Genetic diversity of globe artichoke landraces from Sicilian small-holdings: implications for evolution and domestication of the species

UNIVERSITÀ DEGLI STUDI DI TORINO

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Abstract. Globe artichoke (*Cynara cardunculus* var. *scolymus*) is native to the Mediterranean Basin, where it grows in close proximity with its ancestor wild cardoon (*C. cardunculus* var. *sylvestris*); its commercial production is mainly based on vegetatively propagated clones which guarantee high yields of marketable product (i.e. immature inflorescence or capitula). A collection of 24 landraces of globe artichoke was made from small-holdings in Sicily, which is assumed to be one of the possible centres of its domestication. These landraces have been cultivated for centuries by local farmers, mainly due to their culinary uniqueness. The collection was characterised for a combination of morphological traits and AFLP, gSSR and cpSSr markers. Molecular analyses included genotypes of wild cardoon collected from different sites in Sicily as well as accessions of the most widely grown Sicilian varietal types: the spiny 'Spinoso di Palermo' and the non-spiny 'Violetto di Sicilia'. The landraces follow a gradient of 'ennoblement' towards either the domesticated spiny or the non-spiny types. 'Cimiciusa di Mazzarino' was an outlier, in that it resembled the cultivated forms with respect to its AFLP fingerprint, but was more closely related to the wild cardoon on the basis of SSR profile. This particular landrace presents an example of an intermediary form in the domestication process, although it could also have derived from introgression from sympatric wild cardoon, followed by farmer selection. The abundant genetic variation present demonstrates the key role of farmers' practice in the maintenance of genetic diversity, which should be preserved because of its potential value for plant breeders.

Key words: *Cynara cardunculus* L., Germplasm, Genetic variability, AFLP, SSR

Introduction

The genus *Cynara* is native to the Mediterranean region, sharing its distribution with the olive (*Olea europea*). The members of the genus are the *C. cardunculus* L. species complex, consisting of the globe artichoke [var. *scolymus* (L.) Fiori], the cultivated cardoon (var. *altilis* DC.) and the wild cardoon [var. *sylvestris* (Lamk) Fiori], and other six wild species: *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) (Wiklund 1992; Rottenberg and Zohary 1996; 2005). The three *C. cardunculus* forms are fully cross-compatible with one another, and form fertile inter-varietal hybrids (Basnizki and Zohary, 1994). However, reproductive barriers separate the *C. cardunculus* complex from the other *Cynara* species (Rottenberg et al., 1996). The crosses between *C. cardunculus* and any of the other species *C. syriaca*, *C. algarbiensis*, *C. baetica* or *C. humilis* do all produce few seeds, although the hybrids are generally sterile. These four wild *Cynara* species are therefore regarded as members of the secondary wild gene pool of globe artichoke and cardoon (Rottenberg and Zohary, 2005). On both morphological (Wiklund, 1992) and cytogenetic (Rottenberg et al., 1996) grounds, the closest of these species to the cultivated complex is *C. syriaca*. The monophyly and evolution of the *Cynara* spp. have been investigated by sequence comparisons between various ITS (internal transcribed spacer) and ETS (external transcribed spacer) regions (Robba et al., 2005; Sonnante et al., 2007), leading to the suggestion that the *cardunculus* complex is more differentiated and evolved than the other wild species.

In southern Europe, globe artichoke production contributes significantly to regional economic stability, with Italy the leading global producer (50,000 ha, 470,000 t mean annual production) (ISTAT data 2007: http://www.istat.it/). Some cultivation also occurs in the Middle East, North Africa, South America and the United States, and of recent years, its

popularity has also increased in China (FAO data 2006: http://faostat.fao.org/). The prime globe artichoke product consists of the immature inflorescence (capitulum), which can be consumed in fresh, canned or frozen form, and is an ingredient of many traditional Mediterranean dishes. Each plant produces a number of capitula, the largest of which (the main capitulum) emerges from the apex of the central stem, while the smaller ones are produced on lateral branches. Renewed interest in the crop has been sparked by the presence of a series of alternative products, including inulin from the roots (Lattanzio et al., 2001), various biopharmaceuticals from the leaves (Gebhardt, 1997; Brown and Rice-Evans, 1998; Perez-Garcia et al., 2000; Wang et al., 2003), oil from the seeds (achenes) and feed from the residual flour (Maccarone et al., 1999), fresh biomass for forage (Montemurro and Cianci, 1976), and dry biomass for paper pulp or biofuel (Foti et al., 1999; Gominho et al., 2001).

Most commercial production of globe artichoke is based on the cultivation of perennial, vegetatively propagated clones (Mauromicale, 1987). The total number of genetically distinct clones under cultivation is difficult to determine with accuracy, but only 11-12 are considered to be of any major commercial importance (Basnizki and Zohary, 1994). These have been discriminated from one another on the basis of either capitulum morphology (shape, size, presence/absence of spines: Dellacecca et al., 1976; Porceddu et al., 1976; Vanella et al., 1981) and/or harvest time (Mauromicale and Ierna, 2000). Traditionally, Sicilian farmers use small plots to cultivate populations of landraces, which typically yield less than commercial varieties, but are well suited for specific end-uses, are tolerant of environmental stress, and are adapted to a low input farming system. The most popular Sicilian varietal types (‘Spinoso di Palermo’ and ‘Violetto di Sicilia’) are thought to have emerged from such small-holdings (Nicolosi Gallo, 1880; Viani, 1929). Of late, some effort has also been directed at the breeding of seed-propagated cultivars, and these are steadily gaining in popularity.

Our goal was to collect globe artichoke landraces from small-holdings, and to assess the

genetic variation present in these materials, at both the molecular and morphological level. Molecular fingerprinting was carried out by applying both the multi-locus AFLP (amplified fragment length polymorphism) and the single-locus microsatellite (SSR- simple sequence repeats) markers, in order to provide more robust and reliable data than those based on a single technique. Furthermore, the complementation of molecular and morphological (biometric) data strengthen our understanding of evolution and domestication of the crop.

Materials and Methods

Plant materials and research site

During the early summers of 2004 and 2005, 24 globe artichoke accessions were collected from Sicilian small-holdings located at sites varying in altitude from 12 to 1,000 m a.s.l. (listed in Table 1). For most of these sites, there was no intensive cultivation of artichoke (Fig. 1). Semi-dormant offshoots (‘ovoli’) were sampled, and these were transplanted into an experimental field near Siracusa (37°03’N, 15°18’E, 15 m a.s.l.), where the climate is typically Mediterranean (mild winters and hot dry summers) and the soil characteristics were: 35% sand, 25% silt, 30% clay, pH 8.4, 2.0% organic matter, 1.8 g Kg^{-1} total N, 78 mg Kg^{-1} available P, 337 Kg ha^{-1} exchangeable K. At least 20 plants per each accession were grown. The “ovoli” were planted 0.80 m apart within each row, with an inter-row spacing of 1.25 m, to give an overall density of 1 plant m^{-2}. The material was arranged in a randomised block design with four replications, with each experimental unit consisting of five plants. Crop management practices (fertilization, irrigation, weed and pest management) were performed as per local practice.

Morphological characterization

Each globe artichoke accession, along with three wild cardoon accessions (‘Naro’, ‘Kamaryna’ and ‘Marsala’) for reference was assessed over two seasons. The following traits were determined: fresh weight (FW) of the main and the first order capitulum, the ratio between longitudinal and transverse diameter of the capitulum (L/D), ratio weight receptacle/capitulum in percentage (RW), total yield = FW of all capitula (Y), the number of

days to first harvest (DFH), the number of first order capitula per plant (N° P^{-1}), and percentage of Y contributed by the first order capitula (%Y)

DNA extraction and genotyping

One young leaf was collected from each of three plants per accession, and pooled to obtain DNA, extracted following Lanteri et al. (2001). DNA standards were provided by 14 globe artichoke samples (six from the variety 'Spinoso di Palermo' and eight from 'Violetto di Sicilia') and four wild cardoon samples ('Roccella', 'Palazzolo', 'Bronte' and 'Piano Tavola'). The new accession and the reference DNAs were subjected to a combination of microsatellite and AFLP assays. The genomic microsatellite (gSSR) assays consisted of the four globe artichoke primer pairs CDAT-01, CELMS-09, CELMS-14 and CELMS-40 (Portis et al., 2005a, Acquadro et al., 2007). Amplification and detection were performed following Acquadro et al. (2003). Chloroplast microsatellite (cpSSR) assays were performed with the eight primer pairs CCMP-7, NTCP-7, NTCP-9, NTCP-18, NTCP-30, NTCP-40, (Wills et al. 2005), trnT-trnL, psbC-trnS (Nielsen et al. 2003). The PCR conditions applied for these assays were as for the gSSR assays. The NTCP-7 and NTCP-40 amplicons from 12 templates (three individuals of 'Violetto di Sicilia', three of 'Spinoso di Palermo', three of 'Cimiciusa di Mazzarino', and one each of the wild cardoons 'Naro', 'Kamaryna' and 'Marsala') were purified and sequenced (BMR-Genomics, Padua, Italy). Sequence similarity was analysed with the ClustalW algorithm. (http://www.ebi.ac.uk). All sequences have been deposited in the NCBI database (accessions EU431090 through EU431113). AFLP assays used a protocol adapted from Vos et al. (1995), as described by Lanteri et al. (2003). On the basis of previous experiments (Lanteri et al. 2004a, 2004b, Portis et al. 2005b, 2005c), the AFLP primer combinations (PCs) selected were E+ACA/M+CAT, E+ACG/M+CAA, E+ACT/M+CAA,

E+ACT/M+CAT, E+ACT/M+CTT. Amplicons were electrophoresed on a LI-COR Gene ReadIR 4200 in 6.5% polyacrylamide gels, as described by Jackson and Matthews (2000).

Data analysis

Morphological data were first tested for homoscedasticity using Bartlett's test, then subjected to ANOVA (Snedecor and Cochran, 1989). Means were separated using Tukey's HSD test, with a minimum level of acceptance of $P \leq 0.05$. Percentage data were Bliss-transformed before ANOVA. The coefficient of variation (CV%) and Pearson's correlation coefficient between parameters were calculated by standard methods (Camussi et al., 1995). Z-transformation was applied to mean values of the quantitative traits to meet the requirements of independence and normal distribution with a zero mean (Sneath and Sokal, 1973). Standardized trait values were subjected to principal component analysis (PCA) to determine which traits were the most effective in discriminating between accessions. The first two components explaining the maximum variance were selected for the ordination analysis, and the correlation between the original traits and the respective principal component was calculated. Characters with a correlation >0.6 were considered as relevant for that component (Matus et al., 1996). All calculations and analyses were made using the appropriate options within SPSS version 12.0 (Apache Software Foundation, Chicago, IL) software.

SSR and AFLP data were used to determine the polymorphic information content (PIC), calculated by equating the expected heterozygosity to 2f (1-f) [where f is the percentage of plants carrying a particular marker - Anderson et al. (1993)]. Each amplified fragment (60-650bp) was assumed to represent a single bi-allelic locus, so that fingerprints were scored as the presence (1) or absence (0) of each polymorphic band. A marker index (MI) was calculated for the AFLP data, by multiplying the PIC by the effective multiplex ratio

(EMR), following Powell et al. (1996). The EMR of each PC was defined as ßn, where ß is the percentage of polymorphic fragments and n the number of fragments detected (Milbourne et al., 1997). The two binary matrices were imported into NTSYS-pc software (Rohlf 1993) for cluster analysis. Genetic similarities (Nei and Li, 1979; Jaccard, 1908) were calculated for the SSR and AFLP data, and used to construct UPGMA-based dendrograms. Co-phenetic matrices were produced using hierarchical clustering, and these were correlated with the raw distance matrices for SSR and AFLP data, in order to search for any association between the clustering and the similarity matrices. The strength of each dendrogram node was tested by bootstrapping, using the NEIGHBOR and CONSENSE subroutines within the PHYLIP 3.5 package (Felsenstein, 1993; http://evolution.genetics.washington.edu/phylip.html).

Results

Plant morphology

The ecotypes varied widely for the morphological traits (Table 1). Mean capitulum FW was a highly discriminant character. 'Belpasso' and 'Quartarella' produced the heaviest capitula, both main (414.2 and 429.0 g) and first order (210.5 and 215.7 g). The three accessions of 'Cimiciusa di Mazzarino' produced the smallest main (152.7, 142.1 and 153.5 g) and first order (125.8, 109.1 and 128.0 g) capitula. A significant correlation linked the FW of the first order heads and Y ($r = 0.28 \pm 0.11$*). The accessions also differed widely with respect to both the L/D ratio and WI % (Table 1). There was a predominance of late-flowering accessions (mean DFH 227 d), although some early ('Caltagirone' and 'Spinoso di Sciara' - mean DFH 133 d), and medium-late producing ('Bellocozzo', 'Modica Mauto', 'S. Giacomo' 1 and 3 and 'Vizzini' - mean DFH 188 d) types were also present. A strong positive correlation between DFH and Y was noted ($r = 0.32 \pm 0.11$**). Y varied from 1,340 ('Femminello di Marsala') to 681g plant^{-1} ('S. Giacomo' 1). Y was strongly correlated with N° P^{-1} ($r = 0.60 \pm 0.10$***). %Y was a stable trait (CV% = 12.6), varying from 41 ('Spinoso di Sciara') to 68 ('S. Giacomo' 2).

Microsatellite genotype

All the primer pairs generated robust amplification profiles, consisting of either one or two alleles, as expected from a diploid template. The four gSSR primers detected 32 alleles across the 45 accessions (7 to 9 alleles/locus) (Table 2). Of these, 19 (4-6 per locus) were represented among the globe artichokes and 20 among the wild cardoons (range 3-7). Twelve taxon-specific alleles were noted for globe artichoke and 13 for wild cardoon, with seven shared

between the two taxa. Of the latter, three (CDAT-01, CELMS-09 and CELMS-14) were only present in globe artichoke in ‘Cimiciusa di Mazzarino’. The PIC values ranged from 0.611 to 0.818 (mean 0.676). The genomic SSR set recognised 20 genotypes, consisting of 13 globe artichokes and all seven wild cardoons (Table 2). The eight cpSSR primers produced only monomorphic profiles. No sequence variation was observed in the 192bp of the NTCP-7 or the 161bp of the NTCP-40 amplicons.

AFLP genotype

A total of 121 polymorphic fragments (34.5% of the total number visible on the gels), ranging in size from 40 to 850bp, were scored. The average number of polymorphic fragments per PC was 24.2 (range 20 - 30). In globe artichoke, 100 of these fragments were polymorphic (range 14-18 per PC) and 68 were taxon-specific; while in wild cardoon only 39 varied (range 5-13) and 19 were taxon-specific (Table 3). Of the 34 shared fragments, seven were restricted among the globe artichokes to ‘Cimiciusa di Mazzarino’ (Table 3). PIC values ranged from 0.231 to 0.275 (mean 0.247), and MI from 4.61 to 7.36 (mean 5.99). The E+ACT/M+CTT PC produced the highest PIC, and E+ACT/M+CAT the highest MI. The lowest PIC and MI were obtained with E+ACG/M+CAA. The number of distinct profiles generated by a single PC varied from 22 to 35, and all the 45 genotypes could be discriminated from one another on the basis of a combined AFLP profile (Table 3).

PCA and genetic relatedness

The first two principal components gave eigenvalues greater than 1, and together accounted for more than 70% of the total variance (Table 4). The first component, which explained

51.2% of the total variance, was positively and strongly correlated with FW of either the main or the first order capitula, and to a lesser extent with Y and %Y. On the other hand, the first principal component was negatively correlated with L/D. The second component was positively correlated with $N°P^{-1}$ and DFH (Table 4). The PCA centroids in the first two principal coordinate dimensions are illustrated in Figure 2. The first axis efficiently separates the landraces from the wild cardoon accessions, mainly on the basis of productivity (Y and FW); among the globe artichokes, 'Quartarella' (17) and 'Belpasso' (2) (see Fig. 2), which produce the highest FW, appear as outliers. The second axis separates the late-flowering landraces bearing many first order capitula, landraces 'Femminello di Marsala' (11), 'Cimiciusa di Mazzarino' (6, 7 and 8) and 'Domestica di Castelvetrano' (9). from the early-flowering accessions yielding few first order capitula.

The co-phenetic correlation coefficient (r-value) between the gSSR-based data dendrogram (Fig. 3a) and the similarity matrix clustering was 0.95, and thus there is an excellent fit between these two representations of the genetic relationships between accessions. Three major clades were distinguished, in which the seven wild cardoons and the three 'Cimiciusa di Mazzarino' accessions cluster together with a bootstrap probability of 94% (Fig. 3a, cluster C), while showing a mean genetic similarity of only ~10% with the other accessions. The remaining globe artichoke accessions can be separated into two main clades (with bootstrap probabilities higher than 91%). The first ("A") includes the spiny types, and the second ("B") the non-spiny types. 'Spinoso di Palermo' and 'Spinoso di Sciara' were not distinguishable from one another within "A", analogously, a unique fingerprint was obtained for the 'Violetto di Sicilia' and ten of the landraces within "B". The co-phenetic correlation coefficient (r-value) between the AFLP-based data dendrogram (Fig. 3b) and the similarity matrix clustering was 0.91, again demonstrating a good fit between the dendrogram clusters and the similarity matrix from which they were derived. The seven wild cardoon

accessions clustered with a bootstrap probability of 89% (Fig. 3b, cluster “D”) and differ substantially from the globe artichoke accessions. The three accessions of ‘Cimiciusa di Mazzarino’ formed a distinct cluster (Fig. 3b, cluster “C”). The remaining globe artichokes clustered into two main clades: “A”, which included the spiny types, and “B”, the non-spiny types.

Discussion

The earliest report of the presence of *C. cardunculus* in Sicily and Greece dates back to Theophrastus (371-287 BCE), while in 77 CE, the Roman naturalist Pliny the Elder mentioned its use for medicinal purposes. In the opinion of De Candolle (1886) the 'cardoon', but not the 'globe artichoke', was known by ancient writers such as Athenaeus. Indeed little is known either of the process of domestication or the subsequent diversification of the two taxa, but a best guess is that the globe artichoke was domesticated and transformed into the plant which we know today, most probably between 800 and 1500 CE in family or monastery gardens. Phenotypic considerations (Foury 1987), along with inferences based on isoenzyme (Rottenberg et al., 1996) and DNA-based marker (Lanteri et al., 2004a; Acquadro et al., 2005) alleles suggest that both the globe artichoke and the cultivated cardoon are closely related to the wild cardoon. Along with their sexual compatibility (Zohary and Basnizki, 1975; Basnizki and Zohary, 1994), this evidence has been taken to conclude that the wild cardoon is the progenitor of the two domesticated forms. From this wild relative, divergent selection criteria - one for the width of the foliar midrib, and the other one for a large capitulum - have led to the present day cultivated cardoon and globe artichoke (Lanteri et al., 2004a; Portis et al., 2005b).

Only recently has the genetic diversity of *C. cardunculus* been critically assessed using DNA-based markers (reviewed by Lanteri and Portis 2008). Here, we have described a collection of Sicilian globe artichoke landraces. These accessions are characterised by a set of particular traits, which have been maintained over time by vegetative propagation and their isolation from large, more uniform cultivation areas. We included in our molecular analyses all the ecotypes identified, along with accessions of wild cardoon collected from different sites in Sicily and accessions of the two most widely cultivated Sicilian varietal types: the

spiny ‘Spinoso di Palemo’, which is grown on the western side of the island, and the non-spiny ‘Violetto di Sicilia’ confined to its eastern side (Mauromicale et al., 2004). The DNA marker data confirm our earlier finding that wild cardoon is genetically highly diverse (Portis et al., 2005a). Although to a lesser extent, significant genetic variation also exists between accessions of the two varietal types, mainly as a consequence of their multiclonal composition due to the limited selection adopted by farmers during vegetative propagation (Lanteri et al., 2001). The landrace material has managed to retain much of the variability present in the wild progenitor. Nevertheless, the globe artichoke landraces remain genetically well differentiated from the wild cardoon.

Cluster analysis based on AFLP markers identified four main clusters: the first includes the spiny types ‘Spinoso di Palermo’ and ‘Spinoso di Sciara’, and at a higher level of genetic differentiation, the landraces ‘Giarratana’, ‘Vizzini’ and ‘Chiaramonte spinoso’; the second groups the non-spiny types, including ‘Violetto di Sicilia’; the third is a small group containing only three accessions of the landrace ‘Cimiciusa di Mazzarino’ from the Mazzarino region of central south Sicily, and the fourth represents wild cardoon. Most of the landrace material, on the basis of both AFLP and SSR genotype, clustered with one or other of the two varietal types widespread in cultivation (spiny or non-spiny), with geographic origin being largely irrelevant. Thus ‘Giarratana’ and ‘Chiaramonte spinoso’ clustered with ‘Spinoso di Palermo’, while ‘S. Giacomo’, ‘Bellocozzo’, ‘Quartarella, ‘Modica Mauto’, ‘Chiaramonte inerme’ and ‘Monterosso Almo’ grouped with ‘Violetto di Sicilia’, even though all of these landraces were collected from around Ragusa in the southern part of Sicily. Barbieri (1959) hypothesised that the non-spiny types evolved from the spiny types, thus representing a further step of domestication. However, as previously reported by Lanteri et al. (2004a), the high genetic differentiation we detected between spiny and non-spiny types does not exclude the possibility of separate domestication events.

The genetic differentiation between landraces and cultivated forms was not found associated with variation in soil or climatic conditions; the former appear to represent the outcome of an array of selection criteria adopted by farmers to suit local tastes and applications, and are intermediate forms between the modern spiny and non-spiny types. A good example of this is ‘Cimiciusa di Mazzarino’ which, while on the basis of AFLP genotype forms a well defined cluster allied to the cultivated forms, on the basis of SSR genotype is more closely related to wild cardoon.

Powell et al. (1996) compared the efficacy of RFLP, RAPD, AFLP and SSR markers for germplasm analysis in soybean, and demonstrated that outcomes based on RFLPs, AFLPs and SSRs are highly correlated to one another. In a similar comparison in maize, Garcia et al. (2004) showed that AFLP was the optimal assay for fingerprinting and assessing genetic relationships among inbred lines. The discrimination power of the three AFLP PCs was sufficient to uniquely fingerprint all the *Cynara* accessions, which was not possible with the set of SSRs used here. Nevertheless, both led to a phylogeny which grouped the cultivated and wild accessions in the same way as was established in earlier investigations (Lanteri et al., 2004a, Acquadro et al., 2005).

The origin of the ‘Cimiciusa di Mazzarino’ landrace is intriguing, as it stands out as an outlier from the other landraces. Although chloroplast sequence is frequently associated with considerable levels of polymorphism, the chloroplast SSR assays delivered neither any amplicon size variation, nor any SNPs in the flanking regions among the 12 accessions representative of both wild and domesticated forms. ‘Cimiciusa di Mazzarino’ yields well compared to the other landraces, mainly thanks to the large number of capitula it produces. This latter trait is characteristic of wild forms. Thus, this landrace could represent an early stage of the domestication process. Alternatively it may be a product of introgression following hybridization with wild cardoon, with which it grows in close proximity. Such a

hybridization must have occurred a long time ago, and been followed by generations of farmer selection, since, in our experience, the phenotype of synthetic globe artichoke x wild cardoon progenies strongly resembles that of the wild type.

Conclusions

We have addressed the pattern of genetic diversity of a collection of Sicilian globe artichoke landraces (Sicily is considered to be one of the possible centres of its domestication). These landraces have been cultivated for a number of centuries by local farmers, and have been retained on the strength of their culinary uniqueness. Successive generations of vegetative propagation have minimised the introgression of genes from other populations. Considerable diversity both for a number of morphological traits and at the AFLP level allowed each accession to be distinguished from all others, underlining the role of farmers' practice in the maintenance of landrace identity. The domestication and subsequent selection of globe artichoke seems have avoided the genetic bottleneck suffered by many crop species. One of the landrace selections appears to represent an early stage of the domestication process, although it may also have derived from hybridization with wild cardoon, followed by farmer selection. The importance of preserving these artichoke landraces as a resource for broadening the genetic base of globe artichoke lies in their acting as novel sources of disease resistance and tolerance to biotic stresses. For this reason, a living collection is being maintained in the experimental fields of the University of Catania.

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Table1. Biometrical characterization of the collected ecotypes (data are average of two years). Different letters on the same column indicate significance at Tukey's HSD, at $P \leq 0.05$

Code	Ecotype	Yield (g plant^{-1})	Main Capitulum				First Order Capitula		
			DFH (d)	FW (g)	RW (%)	L/D	N°P^{-1}	FW(g)	%Y (%)
1	Bellocozzo	686 j	186 ij	229.5 h	19.8 jk	1.30 df	2.2 gh	147.6 k	52 fi
2	Belpasso	1047 cd	239 a	414.2 b	28.2 d	1.20 fh	3.0 dg	210.5 b	59 bc
3	Caltagirone	705 ij	129 k	158.7 p	19.6 jk	1.33 ce	3.6 be	116.6 p	60 bc
4	Chiaramonte inerme	691 j	214 dg	235.7 gh	31.2 c	1.13 h	2.3 fh	156.2 hi	53 di
5	Chiaramonte spinoso	881 fh	232 ac	267.2 d	28.1 d	1.33 ce	3.2 cf	161.7 ef	60 bc
6	Cimiciusa di Mazzarino 1	1098 bc	234 ac	152.7 p	35.0 a	1.37 bd	4.3 ab	125.8 o	49 hi
7	Cimiciusa di Mazzarino 2	932 dg	237 ab	142.1 q	34.1 ab	1.13 h	5.0 a	109.1 q	57 bf
8	Cimiciusa di Mazzarino 3	1115 bc	243 a	153.5 p	32.1 c	1.23 eh	4.3 ab	128.0 o	49 i
9	Domestica di Castelvetrano	1219 ab	226 ad	214.0 ik	35.4 a	1.37 bd	4.2 ac	163.2 e	56 bg
10	Donnafugata	748 ij	202 fi	254.9 e	31.9 c	1.27 dg	2.3 fh	164.0 e	53 di
11	Femminello di Marsala	1340 a	238 ab	192.1 m	23.9 fg	1.50 a	4.9 a	144.9 l	53 ei
12	Giarratana	1020 ce	233 ac	215.8 ij	25.3 ef	1.43 ac	3.6 be	159.7 fg	57 bf
13	Monterosso Almo	878 fh	219 cf	237.0 g	34.6 ab	1.17 gh	2.9 dh	182.8 c	61 b
14	Modica Mauto	694 j	178 j	186.4 mo	25.9 e	1.30 df	2.8 eh	142.4 m	58 bd
15	Naro 1	1101 bc	221 be	245.7 f	22.8 gh	1.17 gh	3.8 bd	151.1 j	52 ei
16	Naro 2	907 eg	229 ad	346.3 c	24.4 eg	1.20 fh	1.9 h	160.7 f	34 k
17	Quartarella	1289 a	237 ab	429.0 a	22.0 hi	0.91 i	3.0 dg	215.7 a	51 gi
18	S. Giacomo 1	681 j	192 hj	208.6 jl	20.8 ij	1.30 df	2.3 fh	150.4 j	52 ei
19	S. Giacomo 2	827 gi	214 dg	217.8 i	16.4 l	1.37 bd	3.6 be	156.0 hi	68 a
20	S. Giacomo 3	686 j	197 gi	207.2 kl	15.1 l	1.23 eh	2.3 fh	155.0 i	54 ch
22	S. Giacomo 4	771 hj	205 eh	205.7 l	18.8 k	1.33 ce	2.7 eh	157.8 gh	58 be
21	Santa Domenica Vittoria	773 hj	241 a	184.1 no	21.1 hj	1.23 eh	3.6 be	131.7 n	60 b
23	Spinoso di Sciara	1245 a	138 k	181.7 o	32.9 bc	1.30 df	3.0 dg	169.7 d	41 j
24	Vizzini	998 cf	187 ij	190.9 mn	24.8 ef	1.47 ab	3.6 be	151.9 j	54 ci
	MEAN	**931**	**211**	**227.9**	**26.0**	**1.27**	**3.3**	**154.7**	**54**
	CV(%)	**23.6**	**15.0**	**32.0**	**23.9**	**11.0**	**29.9**	**15.8**	**13.4**
	HSD ($P \leq 0.05$)	**129**	**17**	**7.5**	**1.8**	**0.12**	**1.0**	**2.5**	**6**

Table 2. Characteristics of the four microsatellite loci utilized. N°A: number of alleles amplified in the whole sample (artichoke/wild cardoon accessions); PIC: Polymorphic Information Content; N°G: number of genotypes fingerprinted (artichoke / wild cardoon accessions)

Locus	**N° A**	**PIC**	**N° G.**
CDAT-01	7 (5/3)	0.818	12 (8/4)
CELMS-09	7 (4/4)	0.635	11 (5/6)
CELMS-14	9 (6/7)	0.611	14 (7/7)
CELMS-40	9 (4/6)	0.642	10 (5/5)
total	32 (19/20)		20 (13/7)
average	8 (4.7/5.0)	0.676	

Table 3. Summary of AFLP primer combination (PC) characteristics. TNB: total number of bands; NPB: number of polymorphic bands (within artichoke / wild cardoon accessions); P%: percentage of polymorphic bands; PIC: Polymorphic Information Content; MI: Marker Index; N°G: number of genotypes fingerprinted (artichoke / wild cardoon accessions)

PC	TNB	NPB	P%	PIC	MI	N°G.
E+ACA/M+CAT	66	22 (18/7)	33.3	0.240	5.28	22 (15/7)
E+ACG/M+CAA	69	20 (17/5)	29.0	0.231	4.61	26 (19/7)
E+ACT/M+CAA	73	30 (15/13)	41.1	0.236	7.08	35 (28/7)
E+ACT/M+CAT	75	28 (16/7)	37.3	0.263	7.36	33 (26/7)
E+ACT/M+CTT	68	21 (14/7)	30.9	0.275	5.77	25 (20/5)
total	351	121 (100/39)	34.5			45 (38/7)
average	70.2	24.2 (20.0/7.8)		0.247	5.99	

Table 4. Correlation coefficients for each trait with respect to the first two principal components, eigen-values, relative and cumulative proportion of total variance

TRAITS		**Common principal component coefficients**	
		First	**Second**
	Yield	0.778	0.466
Main Capitulum	**DFH**	0.091	0.603
	FW	0.903	-0.152
	RW	0.574	0.481
	L/D	-0.860	0.052
First Order Capitula	**N°P^{-1}**	-0.281	0.883
	FW	0.973	-0.083
	%Y	0.738	0.091
Eigenvalue		4.095	1.608
Variability %		51.2	20.1
Accumulated variability		51.2	71.3

Figure 1. Geographic location of Sicilian ecotypes in study.

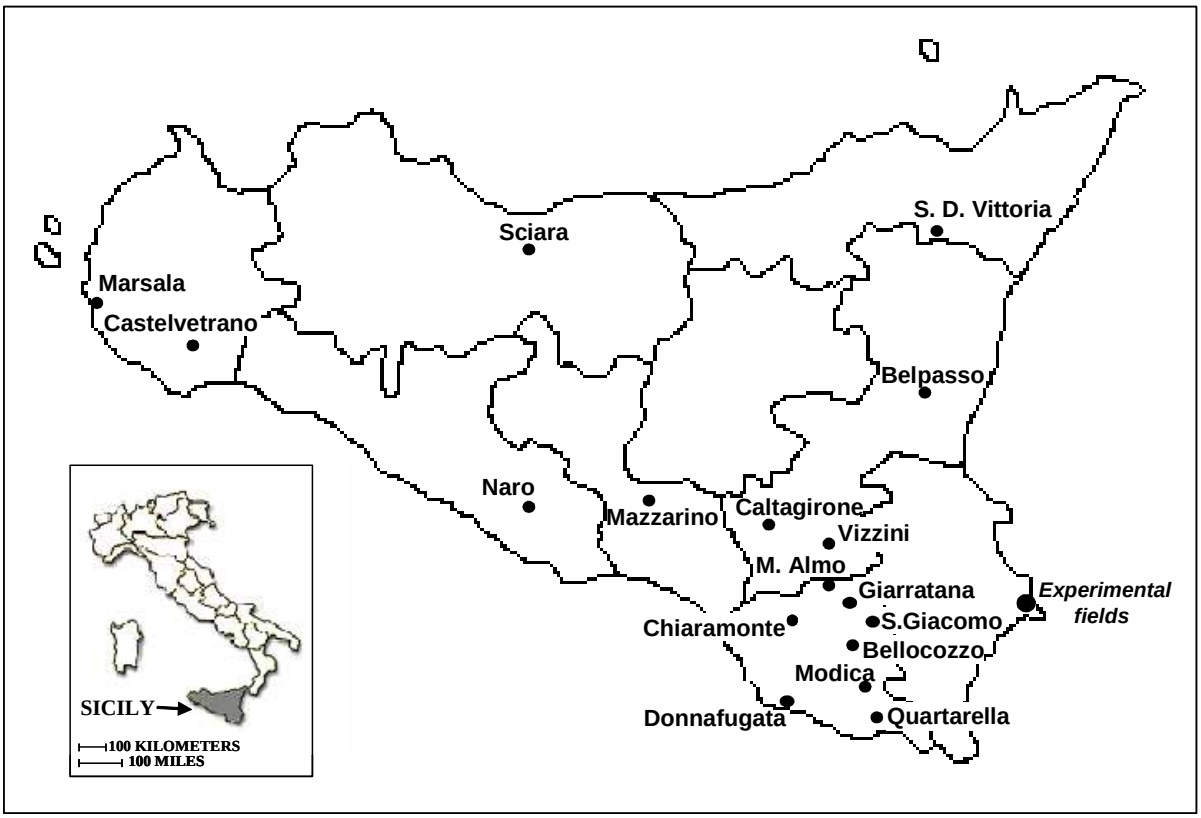

Figure 2. The Principal Components Analysis based on the quantitative traits of the globe artichoke ecotype in study and three wild cardoon genotypes.

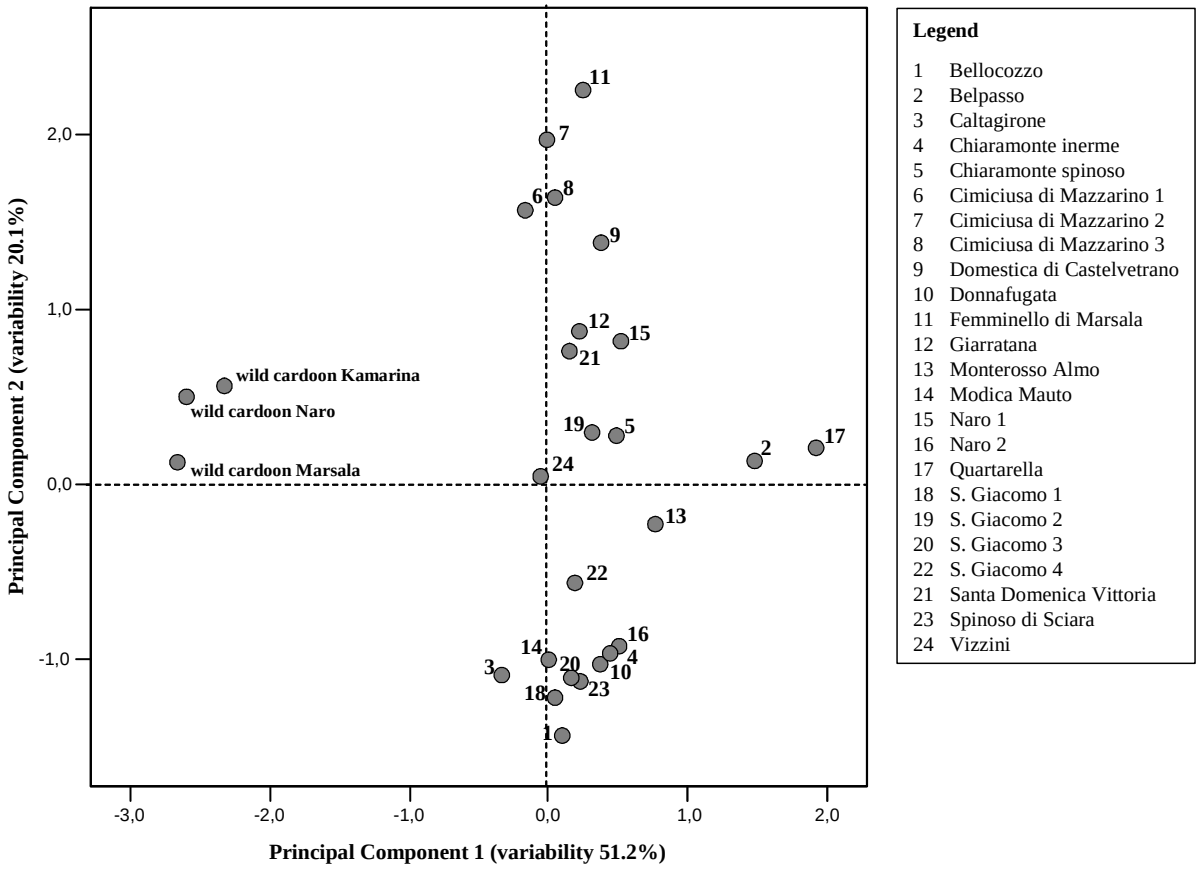

Figure 3. Dendrograms obtained from UPGMA cluster analysis of: (A) SSR data and Nei-Li's genetic distance; (B) AFLP data and Jaccard's similarity index.