4. Locale di Mola | Bari, Italy | CAT |
| 5. Di Ogni Mese | Pisa, Italy | CAT |
| 6. Masedu | Sassari, Italy | CAT |
| 7. Precoce Violetto di Chioggia | Padova, Italy | VIO |
| 8. Moretto | La Spezia, Italy | VIO |
| 9. Violetto di Toscana | Pistoia, Italy | VIO |
| 10. Testa di Ferro | Pistoia, Italy | |
| 11. Violetto di Maremma | Grosseto, Italy | VIO |
| 12. Mazzaferrata | Siena, Italy | |
| 13. Verde di Pesaro | Pesaro, Italy | |
| 14. Locale Tolentino | Tolentino, Italy | ROM |
| 15. Violet de Camargue | France | ROMt |
| 16. Spinoso Violetto di Liguria | Imperia, Italy | SPI |
| 17. Spinoso Sardo | Sassari, Italy | SPI |
| 18. Spinoso di Sciacca | Agrigento, Italy | SPI |
| 19. Spinoso di Gela | Gela, Italy | SPI |
| 20. Spinoso di Palermo | Messina, Italy | SPI |
| 21. Spinoso 2 | Cagliari, Italy | |
| 22. Sakiz | Turkey | |
| 23. Bayrampasa | Turkey | ROMt |
| 24. Mazzaferrata | Pescara, Italy | ROM |
| 25. Castellammare | Roma, Italy | ROM |
| 26. Romanesco | Latina, Italy | ROM |
| 27. Campagnano | Napoli, Italy | ROM |
| 28. Empolese | Arezzo, Italy | ROMt |
| 29. Violetto di Putignano | Bari, Italy | |
| 30. Verde di Putignano | Bari, Italy | |
| 31. Macau | France | ROMt |
| 32. Violetto di Provenza | Sassari, Italy | CAT |
| 33. Escarot | France | CAT |
| 34. Hyerois | Egypt | CAT |
| 35. Banafsigi | Egypt | CAT |
| 36. Baladi | Egypt | CAT |
| 37. Blanco | Argentina | |
| 38. Ñato | Argentina | ROMt |
| 39. Green Globe Thornless | California, USA | ROMt |
| 40. *C. cardunculus* var. *altilis* | Bari, Italy | |
| 41. *C. cardunculus* var. *sylvestris* 1 WILD | Apulia, Italy | |
| 42. *C. cardunculus* var. *sylvestris* 2 WILD | Sicily, Italy | |
| 43. *C. cornigera* WILD | Egypt | |
| 44. *C. humilis* WILD | Spain | |
| 45. *C. syriaca* 1 WILD | Israel | |
| 46. *C. syriaca* 2 WILD | Israel | |

[a] For each accession, the first abbreviation refers to belonging to the groups as in Porceddu *et al.* (1976), precisely: CAT, Catanese; VIO, Violetto; SPI, Spinoso; ROM, Romanesco. ROMt is similar to a 'Romanesco', but not classified by Porceddu *et al.* (1976).

500 ng of DNA were digested simultaneously with EcoRI and MseI and ligated to adaptor pairs at room temperature overnight. The reaction was diluted in 0.1×TE, in order to obtain the appropriate concentration for subsequent polymerase chain reaction (PCR). Four microlitres of the diluted DNA solution were pre-amplified using EcoRI + A and MseI + C preselective primers. PCR was performed in a 9700 Thermal Cycler (Applied Biosystems) programmed to carry out 20 cycles at: 94°C for 20 s, 56°C for 30 s and 72°C for 2 min. Pre-amplified DNA was partially run on a 1.5% agarose gel in 1×TBE buffer in order to check the pre-amplification pattern. The remaining pre-selective product was diluted (1:10) in 0.1×TE for selective amplification. Twenty-four

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primer combinations were initially tested and, finally, the following nine primer combinations from the Applied Biosystems commercial AFLP kit were used for the selective amplification in all samples: E-ACT + M-CTG; E-ACG + M-CTG; E-ACT + M-CTA; E-ACG + M-CTT; E-ACT + M-CAG; E-ACT + M-CTC; E-ACG + M-CTA; E-ACG + M-CTC; E-ACC + M-CTA. PCR temperature cycles were according to the instruction protocol (Applied Biosystems). EcoRI primers used in the selective amplification were fluorescence labelled. The amplification products of each three differently labelled selective amplifications were pooled and electrophoresed on a Perkin-Elmer ABI 310 automatic sequencer together with a size standard (Genescan-500 Rox, Applied Biosystems).

### *Data analysis*

AFLP data were manually scored for the presence (1) or absence (0) of peaks. These data were used to assess genetic relationships based on Jaccard's (1908) similarity index. The similarity matrix was used to construct a UPGMA dendrogram and to derive co-ordinates from the principal co-ordinate (PCO) analysis with the NTSYS-pc package (Rohlf, 2000).

## Results

An initial test using 24 primer combinations with the variety 'Locale di Mola' allowed a choice of nine primer combinations. The number of peaks produced was variable depending on the primer combination used. Some peaks were not considered because of their low height (below 70) or because they were too close to each other (less than 1 s elution). The size of AFLP fragments was in a range from about 40 to about 500 bp, but only fragments between 55 and 380 bp were taken into account in order to avoid scoring problems arising from an excess of primer peaks near the front of the electrophoresed fragments and a decreasing signal for fragments longer than 400 bp. The nine primer combinations selected allowed a total of 758 peaks to be scored; of these, 98 were present only in the cultivated artichoke, whereas 146 were present only in the other taxa. When all samples were considered, 613 fragments (80.9%) were revealed to be polymorphic; when only the cultivated artichoke was taken into account, the number of polymorphic fragments was 513 (83.8% over the total of fragments present in the crop).

The analysis of relationships based on Jaccard's index showed that similarity varied between 0.290 and 0.947. The lowest similarity was observed between *C. cornigera* and cultivated artichoke, whereas the highest similarity value was detected between two cultivated artichokes belonging to the 'Spinosi' group. The minimum similarity value within the cultivated artichoke group was 0.540 between 'Verde di Putignano' and 'Locale di Mola'. In Table 2 the average similarity between taxonomic groups is reported. The highest average similarities were observed within the primary gene pool of artichoke: 0.537 between artichoke and cultivated cardoon, 0.505 between artichoke and wild cardoon, 0.560 between wild and cultivated cardoon. The other wild species were more distantly related to the artichoke and showed an average similarity of 0.311, 0.336 and 0.345 for *C. cornigera*, *C. humilis* and *C. syriaca*, respectively.

The UPGMA dendogram (Fig. 1) based on Jaccard's similarity indicates that the wild species other than *C. cardunculus* are grouped in a basal, separate branch. The other cluster is divided into two main sub-branches at a node of 0.515: one sub-branch contains the cultivated and the two wild cardoons; the other sub-branch includes all the cultivated artichokes in which two main groups can be identified, apart from 'Verde di Putignano' (conic, medium-sized heads with light green external bracts; medium–late production), which stands isolated. The upper cluster includes accessions belonging to the 'Catanesi group', and contains only two varieties which could not be considered typical 'Catanesi': 'Testa di Ferro' and 'Violetto di Putignano', late varieties with an ovoid head of medium size, the former, and of large size, the latter. In the other bigger cluster some subclusters can be identified. The upper one contains small groups including the 'Violetti' or the 'Spinosi' types,

**Table 2.** Mean similarity between taxonomic groups

| Taxon | (a) | (b) | (c) | (d) | (e) |
|---|---|---|---|---|---|
| (a) *C. cardunculus* var. *scolymus* | – | | | | |
| (b) *C. cardunculus* var. *altilis* | 0.537 | – | | | |
| (c) *C. cardunculus* var. *sylvestris* | 0.505 | 0.560 | – | | |
| (d) *C. cornigera* | 0.311 | 0.336 | 0.337 | – | |
| (e) *C. humilis* | 0.336 | 0.349 | 0.360 | 0.355 | – |
| (f) *C. syriaca* | 0.345 | 0.385 | 0.394 | 0.353 | 0.375 |

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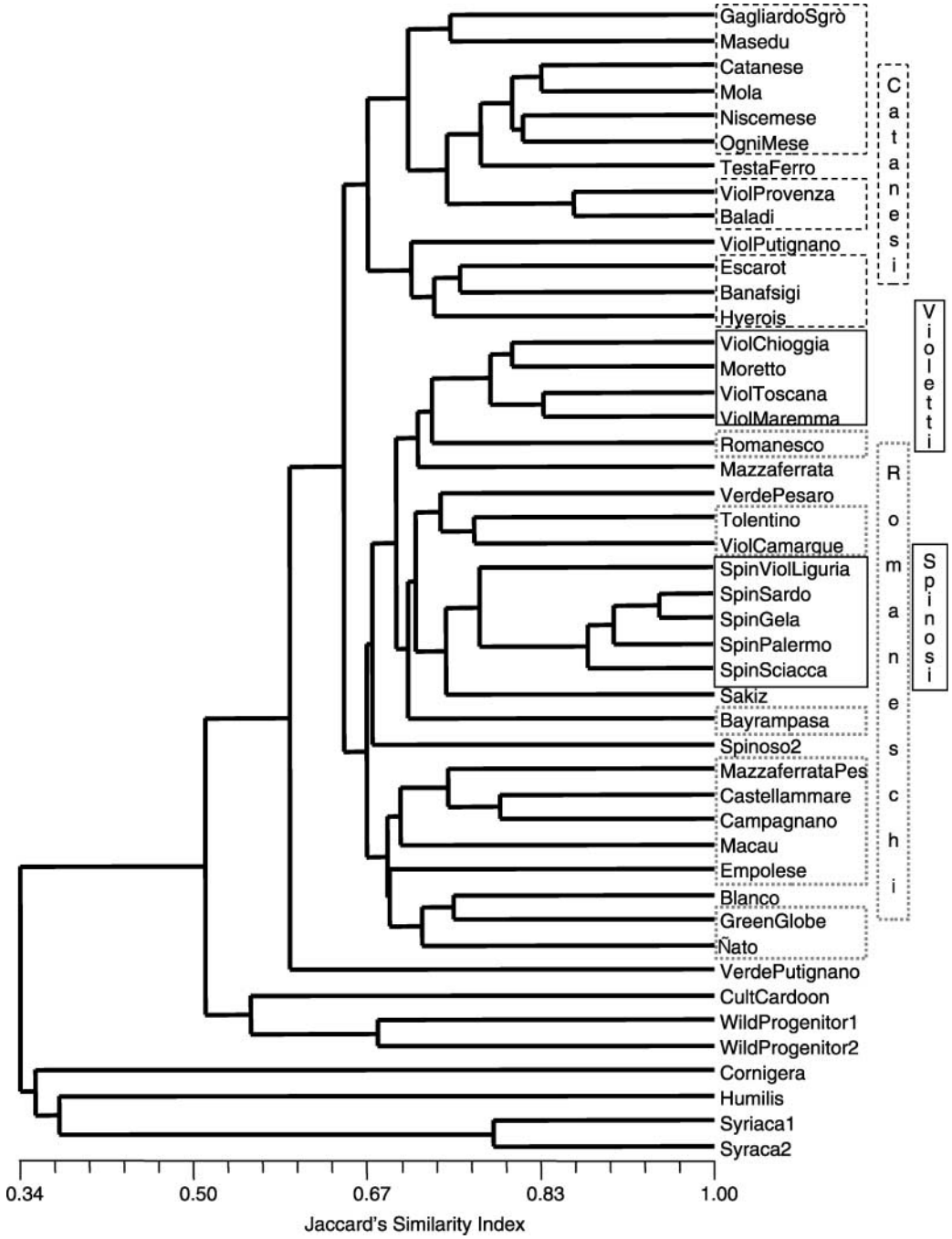


**Fig. 1.** UPGMA dendrogram based on Jaccard's similarity index. Names are as in Table 1. Varieties are framed with the same style as the group to which they belong.

respectively. The lower subcluster includes all artichokes with a big and global head and that can be considered as belonging to the Romaneschi group. In between these latter three groups, other 'Romaneschi' ('Romanesco', 'Tolentino', 'Violet de Camargue', 'Bayrampasa') and different varieties ('Mazzaferrata di Toscana', late, with a big, elongated spherical head; 'Verde di Pesaro', late with a cylindrical, medium-sized head; 'Sakiz', early, with a cylindrical medium-sized head) are distributed.

PCO analysis based on the similarity matrix previously calculated showed a similar trend (Fig. 2). In fact, the wild *Cynara* species occupied a distinct domain in the plot of principal co-ordinate 1 versus 2; the cultivated cardoon was in an intermediate position between the artichokes and the wild progenitor. Finally, the cultivated artichokes occupied a large domain: the groups were quite homogeneous, except for the 'Romaneschi' which were more spread in the plot.

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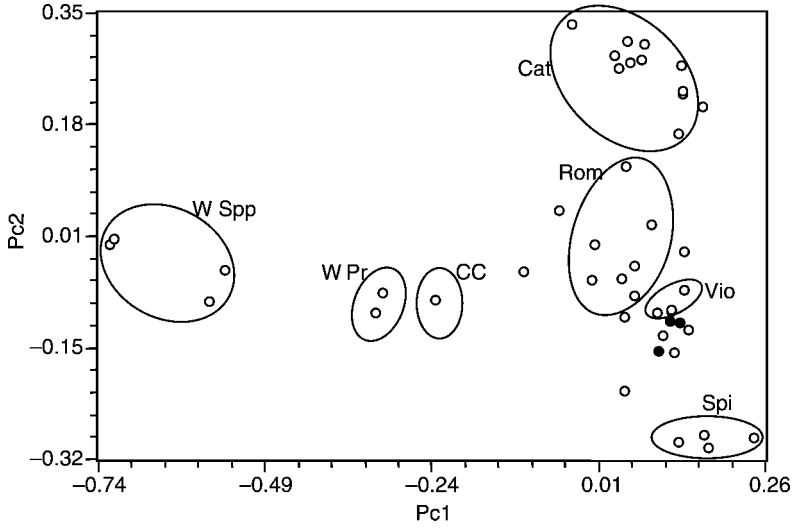


**Fig. 2.** Scatterplot of PCO analysis of artichoke cultivars and the other taxa analysed, using the AFLP data. Pc1, Principal component 1; Pc2, principal component 2; Spi, 'Spinosi types'; Vio, 'Violetti types'; Cat, 'Catanesi types'; Rom, 'Romaneschi types'; solid black circles, other 'Romaneschi'; CC, cultivated cardoon; W Pr, wild progenitor; W Spp, other wild species.

## Discussion

The wild cardoon, *C. cardunculus* var. *sylvestris*, is considered the wild progenitor of the cultigen composed by the leafy cardoon and artichoke crops. In fact, several pieces of evidence support this hypothesis. First of all, the wild cardoon is fully interfertile with the two crops, and studies based on isozyme markers (Rottenberg *et al.*, 1996) have demonstrated that the cultivated artichokes and wild cardoon are closer to each other than to other wild *Cynara* species. Considering these data and also morphological characteristics, the two crops and the wild cardoon were included in a single species and they are now considered varieties of *C. cardunculus*. RAPD markers clearly separated the cultivated artichoke from the leafy cardoon and from the wild cardoon (Sonnante *et al.*, 2002), however, in that study, no other wild *Cynara* species were included. Using AFLP, we detected a closer similarity of wild cardoon to the two crop types than to the other wild species analysed. This is, therefore, further evidence that the wild cardoon is in the lineage of the cultivated cardoon and artichoke.

According to some morphological traits and geographical distribution, Wiklund (1992) recognized two subspecies of wild cardoons: *C. cardunculus* subsp. *cardunculus* and *C. cardunculus* subsp. *flavescens*. They differ for a few morphological traits and have a different distribution in the Mediterranean Basin, the former being diffused in the eastern area, while the second being present in the western region; the two ranges get in contact in southern Italy and Sicily. According to this author, the western-distributed wild cardoons could be the progenitor of the cultivated forms because they share a yellowish margin on the middle involucral bracts, which is not present in the eastern-distributed cardoons. In our analysis we only included two wild cardoon samples from Italy and could not compare to wild western cardoons; for this reason, it is not possible to determine whether one of the two subspecies recognized by Wiklund is genetically closer to the crops compared to the other variety on the basis of AFLP markers.

Cultivated leafy cardoon is grown on a minor scale for its succulent young leaves (Dellacecca, 1990); as stated before, it belongs to the primary gene pool of artichoke and there is a continuous variation between the two crops and the wild cardoon. Sometimes also artichoke leaves are eaten as for cultivated cardoon and heads from wild cardoon are also cooked and eaten and are considered more tasty than the cultivated artichoke (Caneva *et al.*, 1997). The two cultivated forms represent the result of the different selection pressures unconsciously operated by humans for large, non-spiny heads on one side and non-spiny, tender leaves on the other side (Basnizki and Zohary, 1994). Analysing the frequency distribution of the considered characters among the two cultivated forms, it appears that concurrent directional selection was operated for distinct traits, while disruptive selection appears less probable in this case (Solbrig and Solbrig, 1979).

Some authors (Zohary and Basnizky, 1975) hypothesized that *C. syriaca* also contributed to the genetic make-up of the cultivated artichoke. Further studies, however, do not support this hypothesis: crossability studies (Rottenberg and Zohary, 1996) demonstrated that *C. syriaca* belongs to the secondary gene pool of artichoke; relationships based on isozyme analysis

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(Rottenberg *et al*., 1996) showed that the taxon closest to cultivated artichoke was *C. cardunculus* subsp. *cardunculus* (actually corresponding to our *C. cardunculus* var. *sylvestris*) and that *C. syriaca* was more distantly related, as were the other wild *Cynara* species. Also according to our results based on AFLP data, the two samples of *C. syriaca* from Israel displayed a similarity to the cultivated artichoke lower than that observed for the presumed wild progenitor, and similar to that observed for the other wild *Cynara* taxa, i.e. *C. cornigera* and *C. humilis*. Therefore, these data corroborate the hypothesis that *C. cardunculus* var. *sylvestris* is the wild ancestor of the cultivated artichoke, while the other *Cynara* wild species used in this work are more distantly related and thus their belonging to the secondary gene pool of artichoke is confirmed. We only used one or two samples per wild species. This choice was dictated by the unavailability of other samples. Despite this, due to their relatively low similarity to the cultigen, and to each other, this limitation has probably no influence on the pattern of genetic relationships here described. Anyhow, a larger availability of samples of wild species could shed a better light on understanding the real web of relationships within the genus *Cynara*.

The cultivated artichokes can be classified into four main groups based on morpho-productive traits (Porceddu *et al*., 1976). The 'Spinosi' group includes varieties with long thorns on bracts and leaves; the 'Violetti' group comprises types with violet-coloured heads harvested in the early springtime in the Mediterranean area; the 'Romaneschi' group includes the big, spherical-headed varieties harvested late in the spring; the 'Catanesi' group is characterized by early varieties with elongated heads harvested for a longer period, from the late autumn to the spring. Some types cannot be included in any of these groups, since they show intermediate or different characters.

According to the UPGMA tree based on Jaccard's similarity, the artichokes belonging to the 'Catanesi' group were clustered together showing that this represents a homogeneous group, containing only two varieties which could not be considered typical 'Catanesi'. Within the other big cluster of the cultivated artichokes, the 'Violetti' and 'Spinosi' are in separate small clusters and most of the 'Romaneschi' types form a big independent cluster. Therefore, even with some exceptions, the groups observed on a morpho-productive basis are quite homogeneous at the molecular level using AFLP markers. Also PCO analysis corroborates this view, as the distribution of the samples in the scatter plot of the first two principal components reveals. Comparing with the results obtained using RAPDs (Sonnante *et al*., 2002), there is quite good correspondence of the groups of the dendrogram. Of course, in that paper a much lower number of polymorphic fragments was detected and therefore higher values of similarity were observed.

The 'Romaneschi' group is more spread in the dendrogram and also in the PCO scatter plot. This can be explained by the fact that we used a higher number of samples for this group and also that, even though the 'Romaneschi' types used in this analysis have all the characteristics of the group (late production and big, spherical head), differences in the head morphology, plant habit, leaf colour, etc. can be observed and therefore can determine a high variation at the molecular level. Moreover, notwithstanding the use of a very large number of AFLP fragments in this study, it should be considered that these markers are often unevenly spaced on genetic maps and tend to form clusters (Alonso Blanco *et al*., 1998; Winter *et al*., 2000). Another point concerns the biased distribution of AFLP fragments. It has been noted in *Arabidopsis thaliana* (Miyashita *et al*., 1999) and *Vicia sativa* (Potokina *et al*., 2002) that there is a large fraction of fragments present only in one or a few samples, while a smaller amount of fragments are present in nearly all of the plants. We have found several of these cases and this can be an indication of a high mutation rate and low information content of a fraction of AFLP fragments. These rare polymorphisms can produce noise when analysing samples for similarity and can therefore determine a certain rearrangement of the groups. Finally, one has also to consider that the 'Romaneschi' are a more evolved type and therefore it cannot be excluded that they are the result of different unconscious breeding lines which led to plants with similar morphological characteristics but different genetic background.

The accepted evolutive line for artichoke is wild cardoon–'Spinosi'–'Violetti'–'Catanesi'–'Romaneschi', since less spiny and bigger capitula were progressively selected by humans (Foury, 1989; Bianco, 1990). Within this frame it is also interesting to observe that the highest variation for cultivated artichoke is found in Italy (Bianco, 1990) and that Italy is the country where there is the highest concentration of 'Spinosi' types, that is the most primitive group with spiny leaves and bracts. Therefore it could be hypothesized that Italy, and in particular southern Italy and its major islands, Sicily primarily, could be the place were the artichoke was first domesticated. This hypothesis is further strengthened by our own observation that still nowadays it is a customary habit in some regions of southern Italy (Calabria, Sicily, Basilicata, etc.) to collect young heads of wild cardoons in spring to make traditional dishes (Pignone and Sonnante, in press). Further evidence to this working hypothesis can derive from more complete and meticulous sampling of wild cardoons in the south of Italy.

It is interesting to notice that the usefulness of AFLP markers in identifying varieties or groups of varieties

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depends on the morpho-group considered. In fact, in some groups, such as the 'Spinosi' or the 'Catanesi', it is possible to identify specific markers that are generally indicative of the morpho-group, while in the 'Romaneschi' type this assumption is not possible. Once more it could depend on the domestication level of the morpho-group considered; nevertheless it cannot be excluded that by increasing the number of AFLP or other markers used, one could identify some markers able to identify the more variable morpho-groups.

In conclusion, AFLP markers are highly variable in artichoke and therefore could have several interesting applications, such as the resolution of synonymy in germplasm collections, variety identification, the constitution of integrated genetic maps, etc. Further markers, e.g. microsatellite sequences, have proved to be of help in these tasks (Sonnante *et al*., 2003) as well as in clarifying phyletic relationships among wild and cultivated taxa or elucidating the domestication pattern of the crops. However, to meet these needs better, it is also necessary to increase the availability of germplasm, especially of wild cardoons and wild *Cynara* species.

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