Clonal selection in a globe artichoke landrace: characterization of superior germplasm to improve cultivation in Mediterranean environments

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Clonal selection in a globe artichoke landrace: characterization of superior germplasm to improve cultivation in Mediterranean environment

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SUMMARY

The morphological (UPOV descriptors) and field performance of five clones selected from the globe artichoke landrace ‘Spinoso di Palermo’ were determined over two seasons, and their AFLP profiles detected using seven primer combinations. The number of heads produced averaged 13.8 per plant (equivalent to a fresh weight yield of 2.1 kg per plant), but two of the clones produced 15.6 heads per plant (2.4 kg per plant). Three clones produced noticeably larger second order heads (mean of 156 g), and so were considered to be suitable for the production of desirable heads over a prolonged harvesting period. Head yield and the number of heads per plant were associated with a moderate level of broad sense heritability (0.29 – 0.46), implying that these traits could be viewed as primary selection criteria. From the list of 51 UPOV descriptors, 18 varied among the five clones, but variation at just six simply scored ones was sufficient to discriminate completely the examined clones. Full discrimination was also achieved by applying only three of the seven selected AFLP primer combinations. According to AFLP profile, two of the clones were highly similar. The similarity matrices calculated from the UPOV descriptors and the AFLP profiles were highly correlated to one another. The data are optimistic and indicate that the performance of ‘Spinoso di Palermo’ could be much improved via clonal selection.

Running title: Clonal selection in a globe artichoke landrace

INTRODUCTION

Globe artichoke [*Cynara cardunculus* L. var. *scolymus* (L.) Fiori], a diploid (*2n* = *2x* = 34) outbreeding, herbaceous, perennial Asteraceae species, is grown largely for its immature inflorescences (hereafter referred to as "heads"), which represent a popular component of the Mediterranean diet (Bianco, 2011). The global production of ~1,449 kt of heads (Faostat 2011) was achieved from 125 kha of land, sited predominantly within the Mediterranean Basin. Production of the crop is on an upward trend, because of its perceived value as a functional food (Lanteri & Portis 2008; Lattanzio *et al.* 2009; Lombardo *et al.* 2009; Pandino *et al.* 2012). The most important primary gene pool of globe artichoke is in the Mediterranean area (Mauro *et al.* 2007; Ciancolini *et al.* 2012). Also is endemic in Italy and more specifically in the Island of Sicily, which is considered the probable geographic site of its domestication from the species *Cynara cardunculus* L. var. *sylvestris* Lamk (Portis *et al.* 2005; Mauro *et al.* 2009).

Over 120 clonally propagated types are present worldwide, and their heterozygosity ensures that attempts to propagate them by sexual reproduction lead to major segregation for most of agronomic traits (Basnizki & Zohary 1994; Lanteri *et al.* 2012; Portis *et al.* 2009; 2012). For this reason, vegetative propagation has been applied over a centuries to ensure the predictability of the phenotype (Lanteri & Portis 2008). According to Porceddu *et al.* (1976) globe artichoke germplasm was classified into four major morphological types, namely the *Spinosi*, *Violetti*, *Romaneschi* and *Catanesi*. More recently, the application of DNA fingerprinting through AFLP markers has shown that direct selection on specific production traits has been an important tool to determine the variation within the cultivated gene pool (Lanteri *et al.* 2004).

Landraces have been recognized as an important source of genetic variation for crop improvement (Gepts 2006; Hajjar *et al.* 2008; Mercer *&* Perales 2010), but many of them are increasingly being threatened by the diffusion of elite breeding cultivars (Hammer *&* Teklu 2008). In Southern Italy, where ancient, autochthonous landraces have traditionally dominated globe artichoke production, there is a growing spread of both allochthonous landraces and modern, highly productive seed-propagated F_1 hybrids (Mauromicale *&* Ierna 2000). As a result, the area devoted to the cultivation of autochthonous landraces is gradually decreasing. The reflowering landrace 'Spinoso di Palermo' has for many years been an important component of the Southern Italian rural economy (Pandino *et al*. 2012) and is genetically highly heterogeneous (Portis *et al.* 2005).

Into this study, a clonal propagation program was applied aiming to identify elite globe artichoke genotypes from landrace 'Spinoso di Palermo' which would be suitable for cultivation in specific areas of Sicily. Both phenotypic and AFLP-based molecular characterization of the selected clones have been performed.

MATERIALS AND METHODS

Plant materials and research site

A program of germplasm collection was carried out in five locations of Western Sicily, representative for the cultivation area of the 'Spinoso di Palermo' landrace: Buonfornello, Caccamo, Cerda, Licata and Menfi. The geographical coordinates, soil type and meteorological information of each sampling area are listed in Table 1. At each site, a sample of 3–8 plants was selected and labelled in late winter 2007 (in total 30 clones), on the basis of the following traits: floral stem ramifications (an index of yield potential), earliness and colour, firmness and size

of the heads. In August 2008 from each clone, 8–10 semi-dormant offshoots ('ovoli') were obtained for planting in the field of the experimental station South of Siracusa (37° 03' N, 15° 18' E, 10 m asl). The local climate is semi-arid Mediterranean, characterized by mild and wet winters (frost are virtually absent) and warm, dry summers. The soil was a moderately deep Calcixerollic Xerochrepts (USDA soil taxonomy), having the following characteristics: 15.5% clay, 29.1% silt, 55.4% sand, pH 7.6, organic matter 2.0%, total N content 0.17%, available P 100 mg kg^{-1}, exchangeable K 580 mg kg^{-1}.

During the 2007–2008 growing season, 25 clones were discarded and 5 (labelled A_1, A_4, A_6, E_3 and E_7), were chosen on the basis of their higher floral stem ramification and marked violet pigmentation of the heads (as previously reported by Mauro *et al.* 2012). The number of plants per selected clone was then increased to 54 (for a total of 270 plants) by transplanting their lateral offshoots, in order to perform a more reliable morphological characterization during two subsequent growing seasons I (2008-2009) and II (2009-2010). To this end, in early August 2008 'ovoli' from each clone were collected and planted in rows separated from one another by 0.80 m. The inter-row spacing was set at 1.25 m (planting density 1 plant m^{-2}). For the bio-agronomical characterization, 54 plants of the allochthonous varietal type 'Violet de Provence' were also included as reference material. This varietal type is spreading in South Italy in virtue of its earliness, high yielding and long productive cycle, and it is endangering native local landraces. The plots were arranged in a randomized strip-plots design with three replications, each including 18 plants for each genotype, for a total of 108 plants per plot (net of border plants). Fertilization was applied before planting (season I) or awakening (season II) with 80, 180, and 150 kg ha^{-1} of N, P_2O_5 and

K_2O, respectively. On both seasons further two N applications (as ammonium nitrate) were applied at a rate of 80 kg ha^{-1} on early November and late February, respectively, shortly after lateral offshoots removal. Drip irrigation was supplied from August to mid October and on early April, by restituting 100% of maximum evapotranspiration (ET_m), when accumulated daily evaporation, net of rain (measured from an unscreened class A-Pan evaporimeter near the crop) reached 40 mm (corresponding to ~50% of available soil water content at 0.30 m depth). Plant regrowth in season II was induced by applying drip irrigation to field capacity in early August 2009. All the experimental plots were kept weed and insect-free by spraying oxyfluorfen and imidachloprid, respectively, when required. Air temperature, rainfall and evaporation were recorded every 30 minutes from a meteorological station (Multirecorder 2.40; ETG, Florence, Italy) sited about 500 m from the experimental field.

Bio-agronomical and morphological characterization

The bio-agronomical characterization was performed on 54 plants of each genotype (for a total of 324 plants) over two growing seasons. Heads were collected at their marketable dimension before bracts divergence (stage D) (Foury, 1967) and deprived from floral stems in order to determine their fresh weight. A sub-sample of collected heads were reweighed after they were kept in a thermo-ventilated oven at 105 °C for ~72 h. The following variables were calculated: *days to first harvest* (DFH) as the number of days elapsed from transplanting (season I) or awakening (season II) and the harvest of the main head; *duration of harvest period* (DHP) as the number of days elapsed from first to last harvest; *yield* (Y) expressed as kg heads/plant; *number of heads/plant* (NH); *rate of yield* (RY) as an

index of yield synchronicity, expressed as the ratio between Y (expressed on a dry weight basis) and DHP; *weight of main heads* (WM) and *weight of lateral heads* (WL).

The morphological characterization was performed on 5 randomly selected plants of each 'Spinoso di Palermo' selected genotype. Two clones of the landrace 'Violetto di Sicilia' (labelled L_1 and M_3) were also characterized as a reference materials. Fifty-one traits were scored (Supplementary Table 1, Table 2) according to the guidelines provided by the International Union for the Protection of New Varieties of Plants (UPOV 2001: TG/184/3 globe artichoke) for D.U.S. (Distinctness, Uniformity and Stability) and adopting a metric scale according to the descriptor list (Supplementary Table 1). Both qualitative and quantitative traits in UPOV's descriptor list were expressed as discontinuous, the latter having been divided into a number of discrete states for the purpose of description.

DNA extraction and AFLP genotyping.

For molecular analyses DNA was extracted from 2 g of young leaves of two plants of each selected clone on the basis of the protocol described by Lanteri *et al.* (2004); the two DNA samples were analysed separately, in order to confirm the reliability of the AFLP fingerprinting. The latter was also performed on DNA from twelve genotypes, previously identified as representative for genetic variation within 'Spinoso di Palermo' (Portis *et al.* 2005), as well as from two genotypes of 'Violetto di Sicilia' (labelled L_1, and M_3), used as a reference. The AFLP profiling method was based on that described by Vos *et al.* (1995) as modified by Lanteri *et al.* (2004). The template was digested with *Eco*RI/*Taq*I and the seven following primer combinations (PCs), applied in a previous study

(Mauro *et al.* 2012), were used: E35/T79 (ACA/TAA), E35/T81 (ACA/TAG), E35/T82 (ACA/TAT), E35/T84 (ACA/TCC), E38/T81 (ACT/TAG), E38/T82 (ACT/TAT) and E38/T84 (ACT/TCC). The final amplicons were electrophoresed on a DNA analyser Gene ReadIR 4200 (LI-COR) device using a 6.5% polyacrylamide gel, as described by Jackson & Matthews (2000). Each variable fragment, which ranged in size from 60 to 650 bp, was assumed to represent a single biallelic locus, allowing the data to be scored in binary form (1 = presence and 0 = absence) for the same-size DNA bands.

Statistical analysis

Bio-agronomic data were firstly subjected to Levene's test for checking the homoscedasticity, then to a two-way ('clone x season') analysis of variance (ANOVA) related to the experimental layout. Provided that the *F*-test was significant, means were separated on the basis of Fisher's protected least significant difference (LSD) test. For each trait under study in the 'Spinoso di Palermo' selected clones, the broad sense heritability was evaluated as follows: the phenotypic variance for each trait (σ^2_p) was considered to be the sum of the genotypic (σ^2_g) and environmental (σ^2_e) components. Since σ^2_e can be equated to the error expected mean square (EMS), then $\sigma^2_p = \sigma^2_g + EMS_{error}$. σ^2_g was estimated from the expression $1/ry$ ($EMS_{clones} - EMS_{clones\ x\ season}$), equivalent to $1/ry\ [(\sigma^2_e + r\sigma^2_{gy} + ry\sigma^2_g) - (\sigma^2_e + y\sigma^2_{gy})]$, where r represents the number of replicates (3), and y the number of seasons (2). The broad sense heritability (h^2_B) for each trait was evaluated by the ratio σ^2_g/σ^2_p. Genotypic (g_{cv}) and phenotypic (p_{cv}) coefficients of variation of each trait were calculated as follows: $g_{cv} = (\sqrt{\sigma^2_g}/x)\ 100$ and $p_{cv} = (\sqrt{\sigma^2_p}/x)\ 100$ (were x is the mean of each trait). With the

goal to define the relationships among bio-agronomical variables, a correlation analysis was performed for all the genotypes in study.

Phenotypic similarity between pairs of genotypes was calculated using the proportion of shared alleles. As each genotype can have only one state for a given trait, the results obtained by using the proportion of shared alleles similarity formula were identical to those obtained by simple matching coefficient (SM) 1-(*m/n*), following Sneath *&* Sokal (1973), where *m* is the number of morphological traits shared between a pair of genotypes and *n* is the total number of traits.

AFLP data were at first evaluated by means of Polymorphic Information Content (PIC), calculated by setting the expected heterozygosity to 2*f*(1-*f*), following Anderson *et al.* (1993), where *f* represents the proportion of individuals carrying a particular AFLP locus. A similarity matrix was then generated by means of the SM coefficient previously described, where *m* is the number of AFLP fragments shared between a pair of genotypes and *n* is the total number of fragments detected.

The Mantel test (Mantel 1967) was used to establish correspondence between the molecular and morphological similarity matrices; this test provides a correlation index (r), which is a measure of the relatedness between them. Cluster analyses based on both similarity matrices were performed using the unweighted pair-group method (UPGMA; Sneath *&* Sokal 1973) as implemented in NTSYS-pc ver. 2.1 (Rohlf 2000).

RESULTS

Research site & Meteorological data

The total rainfall in season I was low (360 mm) with the 85% of the total (307 mm) fell between October and March (Supplementary Figure 1). In season II total rainfall was 572 mm, mainly concentrated in October (123 mm), January (168 mm) and March (195 mm). Both seasons were characterized by a decreasing mean monthly temperature from August to January (from 26.3 to 11.6 °C, on average), followed by a progressive increase up to July (25.7 °C). The higher mean maxima temperature was recorded in season II as compared with season I (Supplementary Figure 1).

Bio-agronomical characterization

Significant variation was observed in the most of the examined traits among globe artichoke clones. Three of the traits (DFH, DHP and RY) were significantly affected by 'clone x season' interaction, while WM proved to be the most stable (Table 3). In the two growing seasons, the highest variability among genotypes (highest coefficient of variation) was detected for traits related to yield, namely Y, NH and RY (Table 4). As regards DFH, the clones A_4 and E_7, were very similar to 'Violet de Provence', since the period elapsing from transplantation/awakening to the day of main head production, was on average 143 days, thus anticipating by 20 days the latest clone, E_3 (Table 4). During the two seasons the clones A_1 and A_4 showed the highest delay (24 days) in producing the first head (Table 4) but, steadily both showed a significantly longer productive period (DHP = 85 days, on average) in comparison to the others genotypes (Table 4). On season II the clones A_6 and E_7 consistently increased their productive period (DHP) of 28 and 14 days,

respectively (Table 4). The average production was 2.01 kg/plant, with all the selected clones being more productive than 'Violet de Provence'; A_1 and A_6 were the best performing clones (2.42 kg/plant, on average), thus exceeding the yield of 'Violet de Provence' by about 1 kg/plant (Table 4). This result appeared consistent with both the number of heads per plant (NH) and yield rate (RY), as both variables showed the highest values in clones A_1 and A_6 (Table 4). On season II a significant increase in the rate of yield (RY) was observed for clones A_1 and A_4 (by 1.4 and 1.6 mg DM/day/plant, respectively) (Table 4). The average weight of the main head (WM) of the studied genotypes across seasons was 205 g and ranged from 220 (A_6) to 184 g ('Violet de Provence'). As expected the weight of secondary heads (WL) was lower (on average 145 g), ranging from 143 g (clone A_1) to 113 ('Violet de Provence') (Table 4).

Components of variance, traits heritability and phenotypic correlations

The estimated components of variance, the genotypic (g_{cv}) and phenotypic (p_{cv}) coefficients of variation, along with the broad sense heritability of traits (h^2_B) are reported in Table 5. The genotypic and phenotypic variances and their associated coefficients of variation differed greatly from trait to trait, with g_{cv} resulting particularly high for Y (23.8%) and NH (26.5%). Accordingly, these two traits showed the highest h^2_B values (0.44 and 0.46, respectively), followed by WM (0.31), WL (0.29) and DHP (0.28). A low h^2_B value was recorded for DFH (0.09).

Traits correlation matrix is reported in Table 6. According to this, DFH was significantly correlated to RY ($0.73^{P\leq0.001}$) and, to a lesser extent, with NH ($0.44^{P\leq0.01}$) and WL ($0.42^{P\leq0.01}$). A strong correlation was also found between Y and both NH ($0.69^{P\leq0.001}$) and RY ($0.46^{P\leq0.01}$) (Table 6). Significant but less strong

correlations were recorded between DHP and NH ($0.34^{P\leq0.05}$) as well as RY and WL ($0.34^{P\leq0.05}$).

Morphological characterization

Eighteen out of the 51 scored morphological traits were uninformative, as they were not able to detect polymorphisms among the set of globe artichoke genotypes in study. Thirty-three traits were polymorphic, of which 15 between 'Spinoso di Palermo' and 'Violetto di Sicilia' genotypes (underlined in Table 2) while 18 within the 'Spinoso di Palermo' clones (bold in Table 2). On the basis of the latter it was possible to identify all the selected clones; for some characters discrimination was based on just two states, while for others (i.e. number of secondary lobes, hue of green colour of the leaf blade and leaf hairiness on upper side) three states were identifiable (Table 2).

Average phenotypic similarity among the whole globe artichoke genotypes in study, evaluated on the proportion-of-shared-alleles, was 0.702, and ranged from 0.515 (between A_4 and M_3) to 0.864 (between E_3 and E_7). Within the 'Spinoso di Palermo' clones, the average phenotypic similarity was 0.829, ranging from 0.764 (between A_4 and A_6) to 0.864. The UPGMA analysis highlighted a marked morphological differentiation between the two landraces 'Violetto di Sicilia' and 'Spinoso di Palermo' (Figure 1). Furthermore, as within the landrace 'Spinoso di Palermo', 3 clones (A_4, E_3, E_7) showed a mean genetic similarity of about 85%, while clone A_6 was the most genetically differentiated from all the others.

Genetic relationships

The seven PCs amplified 415 fragments of which 88 (21.2%) were polymorphic across the whole set of the 19 genotypes used in this study (12 references and 5 selected clones of Spinoso di Palermo / 2 genotypes from 'Violetto di Sicilia') (Table 7). The mean number of polymorphic fragments per PC was 12.6 (range 10-15). E35/T79 was associated with the highest PIC, while E35/T84 generated the greatest number of polymorphisms, both PCs being able to discriminate between 12 of the 19 templates, including three of the five clonal selections. The lowest PIC was generated by E35/T81, which only discriminated seven of the templates and was not able to discriminate between the selected clone.

As expected, no intra-clonal variation was detected as no AFLP polymorphism was recorded between two randomly chosen plants belonging to the same clone (-a and -b in Figure 2). All 19 genotypes could be discriminated from one another on the basis of three PCs, i.e. E35/T79, E35/T82 and E35/T84. The most similar pair of selected clones was E_3 and E_7 (SM=0.92), and the most dissimilar (SM=0.71) A_4 and A_6.

The AFLP-based UPGMA dendrogram is shown in Figure 2. As expected, the two varietal types formed two clearly separated clusters, with an average low similarity of 0.15 between them. Within 'Spinoso di Palermo' cluster, clones E_3, E_7 and A_1 grouped together with 7 reference template, with a mean genetic similarity of about 70%. The clone A_6 together with one reference 'Spinoso di Palermo' genotype showed the highest genetic differentiation from all the others. To objectively resume the degree of agreement between the morphological and molecular classification of entries, the correlation between the derived UPOV's traits and AFLP molecular similarity matrices was evaluated (by considering only

the genotypes in common between the two evaluation system). The correlation coefficient was 0.913 implying a high fitness between the two methods; in spite of some differences, regarding distances and topologies, both classifications agreed, in grouping clones E_3 and E_7 and in identifying A_6 as the most divergent one.

DISCUSSION

The need to conserve crop landraces *in situ* has been widely recognized. Landraces are not only highly heterogeneous, but are also dynamic and evolving entities. Globe artichoke is a significant component of the agricultural economy in the Mediterranean Basin, and especially for South Italy (Portis *et al.* 2005). The Sicilian globe artichoke landraces, maintained over centuries by local farmers via vegetative propagation (Mauro *et al.* 2011), have been favoured by the consumers for their culinary value and by farmers for their adaptability to local climatic conditions. 'Spinoso di Palermo' has long been grown throughout the Western part of the Island, but the area cultivated with this landrace has been declining as a result of its poor productivity. Genetic variation within the landrace, built up over many generations of vegetative propagation via the accumulation of mutations, is theorized to be as the major cause of this unreliable yield performance. In principle, the identification and clonal propagation of elite individuals within the landrace should reverse the yield decline, while at the same time can retain the desirable characteristics of the landrace. An attempt was made to characterize five selected 'Spinoso di Palermo' clones both by phenotype characteristics and molecular profile. It has been possible to demonstrate the feasibility of using clonal selection to provide producers with material which is competitive with the more productive allochthonous germplasm increasingly being adopted.

Heisey & Brennan (1991) have suggested that yield potential is the most important factor for the farmers' choice of variety, and thus is largely responsible for the substitution of autochthonous landraces by true-breeding or allochthonous cultivars. All the selected 'Spinoso di Palermo' clones yielded more than the genotypes of 'Violet de Provence', thus they represent a promising material for improving globe artichoke cultivation in South Italy. In particular, the two clones A_6 and A_1 yielded 70% more (~2.4 kg/plant) than common populations of 'Violet de Provence' (1.4 kg/plant). When compared to other traits, the higher broad sense heritability values observed for yield (0.44) and number of heads per plant (0.46) are encouraging, and they could be theorized as suitable traits for profitable clonal selection in 'Spinoso di Palermo'. Since there was no correlation between yield and heads weight [as was also the case among clones selected out of the 'Violetto di Sicilia' landrace, see Mauro *et al.* (2012)], the yield potential of 'Spinoso di Palermo' appears to be most strongly determined by the number of heads per plant. There was a significant correlation between number of heads per plant and the harvest period duration, the latter being particularly important for ensuring a stable income for the globe artichoke producer (Mauro *et al.* 2011). Clone A_1 was associated with the best combination of yield and harvest period duration. The 'clone x season' interaction was particularly strong for both days to first harvest and yield rate, so any selection pressure imposed on either of these two traits is unlikely to be effective. As evidence of this, we were unable to identify clones as ealy as 'Violet de Provence'. In contrast, all selected clones performed better than 'Violet de Provence' in terms of weight of heads. Two clones, namely A_4 and A_6, performed outstandingly in terms of both main and lateral heads, traits which highly ranking in the preferences of consumers of the fresh product. Clone E_3

produced rather smaller main heads than the other clones, although its lateral heads developed to a larger than average size and its harvest period duration was particularly remarkable.

In all, 18 traits included on the UPOV descriptor list were variable among the set of five clones, but just six easily scorable ones were sufficient to allow unambiguous discrimination between all the clones. These traits were: the number of leaf lobes, the shape of the lobe tip, the number and shape of the secondary lobes, hairiness on the adaxial surface of the leaf, anthocyanin pigmentation at the petiole base and the colour of the outer bract. Nevertheless, it has been suggested that DNA fingerprinting is a valuable adjunct to morphological characterization for the purpose of varietal identification (Singh *et al.* 1991; Tatinery *et al.* 1996; Koutsos *et al.* 2001). AFLP fingerprinting required the application of only three primer combinations to fully discriminate between the clones. Furthermore, when their molecular characterization was placed in relation to the one performed in representatives of the genetic variation at present in cultivation, they were all included in the clusters defined by the references genotypes. In our study, morphological and molecular similarities between pairs of accessions were calculated and the corresponding UPGMA dendrogram was constructed; encouragingly, the grouping of entries generated by AFLP analysis was consistent with the grouping based on morphological variation. The two data sets were compared via a simple matching coefficient (Sneath & Sokal 1973), for making the data more comparable. Their evaluation through the SMC appears appropriate for the latter but ignores the ordering pattern present in the formers, although the intrinsic structure of covariation between the variables is somehow maintained. However our results revealed a certain degree of correspondence between

morphological and molecular data among clones. Expressing morphological variation in ordinal form can help reduce interference caused by environmental variation, and so improve both its utility for estimating genetic distances and the extent of the correlation between classifications based on phenotypic and genotypic characterization (Babic *et al.* 2012).

CONCLUSIONS

We have demonstrated the feasibility of applying clonal selection for the improvement of key traits in the globe artichoke landrace 'Spinoso di Palermo'. The five traits Y, NH, DHP, WM and WL were identified as potential targets for a successful clonal selection program. A subset of the UPOV descriptors was effective for clonal discrimination in globe artichoke, and the outcome of AFLP fingerprinting was consistent and related to morphological pattern. The data showed that a clonal selection program would be effective for increasing productivity of the vegetatively propagated globe artichoke landrace. At the same time, intraselection within landrace provided the opportunity to identify specific clones that would be more suitable in order to at least partially preserve the genetic variation harboured by the originating landrace, and reduce the risk of genetic erosion.

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REFERENCES

ACQUADRO, A., PAPANICE, M.A., LANTERI, S., BOTTALICO, G., PORTIS, E., CAMPANALE, A., FINETTI-SIALER, M.M., MASCIA, T., SUMERANO P. & GALLITELLI, D. (2010). Production and fingerprinting of virus-free clones in a reflowering globe artichoke. *Plant Cell Tissue and Organ Culture* **100**, 329–337.

ANDERSON, J.A., CHURCHILL, G.A., AUTRIQUE, J.E., SORELLS, M.E. & TANKSLEY, S.D. (1993). Optimizing parental selection for genetic-linkage maps. *Genome* **36**, 181–186.

BABIC M., BABIC, V., PRODANOVIC, S., FILIPOVIC, M. & ANDELKOVIC, V. (2012). Comparison of morphological and molecular genetic distances of maize inbreds. *Genetika*, **44**, 119-128.

BASNIZKI, Y. & ZOHARY, D. (1994). Breeding of seed planted artichoke. *Plant Breeding Reviews* **12**, 253-269.

BIANCO, V.V. (2011). The artichoke: a travelling companion in the social life, traditions and culture. *Acta Horticulturae* **942**, 25-40.

BRUTTI, C., APÓSTOLO, N.M., FERRAROTTI, S.A., LLORENTE, B.E. & KRYMKIEWICZ, N. (2000). Micropropagation of *Cynara scolymus* L. employing cyclodextrins to promote rhizogenesis. *Scientia Horticulturae* **83**, 1-10.

CAMPANELLI, A., RUTA, C., TAGARELLI, A. & MORONE FORTUNATO, I. (2011). Nursery inoculation with the mycorrhizal fungus *Glomus viscosum* and its effect on the growth and physiology of hybrid artichoke seedlings. *Italian Journal of Agronomy* **6**, 159-164.

CIANCOLINI, A., REY, N.A., PAGNOTTA, M.A. & CRINÒ, P. (2012). Characterization of Italian spring globe artichoke germplasm: morphological and molecular profiles. *Euphytica* **186**, 433-443.

FAOSTAT (2011). *FAO Statistical Database*, http://www.faostat.org/.

FOURY, C. (1967). Etude de la biologie florale de l'artichaut (*Cynara scolymus* L.): application à la selection (1er partie). *Annales de l'Amélioration des Plantes* **17**, 357-373.

GEPTS, P. (2006). Plant genetic resources conservation and utilization: the accomplishment and future of a social insurance policy. *Crop Science* **46**, 2278-2292.

HAJJAR, R., JARVIS, D.I. & GEMMILL-HERREN, B. (2008). The utility of crop genetic diversity in maintaining ecosystem services. *Agricultural Ecosystems & Environment* **123**, 261-270.

HAMMER, K. & TEKLU, Y. (2008). Plant genetic resources: selected issues from genetic erosion to genetic engineering. *Journal of Agriculture and Rural Development in the Tropics and Subtropics* **109**, 15-50.

HEISEY, P.W. & BRENNAN, J. (1991). An analytical model of farmers demand for replacement of seed. *American Journal of Agricultural Economics* **73**, 44-52.

JACKSON, J.A. & MATTHEWS, D. (2000). Modified inter-simple sequence repeat PCR protocol for use in conjunction with the Li-Cor gene ImagIR(2) DNA analyzer. *Biotechniques* **28**, 914–917.

KOUTSOS, T., KOUTSIKA-SOTIRIOU, M. & TSAFTARIS, A.S. (1999). Identification of four cabbage open-pollinated populations and an F" hybrid by starch gel electrophoresis of isozymes. *Journal of Vegetable Crop Production* **4**, 9-16.

LANTERI, S. & PORTIS, E. (2008). Globe artichoke and cardoon. In: *Vegetables I: asteraceae, brassicaceae, chenopodicaceae and cucurbitaceae* (Eds. J. Prohens & F. Nuez), pp. 49–74. New York, USA: Springer.

LANTERI, S., PORTIS, E., ACQUADRO, A., MAURO, R.P. & MAUROMICALE, G. (2012). Morphology and SSR fingerprinting of newly developed *Cynara cardunculus* genotypes exploitable as ornamentals. *Euphytica* **184**, 311-321.

LANTERI, S., SABA, E., CADINU, M., MALLICA, G.M., BAGHINO, L. & PORTIS, E. (2004). Amplified fragment length polymorphism for genetic diversity assessment in globe artichoke. *Theoretical and Applied Genetics* **108**, 1534-1544.

LATTANZIO, V., KROON, P.A., LINSALATA, V. & CARDINALI, A. (2009). Globe artichoke: a functional food and source of nutraceutical ingredients. *Journal of Functional Foods* **1**, 131-144.

LOMBARDO, S., PANDINO, G., MAURO, R. & MAUROMICALE, G. (2009). Variation of phenolic content in globe artichoke in relation to biological, technical and environmental factors. *Italian Journal of Agronomy* **4**, 181-189.

MANTEL, N.A. (1967). The detection of disease clustering and a generalized regression approach. *Cancer Research* **27**, 209-220.

MAURO, R., IERNA, A., PORTIS, E., LANTERI, S. & MAUROMICALE, G. (2007). Morphological and molecular characterization of autochthonous Sicilian globe artichokes grown in family gardens. *Acta Horticulturae* **730**, 113-121.

MAURO, R., PORTIS, E., ACQUADRO, A., LOMBARDO, S., MAUROMICALE, G. & LANTERI, S. (2009). Genetic diversity of globe artichoke landraces from Sicilian small-holdings: implications for evolution and domestication of the species. *Conservation Genetics* **10**, 431-440.

MAURO, R.P., LOMBARDO, S., LONGO, A.M.G., PANDINO, G. & MAUROMICALE, G. (2011). New cropping designs of globe artichoke for industrial use. *Italian Journal of Agronomy* **6**, 44-49.

MAURO, R.P., PORTIS, E., LANTERI, S. & MAUROMICALE, G. (2012). Genotypic and bio-agronomical characteristics of an early Sicilian landrace of globe artichoke. *Euphytica* **186**, 357-366.

MAUROMICALE, G. & IERNA, A. (2000). Characteristics of heads of seed-grown globe artichoke [*Cynara cardunculus* L. var. *scolymus* (L.) Fiori] as affected by harvest period, sowing date and gibberellic acid. *Agronomie* **20**, 197-204.

MERCER, K.L. & PERALES, H.R. (2010). Evolutionary response of landraces to climate change in centers of crop diversity. *Evolutionary Applications* **3**, 480-493.

MORONE FORTUNATO, I., RUTA, C., CASTRIGNANÒ, A. & SACCARDO, F. (2005). The effect of mycorrhizal simbiosi on the development of micropropagated artichoke. *Scientia Horticulturae* **106**, 472-483.

PANDINO, G., LOMBARDO, S., MAURO, R.P. & MAUROMICALE, G. (2012). Variation in polyphenol profile and head morphology among clones of globe artichoke selected from a landrace. *Scientia Horticulturae* **138**, 259-265.

PORCEDDU, E., DELLACECCA, V. & BIANCO, V.V. (1976). Classificazione numerica di cultivar di

carciofo. In *Proceedings of the II International Congress on Artichoke* (Ed. Minerva Medica), pp. 1105–1119. Bari, Italy.

PORTIS, E., MAUROMICALE, G., BARCHI, L., MAURO, R. & LANTERI, S. (2005). Population structure and genetic variation in autochthonous globe artichoke germplasm from Sicily Island. *Plant Science* **168**, 1591-1598.

PORTIS, E., MAUROMICALE, G.., MAURO, R., ACQUADRO, A., SCAGLIONE, D. & LANTERI S. (2009).Construction of a reference molecular linkare map of globe artichoke (*Cynara cardunculus* var. *scolymus*). *Theor Appl Genet* **120**, 59-70.

PORTIS, E., SCAGLIONE, D., ACQUADRO, A., MAUROMICALE, G., MAURO, R., KNAPP, S.J. & LANTERI, S. (2012). Genetic mapping and identification of QTL for earliness in the globe artichoke/cultivated cardoon complex. *BMC Research Notes* **5**, 252-266.

ROHLF, F.J. (2000). NTSYS, Numerical Taxonomy and Multivariate analysis System, vers. 2.1. Exeter Software. Applied Biostatistic Inc., NY.

SINGH, S.P., GUTIERREZ, J.A., MOLINA, A., UREA, C. & GEPTS, P. (1991). Genetic diversity in cultivated common bean: II. Marker based analysis of morphological and agronomic traits. *Crop Science* **31**, 23-29.

SNEATH, P.H.A. & SOKAL, R.R. (1973). Numerical taxonomy. W.H. Freeman, San Francisco

TATINERY, V., CANTRELL, R.G. & DAVIS, D.D. (1996). Genetic diversity in elite cotton germplasm determined by morphological characteristics and RAPDs. *Crop Science* **36**, 186-192.

UPOV, 2001. Guidelines for the conduct of tests for distinctness, uniformity and stability, globe artichoke (*Cynara scolymus* L.) (*Cynara cardunculus* var. *scolymus* L.). Document number TG/184/3, available on line at: http://www.upov.int/test_guidelines/en/fulltext_tgdocs.jsp?lang_code=EN&q=globe+artichoke

VOS P., HOGERS, R., BLEEKER, M., REIJANS, M., VAN DE LEE, T., HORNES, M., FRIJTERS, A., POT, J., PELEMAN, J., KUIPER, M. & ZABEAU, M. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* **23**, 4407-4414.

Table 1. *Geographical, meteorological variables (long-term 1971-2000) and soil type of the five areas selected for the globe artichoke clonal collection program.*

	Location				
	Buonfornello	**Caccamo**	**Cerda**	**Licata**	**Menfi**
Geographical coordinates					
Latitude	37°59' N	37°56' N	37°54' N	37°06' N	37°36' N
Longitude	13°53' E	13°40' E	13°49' E	13°56' E	12°58' E
Altitude (m a.s.l.)	54	171	274	8	109
Meteorological variables *					
Minimum air temperature (°C)	14	12	11	14	15
Maximum air temperature (°C)	24	22	21	22	21
Mean air temperature (°C)	19	17	16	18	18
Total rainfall (mm)	570	600	600	428	508
Soil type †	Xerorthents	Xerochrepts or Xerorthents	Xerochrepts or Xerorthents	Chromoxerents or Vertic xerofluents	Xerorthents or Xerofluents

* *average per year.*
† *: according to USDA Soil Taxonomy. The soil texture in mainly clay for all the locations*.

Table 2. Phenotypic variation in the 33 polymorphic UPOV descriptors. Eight-teen descriptors (in bold) are polymorphic within the 5 globe artichoke selected clones from landrace "Spinoso di Palermo"; 15 descriptors (in italics) are polymorphic between 'Spinoso di Palermo' and 'Violetto di Sicilia' genotypes. Character numbers and state score as reported in Supplementary table 1.

Character	Spinoso di Palermo					Violetto	
	A_1	A_4	A_6	E_3	E_7	M3	L1
2. Plant: N° of lateral shoots on main stem	2	1	1	1	1	2	2
10. Leaf: number of lobes	3	2	3	2	3	1	1
11. Leaf :length of longest lobe	2	2	3	2	2	1	1
13. Lobe: shape of tip (excluding terminal)	2	2	1	2	1	1	2
14. Lobe: N° of secondary lobes	2	3	5	2	2	2	3
15. Lobe: shape of tip (secondary lobes)	1	1	2	2	2	3	3
17. Leaf blade: intensity of green color (upper side)	1	1	2	2	1	1	2
18. Leaf blade: hue of green colour	3	1	2	3	3	3	3
20. Leaf: hairiness on upper side	2	3	2	4	3	2	2
22. Petiole: anthocyanin coloration at the base	3	2	2	2	3	2	2
24. Central flower head: diameter	1	1	2	1	1	1	1
30. First flower head on lateral shoot: length	3	3	3	2	3	3	3
31. First flower head on lateral shoot: diameter	1	2	1	1	1	1	1
41. Outer bract: colour	3	2	2	2	2	4	3
42. Outer bract: hue of secondary colour	3	2	2	2	2	4	3
46. Central head: anthocyanic col. of inner bracts	2	3	2	3	3	1	1
47. Central head: density of inner bracts	1	2	1	1	2	1	1
50. Receptacle: shape longitudinal section	1	1	1	1	2	1	1
7. Leaf: long spines	2	2	2	2	2	1	1
8. Leaf: length	3	3	3	3	3	2	3
9. Leaf: incision	2	2	2	2	2	1	1
21. Leaf blade: blistering	2	2	2	2	2	2	3
27. Central flower head: shape of tip	1	1	1	1	1	2	2
29. Central flower head: beginning of opening	2	2	2	2	2	1	1
32. First flower head on lateral shoot: size	1	1	1	1	1	1	2
34. First flower head on lateral shoot: degree of opening	2	2	2	2	2	1	1
37. Outer bract: thickness at base	2	2	2	2	2	1	2
38. Outer bract: main shape	1	1	1	1	1	3	3
39. Outer bract: shape of apex	1	1	1	1	1	2	2
43. Outer bract: reflexing of tip	1	1	1	1	1	2	2
44. Outer bract: size of spines	4	4	4	4	4	1	1
45. Outer bract: mucron	1	1	1	1	1	2	2
49. Receptacle: thickness	2	2	2	2	2	1	1

Table 3. *Mean square values of the main factors and their interaction, according to ANOVA*.

	Mean squares		
	Clone	**Season**	**Clone x Season**
Degrees of freedom	4	1	4
DFH (days)	3097.2 $^{P \leq 0.001}$	7248.1 $^{P \leq 0.001}$	3227.3 $^{P \leq 0.001}$
DHP (days)	1817.7 $^{P \leq 0.001}$	6238.4 $^{P \leq 0.001}$	915.7 $^{P \leq 0.01}$
Y (g/plant)	2.7 $^{P \leq 0.001}$	11.2 $^{P \leq 0.001}$	NS
NH (n/plant)	126.3 $^{P \leq 0.001}$	320.0 $^{P \leq 0.001}$	NS
RY (mg DM/plant/d)	15.2 $^{P \leq 0.001}$	NS	11.7 $^{P \leq 0.01}$
WM (g)	1596.8 $^{P \leq 0.01}$	NS	NS
WL (g)	1835.4 $^{P \leq 0.01}$	1812.0 $^{P \leq 0.05}$	NS

DFH: days to first harvest; DHP: duration of harvest period; Y: yield; NH: number of heads per plant; RY: rate of yield; WM: weight of main heads; WL: weight of lateral heads. (NS) not significant.

Table 4. *Bio-agronomical characterization of the selected globe artichoke genotypes.*

Variable	Clone	A_1	A_4	A_6	E_3	E_7	'Violet de Provence'	CV (%)	LSD ($P \leq 0.05$)	
									Clone	Clone x Season
DFH	Season I	144	130	153	158	140	143			
(days)	Season II	168	154	161	169	150	152			
	Mean	**156**	**142**	**157**	**163**	**145**	**147**	5	4	9
DHP	Season I	87	86	60	67	68	78			
(days)	Season II	81	85	84	75	82	82			
	Mean	**84**	**85**	**72**	**71**	**75**	**80**	8	6	12
Y	Season I	2.24	1.56	2.22	1.67	1.82	1.33			
(kg/plant)	Season II	2.50	2.44	2.70	2.15	2.06	1.47			
	Mean	**2.37**	**2.00**	**2.46**	**1.91**	**1.94**	**1.40**	19	0.25	NS
NH	Season I	15.6	10.2	13.4	11.2	12.2	11.6			
(n/plant)	Season II	17.0	15.0	16.4	12.8	14.0	12.0			
	Mean	**16.3**	**12.6**	**14.9**	**12.0**	**13.1**	**11.8**	13	1.6	NS
RY	Season I	3.5	2.4	5.1	3.5	3.6	2.4			
(mg DM/plant/d)	Season II	4.9	4.0	4.3	3.7	3.4	2.2			
	Mean	**4.2**	**3.2**	**4.7**	**3.6**	**3.5**	**2.3**	23	0.4	0.8
WM	Season I	214	213	222	196	206	187			
(g)	Season II	216	205	217	202	202	181			
	Mean	**215**	**209**	**220**	**199**	**204**	**184**	6	12	NS
WL	Season I	142	147	157	148	144	117			
(g)	Season II	144	157	161	164	146	109			
	Mean	**143**	**152**	**159**	**156**	**145**	**113**	12	9	NS

DFH: days to first harvest; DHP: duration of harvest period; Y: yield; NH: number of heads per plant; RY: rate of yield; WM: weight of main heads; WL: weight of lateral heads. (NS) not significant.

Table 5. *Genotypic and phenotypic components of variance of the traits in study.*

Variable	Value [1]		Variance		CV (%)		h^2_B
	Mean	Range	Genotypic	Phenotypic	g_{cv}	p_{cv}	
DFH (days)	153 ± 16	119 – 197	16.1	169.1	2.6	8.5	0.09
DHP (days)	77 ± 12	56 – 96	112.0	397.1	13.7	25.7	0.28
Y (g/plant)	2.14 ± 0.66	1.60 – 3.10	0.3	0.6	23.8	35.9	0.44
NH (n/plant)	13.83 ± 4.41	10.00 – 19.00	13.3	28.7	26.5	38.9	0.46
RY (mg DM/plant/d)	3.82 ± 0.88	1.79 – 6.82	0.4	2.9	17.1	44.2	0.19
WM (g)	209 ± 19	175 – 225	289.2	937.1	8.1	14.6	0.31
WL (g)	151 ± 11	140 – 181	173.5	597.6	8.7	16.2	0.29

DFH: days to first harvest; DHP: duration of harvest period; Y: yield; NH: number of heads per plant; RY: rate of yield; WM: weight of main heads; WL: weight of lateral heads.
[1]: values are referred to the whole two-seasons experiment.

Table 6. *Coefficients of correlation among the studied traits (n = 40).*

Variable	DFH (days)	DHP (days)	Y (g/plant)	NH (n/plant)	RY (mg DM/plant/d)	WM (g)
DFH (days)	-					
DHP (days)	NS	-				
Y (g/plant)	NS	NS	-			
NH (n/plant)	0.44 $^{P \leq 0.01}$	0.34 $^{P \leq 0.05}$	0.69 $^{P \leq 0.001}$	-		
RY (mg DM/plant/d)	0.73 $^{P \leq 0.001}$	NS	0.46 $^{P \leq 0.01}$	0.69 $^{P \leq 0.001}$	-	
WM (g)	NS	NS	NS	NS	NS	-
WL (g)	0.42 $^{P \leq 0.01}$	NS	NS	NS	0.34 $^{P \leq 0.05}$	NS

(NS) not significant;

Table 7. *Variation in the performance according to AFLP fingerprinting, based on: TNB: total number of fragments amplified, NPB: number of polymorphic fragments amplified, P%: percentage of variable fragments, PIC: polymorphism information content, N°Ge: number of genotypes fingerprinted, N°Cl: number of new clones fingerprinted.*

PC	**TNB**	**NPB**	**P%**	**PIC**	**N°Ge**	**N°Cl**
E35/T79	61	14	23.0	0.414	12	3
E35/T81	58	11	19.0	0.218	7	0
E35/T82	60	13	21.7	0.313	11	3
E35/T84	55	15	27.3	0.347	12	3
E38/T81	62	13	21.0	0.299	9	0
E38/T82	58	12	20.7	0.356	10	2
E38/T84	61	10	16.4	0.301	9	0
Total	415	88			19	5
Average	59.3	12.6	21.2	0.307		

Caption to figures

Fig. 1. UPGMA dendrogam based on 33 morphological traits from UPOV descriptors in 5 globe artichoke clones, selected from 'Spinoso di Palermo' (A_1, A_4, A_6, E_3 and E_7) and 2 selected from 'Violetto di Sicilia' (Violetto M_3 and Violetto L_1).

Fig. 2. UPGMA-based phylogeny of the five selected clones (A_1, A_4, A_6, E_3 and E_7) together with 12 genotypes of 'Spinoso di Palermo' and 2 of 'Violetto di Sicilia' (Violetto M_3 and Violetto L_1) included as references individuals, as derived from AFLP fingerprinting.

Figure 1

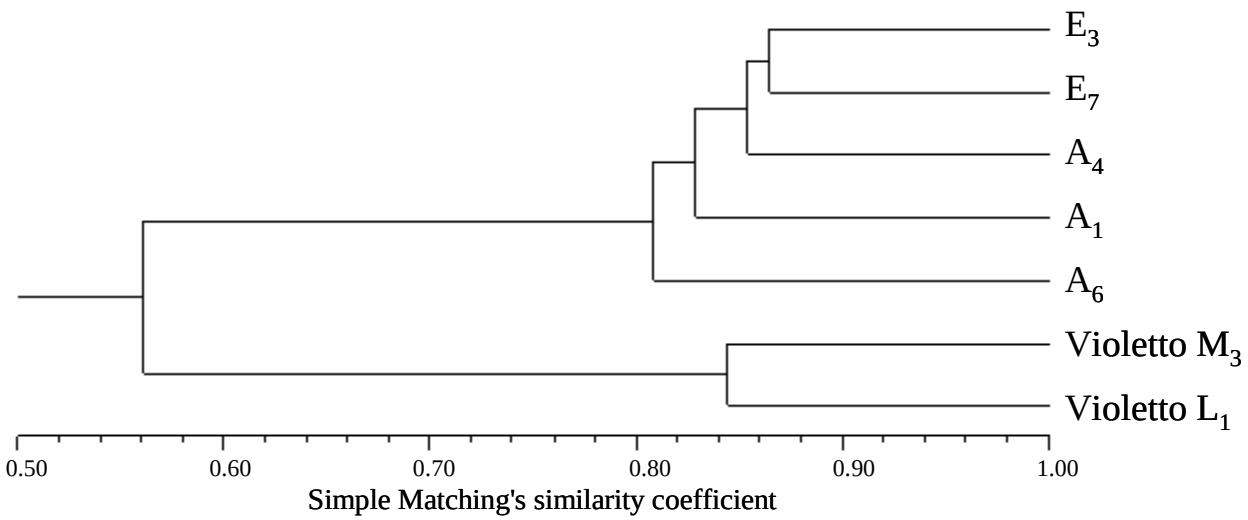
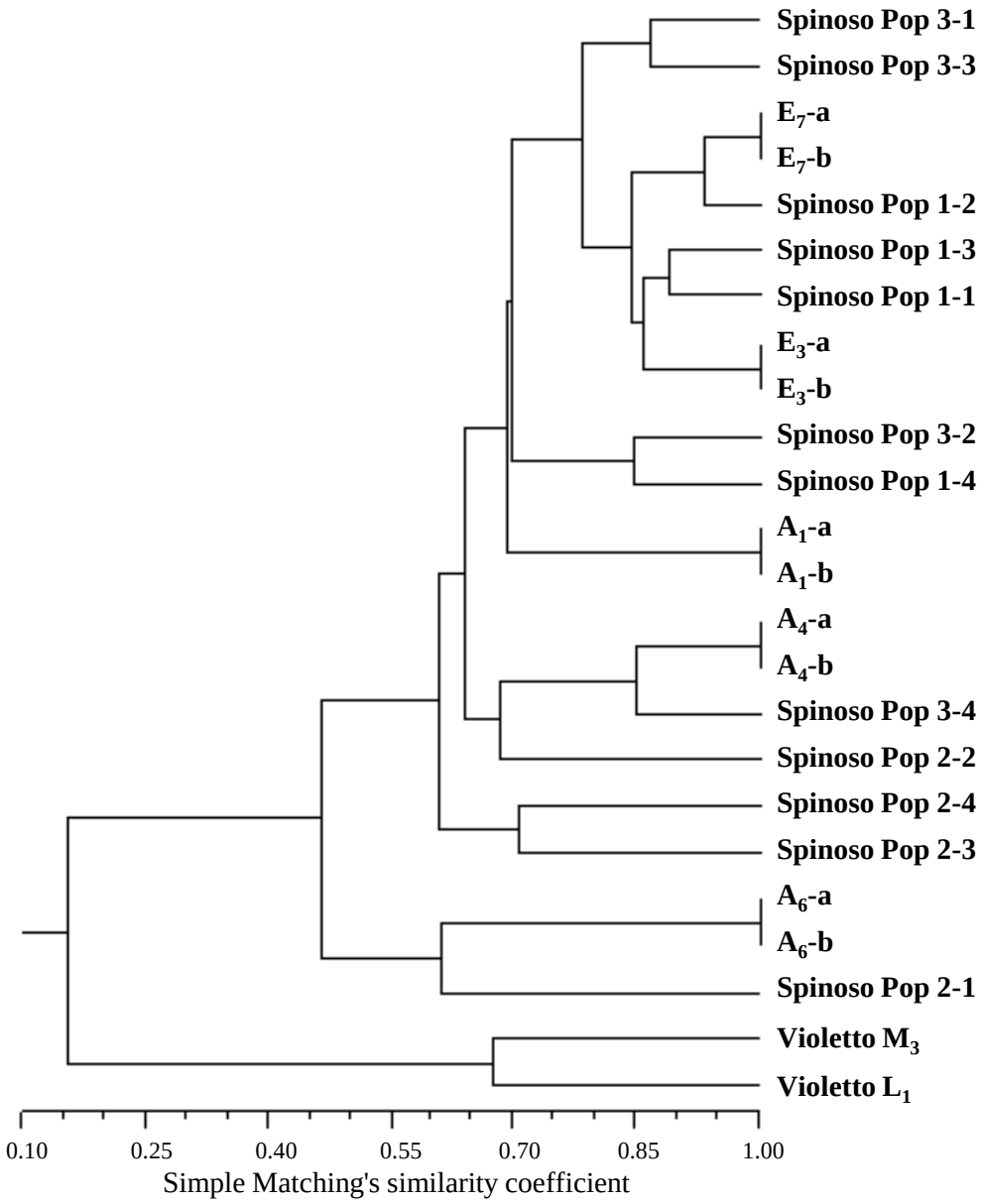

E3
E7
A4
A1
A6
Violetto M3
Violetto L1
0.50
0.60
0.70
0.80
0.90
1.00
Simple Matching's similarity coefficient

Figure 2


Spinoso Pop 3-1
Spinoso Pop 3-3
E7-a
E7-b
Spinoso Pop 1-2
Spinoso Pop 1-3
Spinoso Pop 1-1
E3-a
E3-b
Spinoso Pop 3-2
Spinoso Pop 1-4
A1-a
A1-b
A4-a
A4-b
Spinoso Pop 3-4
Spinoso Pop 2-2
Spinoso Pop 2-4
Spinoso Pop 2-3
A6-a
A6-b
Spinoso Pop 2-1
Violetto M3
Violetto L1
0.10
0.25
0.40
0.55
0.70
0.85
1.00
Simple Matching's similarity coefficient

Supplemental materials

Supplementary Table 1. List of UPOV traits used for morphological characterization. Eighteen traits were uninformative, as they were not able to detect polymorphisms among the set of globe artichoke genotypes in study (underlined traits and states). Eighteen traits were polymorphic within the 'Spinoso di Palermo' clones (traits reported in bold).

Supplementary Figure 1. Meteorological data for temperature (minimum and maximum) and monthly rainfall at the experimental area for 2 growing seasons

Supplementary Table 1.

Character	Scale	Score
1. Plant: height	Short	1
	Medium	2
	Tall	3
2. Plant: N° of lateral shoots on main stem	Few	1
	Medium	2
	Many	3
3. Main stem: height	Short	1
	Medium	2
	Tall	3
4. Main stem: Distance main head - youngest developed leaf	Short	1
	Medium	2
	Tall	3
5. Main stem: diameter	Small	1
	Medium	2
	Large	3
6. Leaf: attitude	Erect	1
	Semi-erect	2
	Horizontal	3
7. Leaf: long spines	Absent	1
	Present	2
8. Leaf: length	Short	1
	Medium	2
	Long	3
9. Leaf: incision	Absent	1
	Present	2
10. Leaf: number of lobes	Few	1
	Medium	2
	Many	3
11. Leaf: length of longest lobe	Short	1
	Medium	2
	Long	3
12. Leaf: width of longest lobe	Narrow	1
	Medium	2
	Broad	3
13. Lobe: shape of tip (excluding terminal)	Acute	1
	Right angle	2
	Obtuse	3
14. Lobe: N° of secondary lobes	Very few	1
	Few	2
	Medium	3
	Many	4
	Very many	5
15. Lobe: shape of tip (secondary lobes)	Acuminate	1
	Acute	2
	Rounded	3
16. Leaf blade: shape in cross section	Flat	1
	V shaped	2
17. Leaf blade: intensity of green color (upper side)	Light	1
	Medium	2
	Dark	3
18. Leaf blade: hue of green colour	Absent	1
	Yellowish	2
	Greyish	3
19. Leaf blade: intensity of grey hue	Weak	1
	Medium	2
	Strong	3
20. Leaf: hairiness on upper side	Very weak	1
	Weak	2
	Medium	3
	Strong	4
	Very strong	5
21. Leaf blade: blistering	Very weak	1
	Weak	2
	Medium	3
	Strong	4
	Very strong	5
22. Petiole: anthocyanin coloration at the base	Very weak	1
	Weak	2
	Medium	3
	Strong	4
	Very strong	5
23. Central flower head: length	Short	1
	Medium	2
	Long	3
24. Central flower head: diameter	Small	1
	Medium	2
	Large	3
25. Central flower head: size	Small	1
	Medium	2
	Large	3
26. Central flower head: shape longitudinal section	Circular	1
	Broad elliptical	2
	Ovate	3
	Triangular	4
	Transverse broad elliptical	5
27. Central flower head: shape of tip	Acute	1
	Rounded	2
	Flat	3
	Depressed	4
28. Central flower head: time of appearance	Early	1
	Medium	2
	Late	3
29. Central flower head: beginning of opening	Early	1
	Medium	2
	Late	3
30. First flower head on lateral shoot: length	Short	1
	Medium	2
	Long	3
31. First flower head on lateral shoot: diameter	Small	1
	Medium	2
	large	3
32. First flower head on lateral shoot: size	Small	1
	Medium	2
	large	3
33. First flower head on lateral shoot: shape in longitudinal section	Circular	1
	Broad elliptic	2
	Ovate	3
	Triangular	4
	Transverse broad elliptic	5
34. First flower head on lateral shoot: degree of opening	Weak	1
	Medium	2
	Strong	3
35. Outer bract: length of base	Short	1
	Medium	2
	Long	3
36. Outer bract: width of base	Narrow	1
	Medium	2
	Broad	3
37. Outer bract: thickness at base	Thin	1
	Medium	2
	Thick	3
38. Outer bract: main shape	Broader than long	1
	As broad as long	2
	Longer than broad	3
39. Outer bract: shape of apex	Acute	1
	Flat	2
	Emarginated	3
40. Outer bract: depth of emargination	Shallow	1
	Medium	2
	Deep	3
41. Outer bract: colour	Green	1
	Green/ violet	2
	Violet/green	3
	Mainly violet	4
	Entirely violet	5
42. Outer bract: hue of secondary colour	Absent	1
	Bronze	2
	Grey	3
43. Outer bract: reflexing of tip	Absent	1
	Present	2
44. Outer bract: size of spines	Absent/very small	1
	Small	2
	Medium	3
	Large	4
	Very large	5
45. Outer bract: mucron	Absent	1
	Present	2
46. Central head: anthocyanin coloration of inner bracts	Absent/very weak	1
	Weak	2
	Medium	3
	Strong	4
	Very strong	5
47. Central head: density of inner bracts	Sparse	1
	Medium	2
	Dense	3
48. Receptacle: diameter	Small	1
	Medium	2
	Large	3
49. Receptacle: thickness	Thin	1
	Medium	2
	Thick	3
50. Receptacle: shape longitudinal section	Flat	1
	Slightly depressed	2
	Strongly depressed	3
51. Tendency to produce lateral shoots at base	Weak	1
	Medium	2
	Strong	3

Supplementary Figure 1