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# UNIVERSITÀ DEGLI STUDI DI TORINO

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**Genotypic and bio-agronomical characterization of an early Sicilian  
landrace of globe artichoke**

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## Summary

In Sicily, the increasing use of exotic globe artichoke germplasm is eroding the presence of autochthonous landraces, including the long established 'Violetto di Sicilia'. Ten clones have emerged from a clonal selection programme in this landrace, and here we describe the variation that they capture both at the level of AFLP-based genotype and phenotypically with respect to key productivity traits, on the basis of two seasons of field evaluation. The clonal selections yielded, on average, 8.9 heads per plant (equivalent to a fresh weight yield of 1.28 kg). Two clones yielded particularly well in both growing seasons (10.6 heads, equivalent to 1.46 kg per plant), while another pair produced particularly large heads (on average 165 g) and a high receptacle incidence (on average 19.3%). Both the number of days to first harvest and the quantity of head dry matter were subject to a significant degree of 'clone x year' interaction. Yield, the number of heads per plant and receptacle incidence were associated with a moderate (0.30-0.53) broad sense heritability, indicating that these traits could be successfully improved by phenotype-based clonal selection. AFLP fingerprinting was able to discriminate between all the clones, based on only three primer combinations. A principal component analysis based on the AFLP fingerprints was used to compare the selected clones with a set of individuals chosen on the basis of maximum genetic diversity. This comparison suggested that the new clone set was representative of the genetic variation present in 'Violetto di Sicilia', because the diversity captured by the two sets was largely overlapping, confirming the possibility of carrying out clonal selection in this globe artichoke landrace without compromising its preservation *in situ*.

**Keywords** *Cynara cardunculus* var. *scolymus*, clonal selection, landrace, germplasm preservation

## Introduction

The globe artichoke [*Cynara cardunculus* L. var. *scolymus* (L.) Fiori] is a herbaceous perennial *Asteraceae* species native to the Mediterranean basin. Its immature inflorescence (referred to as a 'head' or 'capitulum') is used as a vegetable (Bianco and Pace 2009; Marzi and Vanadia 2009). Its global cropping area (concentrated mostly in the Mediterranean basin) of 133 kha produces ~1.5 Mt heads per annum (FAOSTAT 2009). The growing reputation of globe artichoke as a functional food is helping to encourage its cultivation in other parts of the world (Lattanzio et al. 2009; Lombardo et al. 2010; Pandino et al. 2010, 2011). The species is mainly allogamous, and thus tends to be highly heterozygous; as a result it displays plenty of phenotypic variation (Foury 1987; Basnizki and Zohary 1994; Mauromicale and Ierna 2000). Although a small number of seed-propagated cultivars is available, most cultivated germplasm remain vegetatively propagated, employing either semi-dormant or actively growing basal and lateral offshoots, or stump pieces. Since its domestication started around 2,000 years ago (Foury 1989), many well-differentiated landraces have evolved, reflecting a degree of regional variation in growing environment and consumer preference (Mauromicale et al. 2000; Lanteri et al. 2004a, b). Currently, some 120 genotypes are in cultivation, varying with respect to their harvesting time and capitulum traits like dimension, shape, presence/absence of spines, pigmentation of the outer bracts form (Basnizki and Zohary 1994; Lanteri and Portis, 2008). The so-called 'reflowering types' can be induced to produce capitula between autumn and spring, if dormant underground shoots used for propagation are transplanted and watered during the summer; whereas, late flowering types produce capitula only during spring. The most extensive primary globe artichoke gene pool remains in Italy, thought to be the site of its domestication and later diffusion (Foury 1987; Sonnante et al. 2007; Mauro et al. 2009). Dellacecca et al. (1976) showed that as many as 80 of a collection of 115 world cultivars

were of Italian origin, while recently, in a collection of autochthonous landraces collected from Sicilian family gardens, Mauro et al. (2009) were able to demonstrate introgression from wild to cultivated forms.

The survival of traditional landraces in southern Italy is threatened by the introduction of exotic germplasm (e.g. ‘Romaneschi’, ‘Terom’, ‘Tema 2000’, ‘Apollo’, the allochthonous ‘Violet de Provence’ from France) and seed-propagated F<sub>1</sub> hybrids (Ierna and Mauromicale 2004; Lo Bianco et al. 2011). The reflowering landrace ‘Violetto di Sicilia’ has for many years been an important component of the southern Italian rural economy (Mauromicale and Ierna 2000). AFLP (amplified fragment length polymorphism) fingerprinting has shown that this landrace is highly heterogeneous (Portis et al. 2005), giving ample opportunity for a clonal selection programme aimed at identifying elite individuals, while at the same time implementing an *in situ* conservation strategy to guard against genetic erosion.

In this paper, we report the characterization of the phenotypic and genotypic variation present in ten clones of ‘Violetto di Sicilia’, with the goal of improving the efficiency of our ongoing selection programme within this globe artichoke landrace.

## **Materials and Methods**

### *Plant materials and research site*

The four locations sited in eastern Sicily used for plant sampling are representative of the cultivation area of ‘Violetto di Sicilia’. These sites were: Caltagirone (37°14’N 14°31’E, 608 m a.s.l.), Niscemi (37°9’N 14°23’E, 332 m a.s.l.), Ramacca (37°23’N 14°42’E, 270 m a.s.l.) and Rosolini (36°49’N 14°57’E, 154 m a.s.l.). At each site, a sample of 7-10 plants, previously labelled, was taken from a same stand during summer 2006 (in total 36

selections), based on consideration of the number of floral stem ramifications (an index of yield potential), earliness, and head colour, shape and thickness. From each selection, 3-10 semi-dormant offshoots were taken for planting at the University of Catania's experimental station (37°25'N; 15°30'E; 10 m a.s.l.). The local climate consists of mild and wet winters (low probability of frost occurrence) and warm, dry summers. During the 2006-2007 and 2007-2008 growing seasons 26 clones were discarded, while the number of plants of the remaining selections was increased to 60; this allowed the final identification of ten clones (C<sub>1</sub> to C<sub>10</sub>) including at least one plant for each sampled site, which were then characterized in more detail during the 2008-2009 and 2009-2010, by monitoring with respect to a number of head traits and yield potential. In August 2008 ovuli from each clone were collected and planted in rows of 20 plants separated from one another by 0.80 m. The inter-row spacing was set at 1.25 m, so that the overall planting density was one plant per m<sup>2</sup>. The rows were arranged in a randomised strip-plot design with three replications, each of 48 plants (net of border plants). Starter fertilization was done before planting (or awakening) with 70, 180 and 140 kg ha<sup>-1</sup> of N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O, respectively. Further two N applications (as ammonium nitrate) were effected at a rate of 70 kg ha<sup>-1</sup> on early-November and late-February, respectively. On both growing seasons, experimental units were drip irrigated from August to mid October, 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 cm depth). The second growing season (2009-2010) was initiated by applying drip irrigation to field capacity in early August 2009, while weed and pest management were performed as per local custom.

Air temperature (minimum, maximum and mean), relative humidity (minimum, maximum and mean), soil temperature at a depth of 20 cm (minimum, maximum and

mean), wind direction and speed, global radiation, photosynthetically active radiation, rainfall and evaporation were recorded every 30 minutes by means of a meteorological station (Multirecorder 2.40; ETG, Florence, Italy) sited about 200 m from the experimental field. The precipitation during the 2008-2009 season was higher than average, with 85% (519 out of 610mm) falling in the period September to January. The precipitation during 2009-2010 season was 574 mm, with 82% of the rainfall experienced between September and February. Higher mean monthly temperatures were recorded in the 2009-2010 season than in 2008-2009, especially during December (15.4 vs. 12.8°C), February (12.6 vs. 10.3°C) and March (13.0 vs. 12.3°C).

#### *DNA extraction and AFLP genotyping*

DNA was extracted from young leaves collected in mid December, following Lanteri et al. (2004b). Ten DNA samples representative of the genetic variation within 'Violetto di Sicilia' found by Portis et al. (2005) were included. The AFLP protocol applied to these DNAs was that described by Lanteri et al. (2004b). The first amplification was based on the primer combination (PC) *Eco*RI+A / *Taq*I+T, and the second on one of the seven PCs 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 and Matthews (2000). The polymorphic information content (PIC) was calculated by setting the expected heterozygosity to  $2f(1-f)$ , following Anderson et al. (1993). ( $f$  represents the proportion of individuals carrying a particular AFLP locus.) The amplified fragments (of size 60-650bp) were each assumed to represent a single bi-allelic locus, so that the profiles could be assessed in terms of presence or absence of each polymorphic fragment, to produce a



binary genotypic matrix. The effective multiplex ratio (EMR) of each PC was determined as described by Milbourne et al. (1997) and a marker index (MI) was calculated by multiplying the PIC by the EMR (Powell et al., 1996). The binary matrix was imported into NTSYS-pc software (Rohlf 1998) to perform a standard cluster analysis. The genetic similarity between each pair of individuals was estimated from the Jaccard (1908) similarity index (JSI), which was then used as the basis of a principal coordinate analysis (PCoA), in which the first two axes were plotted graphically, according to their extracted eigen vectors. An UPGMA-based dendrogram (Sneath and Sokal 1973) was constructed for the ten clonal selections. Co-phenetic matrices were produced using hierarchical clustering, and these were correlated with the raw distance matrix, in order to identify associations between the clustering and the similarity matrix.

#### *Phenotypic variation and analysis of variance*

The ten clonal selections were characterized over the 2008-2009 and 2009-2010 seasons. Marketable heads were collected just before bract divergence, corresponding to stage D as described by Foury (1967). The fresh weight of the head without the floral stem was determined, and a sample of heads (48 per order) was dried at 105°C for ~72 h in order to measure their dry matter content. The following six variables were documented: days to first harvest (DFH), representing the number of days between transplanting (2008-2009) or awakening (2009-2010) and harvesting of the main head; fresh head yield (Y) per plant; the number of heads per plant (NH), head unitary weight (HW), the incidence of receptacle on head weight (as the ratio weight among receptacle and the corresponding head, IN) and head dry matter content (DM). Collected data were first subjected to Levene's test to check for homoscedasticity, then to a two-way ('clone x season') analysis of variance (ANOVA) related to the experimental layout. Data points recorded as

percentages were subjected to the Bliss' transformation prior to the ANOVA. The data were also subjected to a multiple correlation analysis, followed by a principal component analysis (PCA). The first two principal components were correlated with the original trait data, and those showing a correlation  $>0.6$  were considered as relevant for the ordination analysis (Matus et al. 1996). Variance components were estimated according to a factorial random model with years and clones taken as random factors (Cosentino et al. 2006). The variance for each trait ( $\sigma_p^2$ ) was considered to be the sum of the genotypic ( $\sigma_g^2$ ) and environmental ( $\sigma_e^2$ ) components. Since  $\sigma_e^2$  can be equated to the error expected mean square (EMS), then  $\sigma_p^2 = \sigma_g^2 + \text{EMS}_{\text{error}}$ .  $\sigma_g^2$  was estimated from the expression  $1/ry$  ( $\text{EMS}_{\text{clones}} - \text{EMS}_{\text{clones} \times \text{year}}$ ), equivalent to  $1/ry [(\sigma_e^2 + r\sigma_{gy}^2 + ry\sigma_g^2) - (\sigma_e^2 + r\sigma_{gy}^2)]$ , where  $r$  represents the number of replicates (3), and  $y$  the number of seasons (2). The ratio  $\sigma_g^2 / \sigma_p^2$  was used to estimate the broad sense heritability ( $h_B^2$ ) for each trait. Genotypic ( $g_{cv}$ ) and phenotypic ( $p_{cv}$ ) coefficients of variation were calculated as, respectively,  $(\sqrt{\sigma_g^2/x}) 100$  and  $(\sqrt{\sigma_p^2/x}) 100$ .

## Results

### *Genotype and genetic relatedness*

The seven PCs amplified 433 fragments of which 50 (12%) were polymorphic across the whole set of the 20 genotypes (10 selected and 10 reference) (Table 1). The mean number of polymorphic fragments per PC was 7.1 (range 5-11). E38/T82 was associated with the highest PIC, while E35/T79 generated the greatest number of polymorphisms, produced the highest MI and was able to discriminate between 16 of the 20 templates, including eight of the ten clonal selections. The lowest PIC and MI were generated by E35/T84,

which only discriminated seven of the templates. All 20 templates could be discriminated from one another on the basis of the three PCs E35/T79, E35/T81 and E38/T84. Clonal selections C<sub>2</sub>, C<sub>3</sub> and C<sub>10</sub> each possessed unique fragment(s), which have the potential to be converted into sequence tagged site assays for their simple identification. The most similar pair of clones was C<sub>6</sub> and C<sub>10</sub> (JSI=0.84), and the most dissimilar (JSI=0.14) C<sub>3</sub> and C<sub>4</sub>. The AFLP-based PCoA scatter-plot is shown in Figure 1. The first two principal co-ordinates accounted for, respectively, 39.8% and 27.9% of the genotypic variation; the former identified clone C<sub>3</sub> away from the other clones and from all of the reference templates. The calculated UPGMA-based dendrogram is shown as Figure 2. Clones C<sub>6</sub>, C<sub>10</sub>, C<sub>9</sub> and C<sub>8</sub> clustered together with a mean genetic similarity of about 80%; clones C<sub>3</sub> and C<sub>2</sub> were highly genetically differentiated, sharing the least genetic similarity with the cluster containing all the other clones. The co-phenetic correlation coefficient between the data matrix and the co-phenetic matrix for AFLP data was 0.95, implying a very good fit between the dendrogram clusters and the similarity matrices from which they had been derived.

#### *Phenotypic characterization, variance components and traits heritability*

The mean squares relating to 'clone', 'season' and the 'clone x season' interaction are reported in Table 2, and traits performances summarized in Table 3. All traits were significantly variable and, with the exception of HW, were all season-dependent as well. There was a significant 'clone x season' interaction for DFH and DM. The coefficients of variation were 3.3% for DFH, and 12.4% for NH. DFH, averaged over the two growing seasons, was 163 days. C<sub>6</sub> and C<sub>10</sub> were the latest maturing clones (DFH of 171 days on average), while the earliest ones were C<sub>5</sub>, C<sub>7</sub> and C<sub>9</sub> (157 days on average). The difference between these two groups of clones was ten days in the first season and 17 in the second

(data not reported). The average Y was 1.28 kg plant<sup>-1</sup>, with the best performing clones achieving 1.51 (C<sub>6</sub>), 1.40 (C<sub>3</sub>) and 1.35 kg plant<sup>-1</sup> (C<sub>1</sub>); clones C<sub>5</sub>, C<sub>7</sub> and C<sub>8</sub> were the poorest yielders (1.18 kg plant<sup>-1</sup> on average). The average NH was 8.9, ranging from 7.5 (C<sub>4</sub>) to 10.9 (C<sub>3</sub>), with an overall difference of 45% between the extremes. For HW (averaging 151 g across clones and seasons), the performance ranged from 139 (C<sub>1</sub>) to 177 g (C<sub>4</sub>), while IN ranged from 14.3 (C<sub>6</sub>) to 19.8 g 100 g<sup>-1</sup> fresh weight (C<sub>4</sub>). The average DM was 14.2 g 100 g<sup>-1</sup> fresh weight, varying from 13.2 (C<sub>3</sub> and C<sub>6</sub>) to 15.9 (C<sub>5</sub>). Table 4 reports the estimated variance components, along with  $g_{cv}$ ,  $p_{cv}$  and  $h^2_B$ . The genotypic and phenotypic variances and their associated coefficients of variation differed greatly from trait to trait, while  $h^2_B$  varied from 0.23 (DFH, DM) to 0.53 (NH and IN). Both Y (0.30) and HW (0.27) showed intermediate levels of heritability.

#### *Phenotypic correlations and principal component analysis*

Table 5 shows the trait correlation matrix. DFH was positively correlated with Y ( $r=0.798^{**}$ ) and NH ( $0.702^*$ ), but negatively with IN ( $-0.729^{**}$ ). Meanwhile, Y was strongly and positively correlated with NH ( $0.842^{***}$ ) and negatively with both the IN ( $-0.805^{**}$ ) and DM ( $-0.758^{**}$ ). A very strong correlation was recorded between NH and IN ( $-0.880^{***}$ ), as well as between the latter and DM ( $0.890^{***}$ ). The first two principal components gave eigenvalues  $>1$ , and together accounted for 89.8% of the total variance (Table 6). NH, Y and DFH contributed strongly and positively to the first principal component (74.7% of variance), while IN, DM and, to a lesser extent, HW made a negative contribution. The second component was influenced by HW. The PCA scatter plot is illustrated in Figure 3. The first axis identified a cluster of four clones (C<sub>1</sub>, C<sub>3</sub>, C<sub>6</sub> and C<sub>10</sub>) showing high values for Y and NH, a low IN and a high DFH. Clones C<sub>10</sub> and C<sub>6</sub>, which are genetically rather similar to one another, were distinguishable from the other

two on the basis of their higher HW. Apart from C<sub>4</sub>, the remaining clones clustered largely on the basis of having a low NH, a high IN and a low DFH. Clone C<sub>4</sub> was somewhat of an outlier, thanks to its high HW.

The cophenetic correlation between the phenotypic and the genetic variance was low (0.261), but significant ( $p=0.002$ ). The genotype-based clustering shared some similarity with that based on phenotype: specifically the pairing of C<sub>6</sub> with C<sub>10</sub> and of C<sub>9</sub> with C<sub>8</sub> was discernible in both data sets; however C<sub>1</sub> and C<sub>3</sub>, which appeared to be genotypically well differentiated from one another (Fig. 2), fell into the same phenotypic cluster (Fig. 3)

## **Discussion**

One effect of the increasing globalization of globe artichoke cultivation will be a steady substitution of autochthonous germplasm with exotic cultivars (Sonnante et al. 2007; Lanteri and Portis 2008). Clonal selection within a traditional landrace has been proposed as a strategy which could allow for crop improvement while simultaneously implementing an process of *in situ* conservation (Mauromicale and Ierna 2000). Here, we have characterized a set of ten globe artichoke clones selected from the landrace ‘Violetto di Sicilia’. The clones were distinguishable from one another on the basis of their AFLP profile. This form of genotypic profiling frequently provide a particularly efficient means of discriminating between sets of closely-related individuals, thanks to the large number of genetic loci which it assays in one reaction (Lanteri et al. 2004a; Portis et al. 2005; Acquadro et al. 2010). Only three AFLP PCs (generating 25 polymorphic fragments) were needed to fully separate all ten clonal selections. As some of these fragments were clone-

specific, the possibility arises of converting the rather cumbersome AFLP assay into a much simpler PCR-based test of clone identity.

Consistent with the known complex inheritance of the specific traits studied here, the seasonal influence over trait expression was high; nevertheless, the relative performance of the clonal selections was stable for four of the six traits, specifically Y, NH, HW and IN. All four of these traits were associated with high genetic ( $g_{cv}$  ranging from 12.9 to 26.1) and phenotypic ( $p_{cv}$  of 25.0-36.0) coefficients of variation, indicating that genetic progress should be readily achievable in the 'Violetto di Sicilia' population. On the other hand, for both DFH and DM there was a significant 'clone x season' interaction, meaning that selection on the basis of these two traits is likely to be less effective. Most importantly perhaps, Y varied significantly between clones (by up to 0.33 g plant<sup>-1</sup>) and its intermediate level of  $h^2_B$  implies that there is potential for improvement through clonal selection. Indeed, we have identified two clones (C<sub>6</sub> and C<sub>3</sub>) yielding particularly well (almost 15 t ha<sup>-1</sup>), a level which is considerably higher (~50%) than that of 'Violetto di Sicilia' itself (Mauromicale and Copani 1989).

In a study based on a diverse set of globe artichoke clones, López Anido et al. (1998) were able to demonstrate the possibility of enhancing both Y and its associated traits NH and HW. Similarly, Mauro et al. (2009) showed that Y was positively correlated with both NH and the weight of the secondary heads. In our experiment, whereas Y was strongly dependent on NH, there was no significant relationship between Y and HW, which is taken to indicate that the yield of 'Violetto di Sicilia' is most closely associated with the plants' capacity to produce heads from secondary stems (data not shown), rather than the weight of each head *per se*. On the other hand, there was an inverse relationship between Y and both earliness and DM, reflecting the effect of source/sink competition, a typical feature of many plants with a determinate growth habit. With respect to the heads

characteristics, high  $g_{cv}$  and  $p_{cv}$  values were recorded for both HW and IN, the latter showing in addition a particularly high value of  $h^2_B$  (0.53). Together with the positive, significant association between these traits, we can conclude that genetic progress can be made in the end-use quality of 'Violetto di Sicilia' heads via clonal selection. This outcome differs from the experience in the same globe artichoke landrace reported by Mauromicale and Copani (1989). A possible explanation for this apparent discrepancy is that the genetic base of the present set of clones may have been wider than the one in the previous study. Despite their lower level of productivity, clones C4 and C5 appear particularly promising in any case, as they showed a favourable combination between HW and IN, which are extremely important in influencing consumer preference.

The phenotypic and the genotypic data were only marginally correlated with one another, most probably because most of the AFLP loci were sited in the non-coding portion of the genome, and therefore have little or no impact on phenotype. At the same time, the expression of quantitative traits is typically much affected by environmental conditions, and this component of variation cannot be expected to be correlated to any variation at the genotypic level (Kwon et al. 2005). This degree of disagreement between the genotypic and the phenotypic distances means that conclusions reached on the grounds of similarity (or distinctiveness) will depend on the particular trait in question and how they have been treated, so that establishing correlations between phenotypic and the genotypic data becomes dependent on the number of DNA markers and traits available for comparison (Bernet et al. 2003). Genotypic characterization allows for a much greater resolution in discriminating individuals than does phenotypic characterization (e.g., Dillmann et al. 1997; Tatineni et al. 1996; Bernet et al. 2003), as we have found as well. The advantage of genotypic data lies primarily in the much larger number of independent

variables (in this case genetic loci) which can be assayed, and also that there is zero interference from the environment.

The AFLP-based PCoA of the ten clonal selections and a set of ten individuals chosen from the same landrace on the basis of maximum genetic diversity (Portis et al. 2005) showed that the new clone set was representative of the genetic variation present in ‘Violetto di Sicilia’, because the diversity captured by the two sets was largely overlapping. This confirms the viability of performing clonal selection within the landrace, without compromising its long term preservation.

## **Conclusions**

We have demonstrated here the success of the clonal selection strategy as a means of improving certain traits of ‘Violetto di Sicilia’ without endangering its *in situ* conservation. In particular, the four traits Y, NH, HW and IN can be regarded as suitable targets for the improvement of ‘Violetto di Sicilia’. The peculiarities of these clones could enhance the convenience of those techniques such as micropropagation, nursery production or mycorrhization (Morone Fortunato et al. 2005; Acquadro et al. 2010), in the double perspective to improve the globe artichoke cultivation in the Mediterranean environment and protect, at the same time, the traditional germplasm against the growing threat of genetic erosion.

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Table 1. Variation in the performance of the AFLP primer combinations used. TNB: total number of fragments amplified, NPB: number of polymorphic fragments amplified, P%: percentage of variable fragments, PIC: polymorphism information content, MI: marker index, N°Ge: number of genotypes fingerprinted, N°Cl: number of new clones fingerprinted.

<b>PC</b>	<b>TNB</b>	<b>NPB</b>	<b>P%</b>	<b>PIC</b>	<b>MI</b>	<b>N°Ge</b>	<b>N°Cl</b>
<b>E35/T79</b>	<b>68</b>	<b>11</b>	<b>0.16</b>	<b>0.373</b>	<b>15.54</b>	<b>16</b>	<b>8</b>
<b>E35/T81</b>	<b>61</b>	<b>7</b>	<b>0.11</b>	<b>0.398</b>	<b>7.40</b>	<b>11</b>	<b>6</b>
<b>E35/T82</b>	<b>66</b>	<b>7</b>	<b>0.11</b>	<b>0.316</b>	<b>5.37</b>	<b>8</b>	<b>6</b>
<b>E35/T84</b>	<b>57</b>	<b>6</b>	<b>0.11</b>	<b>0.203</b>	<b>3.04</b>	<b>7</b>	<b>5</b>
<b>E38/T81</b>	<b>59</b>	<b>7</b>	<b>0.12</b>	<b>0.228</b>	<b>4.32</b>	<b>9</b>	<b>7</b>
<b>E38/T82</b>	<b>55</b>	<b>5</b>	<b>0.09</b>	<b>0.420</b>	<b>4.35</b>	<b>9</b>	<b>6</b>
<b>E38/T84</b>	<b>67</b>	<b>7</b>	<b>0.10</b>	<b>0.313</b>	<b>5.29</b>	<b>10</b>	<b>6</b>
<b>total</b>	<b>433</b>	<b>50</b>				<b>20</b>	<b>10</b>
<b>average</b>	<b>61.9</b>	<b>7.1</b>	<b>0.12</b>	<b>0.324</b>	<b>6.24</b>		

Table 2. Analysis of variance at the phenotypic level. Mean square values relating to the main factors and their interaction are shown. DFH: number of days to first harvest, Y: yield, NH: number of heads per plant, HW: heads weight, IN: incidence of receptacle on head weight, DM: head dry matter content.

Variable	Mean squares		
	Clone	Season	Clone x Season
Degrees of freedom	9	1	9
DFH (days)	883 ***	11107 ***	478 *
Y (kg plant <sup>-1</sup> )	0.38 ***	2.12 ***	NS
NH (n plant <sup>-1</sup> )	36 ***	90 ***	NS
HW (g)	3411 ***	NS	NS
IN (g 100 g <sup>-1</sup> FW)	87 ***	111 **	NS
DM (g 100 g <sup>-1</sup> FW)	24 ***	89 ***	13 *

NS: not significant; \*, \*\*, \*\*\*: significant at  $P \leq 0.05$ , 0.01 and 0.001, respectively.

Table 3. Phenotypic characterization of clonal selections. Each datum represents the mean  $\pm$  standard error of the mean averaged over two growing seasons. SED: standard error of the difference. DFH: number of days to first harvest, Y: yield, NH: number of heads per plant, HW: heads weight, IN: incidence of receptacle on head weight, DM: head dry matter content.

Clone	DFH (days)	Y (kg plant <sup>-1</sup> )	NH (n plant <sup>-1</sup> )	HW (g)	IN (g 100 g <sup>-1</sup> FW)	DM (g 100 g <sup>-1</sup> FW)
C <sub>1</sub>	166 $\pm$ 2	1.35 $\pm$ 61	9.8 $\pm$ 0.4	139 $\pm$ 5	15.8 $\pm$ 0.9	13.6 $\pm$ 0.4
C <sub>2</sub>	161 $\pm$ 3	1.26 $\pm$ 56	8.8 $\pm$ 0.3	148 $\pm$ 6	17.2 $\pm$ 0.7	14.0 $\pm$ 0.5
C <sub>3</sub>	166 $\pm$ 4	1.40 $\pm$ 49	10.9 $\pm$ 0.8	140 $\pm$ 6	15.1 $\pm$ 0.6	13.2 $\pm$ 0.3
C <sub>4</sub>	161 $\pm$ 3	1.25 $\pm$ 59	7.5 $\pm$ 0.3	177 $\pm$ 6	19.8 $\pm$ 0.6	15.4 $\pm$ 0.4
C <sub>5</sub>	156 $\pm$ 3	1.13 $\pm$ 55	7.8 $\pm$ 0.3	152 $\pm$ 7	18.8 $\pm$ 0.7	15.9 $\pm$ 0.4
C <sub>6</sub>	171 $\pm$ 3	1.51 $\pm$ 62	10.2 $\pm$ 0.4	150 $\pm$ 5	14.3 $\pm$ 0.6	13.2 $\pm$ 0.5
C <sub>7</sub>	157 $\pm$ 4	1.18 $\pm$ 62	8.3 $\pm$ 0.3	147 $\pm$ 6	18.4 $\pm$ 0.5	14.4 $\pm$ 0.7
C <sub>8</sub>	161 $\pm$ 3	1.22 $\pm$ 54	8.4 $\pm$ 0.3	147 $\pm$ 8	16.9 $\pm$ 0.7	14.2 $\pm$ 0.6
C <sub>9</sub>	158 $\pm$ 4	1.23 $\pm$ 57	8.1 $\pm$ 0.3	156 $\pm$ 7	16.6 $\pm$ 0.7	13.7 $\pm$ 0.3
C <sub>10</sub>	171 $\pm$ 2	1.28 $\pm$ 63	9.0 $\pm$ 0.4	149 $\pm$ 6	16.3 $\pm$ 0.5	14.3 $\pm$ 0.4
CV (%)	3.3	8.8	12.4	7.0	10.0	6.2
SED	3.9	0.09	0.6	8.5	0.9	0.6

Table 4. The genotypic and phenotypic components of the variance. DFH: number of days to first harvest, Y: yield, NH: number of heads per plant, HW: heads weight, IN: incidence of receptacle on head weight, DM: head dry matter content.

Variable	Value		Variance		CV (%)		$h^2_B$
	Mean	Range	Genotypic	Phenotypic	$g_{cv}$	$p_{cv}$	
DFH (days)	163 ± 2	145 – 178	67.5	291.6	5.0	10.5	0.23
Y (kg plant <sup>-1</sup> )	1.28 ± 0.04	1.06 – 1.67	0.04	0.13	15.4	28.3	0.30
NH (n plant <sup>-1</sup> )	8.9 ± 0.3	6.8 – 11.5	5.4	10.3	26.1	36.0	0.53
HW (g)	146 ± 2	130 – 161	378.0	1416.1	12.9	25.0	0.27
IN (g 100 g <sup>-1</sup> FW)	16.9 ± 0.4	13.8 – 20.4	14.5	27.4	22.5	30.9	0.53
DM (g 100 g <sup>-1</sup> FW)	14.2 ± 0.3	12.4 – 16.3	1.8	7.8	9.4	19.6	0.23



Table 5. Trait correlation coefficients (n = 144). DFH: number of days to first harvest, Y: yield, NH: number of heads per plant, HW: heads weight, IN: incidence of receptacle on head weight, DM: head dry matter content.

Trait	DFH (days)	Y (g plant <sup>-1</sup> )	NH (n plant <sup>-1</sup> )	HW (g)	IN (g 100 g <sup>-1</sup> FW)
DFH (days)	-				
Y (kg plant <sup>-1</sup> )	<b>0.798</b> **	-			
NH (n plant <sup>-1</sup> )	<b>0.702</b> *	<b>0.842</b> ***	-		
HW (g)	<b>-0.274</b> <sup>NS</sup>	<b>-0.275</b> <sup>NS</sup>	<b>-0.693</b> *	-	
IN (g 100 g <sup>-1</sup> FW)	<b>-0.729</b> **	<b>-0.805</b> **	<b>-0.880</b> ***	<b>0.663</b> *	-
DM (g 100 g <sup>-1</sup> FW)	<b>-0.550</b> <sup>NS</sup>	<b>-0.758</b> **	<b>-0.817</b> **	<b>0.586</b> *	<b>0.890</b> ***

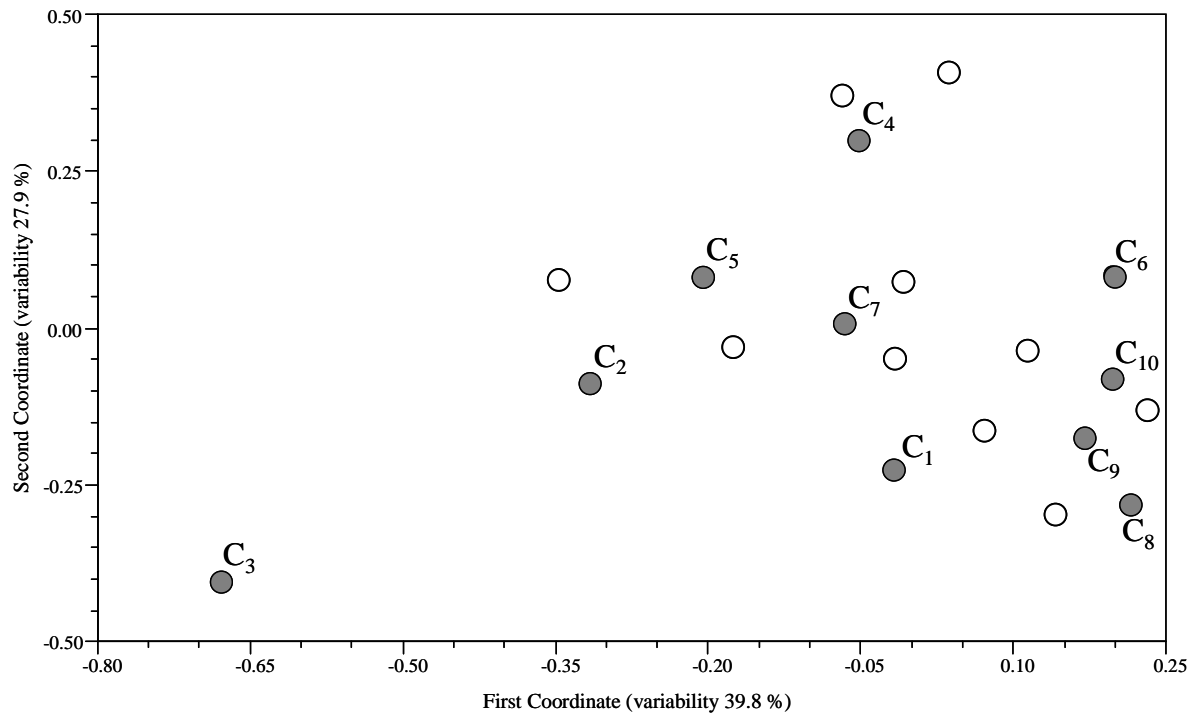
NS: not significant; \*, \*\*, \*\*\*: significant at  $P \leq 0.05, 0.01$  and  $0.001$ , respectively.

Table 6. Correlation coefficients for each trait with respect to the first two principal components, eigenvalues and the relative and cumulative proportions of the variance explained. DFH: number of days to first harvest, Y: yield, NH: number of heads per plant, HW: heads weight, IN: incidence of receptacle on head weight, DM: head dry matter content.

Trait	Common principal component coefficients	
	First	Second
DFH (days)	0.790	0.456
Y (kg plant <sup>-1</sup> )	0.882	0.398
NH (n plant <sup>-1</sup> )	0.957	-0.078
HW (g)	-0.656	0.717
IN (g 100 g <sup>-1</sup> FW)	-0.965	0.065
DM (g 100 g <sup>-1</sup> FW)	-0.898	0.116
Eigenvalue	4.49	1.21
Explained variability	74.7	15.1
Accumulated explained variability (%)	74.7	89.8

Figure 1:

Principal coordinate analysis (PCoA) based on AFLP data, depicting genetic relatedness between 20 genotypes from 'Violetto di Sicilia'. Clonal selections shown as grey circles and the reference individuals as white circles.



*Figure 2:*

UPGMA-based phylogeny of ten clonal selections from 'Violetto di Sicilia', as derived from AFLP genotyping.

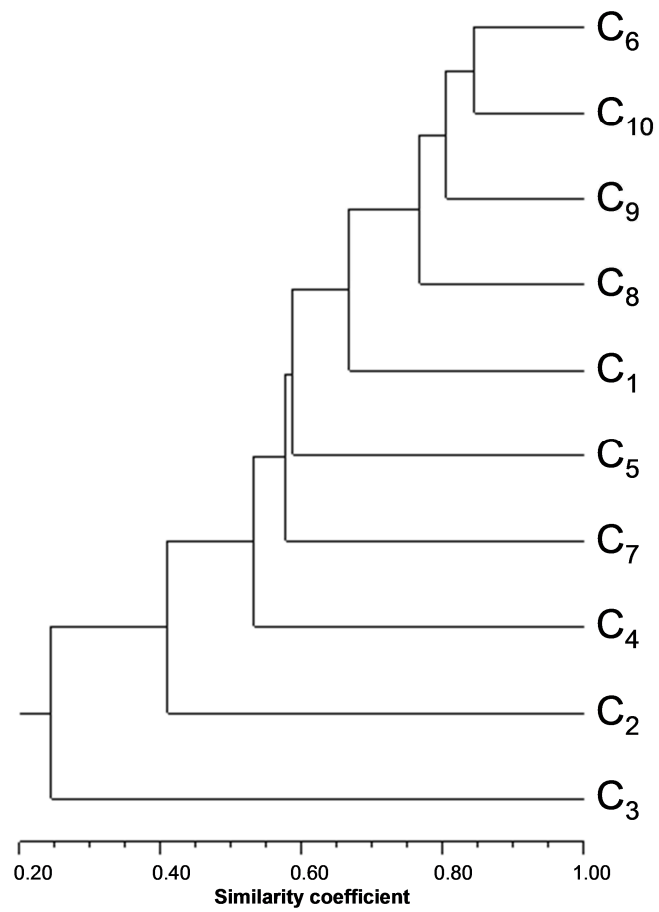


Figure 3:

Scatter plot of the ten clonal selections from 'Violetto di Sicilia', based on six plant traits.

