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# DNA Fingerprinting and Quality Traits of Corbarino Cherry-like Tomato Landraces

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Twenty-five landraces of the cherry-like tomato named Corbarino, a typical niche product grown in the Sarno valley of the Campania region, have been characterized. The landraces used have been compared to eight cultivars widely spread in the same area of cultivation. The genetic diversity within and between landraces was evaluated through the comparison of DNA fingerprints obtained with $(GATA)_4$ probe hybridized to TaqI digested genomic DNA. Twenty-two of the Corbarino landraces were homogeneous and were unequivocally characterized by their DNA fingerprints. The others are probably "population varieties" in that within each of them polymorphic DNA fragments were identified. According to the characterization of fruit shapes, four groups were identified. One landrace from each group, harvested at the same ripening degree, was further characterized for yield, fruit quality components, antioxidant activities, and carotenoid contents and compared to Faino $F_1$ and Tomito $F_1$ hybrids. The Corbarino landraces were all characterized by high yield, a high level of carbohydrates, and high levels of soluble and total solids. Interestingly, the biotype named ISCI 05 was the most suitable for canning. Landraces ISCI 07 and ISCI 05 gave the highest value of antioxidant activities and carotenoid content. These data, together with previously published results, suggested that ISCI 05 should be proposed for the institution of a Label of Origin.

**KEYWORDS: Antioxidants; carotenoid content; genetic variability**

## INTRODUCTION

The changes that have occurred in European agricultural policy in the past 15 years determined important modifications in food consumption of modern societies. Major changes are a decreased attention for the price of food and an increased attention toward the healthy and tasty food grown in environmentally friendly conditions. This was proved by a market survey in which, from a consumer point of view, the food sensory quality and its nutritional value were ranked higher than price (*1*). The depicted scenario offers new opportunities to Mediterranean agriculture, which may be considered the European garden. The horticultural production of southern Italy plays an important role for a relevant number of vegetables. These productions represent a precious patrimony of genetic variability and food diversification. As a matter of fact, the commercialization, as niche products, of landraces combining good taste with excellent health and nutritional properties, is emerging as a new area of Italian food production.

A typical niche product is represented by Corbarino, a small cherry-like tomato grown in the same area where the more famous San Marzano is produced. Corbarino includes several landraces showing at least four different fruit shapes (*2*). They may have different market destinations—fresh or canned or kept aside as a hanging tomato, thus offering multiple possibilities of commercialization. The nice and typical taste of these tomatoes is having great success, with a consequent increase of the market demand as both fresh vegetable and canned products. It is worth noting that the high demand of canned Corbarino (whole, not peeled tomatoes) represents a good opportunity for the local canning industry as this product may be sold at a price higher than that of other tomato products.

In an attempt to characterize the Corbarino landraces and to monitor some quality aspects of the fruits, the Regional Office for Agricultural Development operating in Campania sponsored the collection of Corbarino landraces spread in this region. Agronomic studies proved that in some cases the yield of some landraces was comparable with that normally recorded in the area with standard varieties (*3*). In addition, some preliminary results on the nutritional value of Corbarino landraces, as well

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**Table 1.** Main Morphological Characteristics of Tested Landraces and Cultivars

| genotype | code | plant growth | fruit shape | fruit apex[a] | fruit size[b] |
|---|---|---|---|---|---|
| Nocera Inferiore | 1 | half-determinate | elongate | – | 4 |
| Scafati 1 | 2 | indeterminate | oval-elongate | + | 4 |
| Scafati 2 | 3 | indeterminate | oval-elongate | ++ | 4 |
| Sarno 1 | 4 | indeterminate | oval-elongate, pear-shaped | – | 2 |
| Sarno 2 | 5 | half-determinate | oval-elongate, pear-shaped | – | 2 |
| Corbara 1 | 6 | indeterminate | elongate, pear-shaped | – | 4 |
| Corbara 2 | 7 | indeterminate | oval, pear-shaped | ++ | 5 |
| Grottaminarda | 11 | indeterminate | round-oval | ++ | 5 |
| Mercato S. Severino | 12 | indeterminate | oval | – | 2 |
| Agro Nocerino - Sel. ISCI 01 | 16 | indeterminate | elongate, pear-shaped | ++ | 4 |
| Agro Nocerino - Sel. ISCI 02 | 17 | indeterminate | elongate, pear-shaped | – | 3 |
| Agro Nocerino - Sel. ISCI 03 | 18 | indeterminate | round | ++ | 3 |
| Agro Nocerino - Sel. ISCI 04 | 19 | indeterminate | oval | ++ | 4 |
| Agro Nocerino - Sel. ISCI 05 | 20 | indeterminate | oval, pear-shaped | – | 4 |
| Agro Nocerino - Sel. ISCI 06 | 21 | indeterminate | oval, pear-shaped | – | 2 |
| Agro Nocerino - Sel. ISCI 07 | 22 | indeterminate | round-oval | – | 2 |
| Agro Nocerino - Sel. ISCI 08 | 23 | indeterminate | elongate, pear-shaped | + | 3 |
| Agro Nocerino - Sel. ISCI 09 | 24 | indeterminate | elongate, pear-shaped | ++ | 3 |
| Agro Nocerino - Sel. ISCI 10 | 25 | indeterminate | elongate, pear-shaped | – | 3 |
| Agro Nocerino - Sel. ISCI 12 | 26 | indeterminate | oval-elongate | ++ | 3 |
| Agro Nocerino - Sel. ISCI 13 | 27 | indeterminate | elongate, pear-shaped | – | 3 |
| Agro Nocerino - Sel. ISCI 14 | 28 | determinate | elongate | – | 3 |
| Agro Nocerino - Sel. ISCI 16 | 29 | half-determinate | oval-elongate | – | 4 |
| Agro Nocerino - Sel. ISCI 18 | 30 | indeterminate | oval-elongate, pear-shaped | – | 4 |
| Agro Nocerino - Sel. ISCI 19 | 31 | indeterminate | oval-elongate, pear-shaped | – | 4 |
| Principe Borghese | | indeterminate | round-oval | ++ | 5 |
| Fiaschetto | | determinate | oval-elongate | ++ | 4 |
| Faino $F_1$ | | indeterminate | oval | ++ | 4 |
| Remo $F_1$ | | indeterminate | oval | ++ | 4 |
| Red Cherry $F_1$ | | indeterminate | round | – | 1 |
| Tomito $F_1$ | | determinate | round | – | 2 |
| Tondino PS | | determinate | round-oval | – | 1 |
| Naomi $F_1$ | | indeterminate | round | – | 4 |
| San Marzano (Smec 20) | | indeterminate | elongate | | 5 |

[a] ++, normal apex; +, small apex; –, absence of apex. [b] 1, very small (<12 g); 2, small (12.1–15 g); 3, medium (15.1–18 g); 4, large (18.1–21 g); 5, very large (>21.1 g).

as of other cherry tomatoes, suggested that they may have an antioxidant activity higher than that reported for tomatoes of larger size (*4*). These observations suggest that Corbarino landraces may represent an interesting typical product of Campania to promote for commercialization.

However, to successfully achieve this goal, the various landraces should be distinguished from other similar types. In addition, each landrace should have a reasonable level of genetic uniformity to guarantee yield and quality stability in a definite environment.

The aims of the present study were (1) to monitor the genetic variability, through DNA fingerprinting, within and between the various Corbarino landraces and between them and some cherry-type varieties from the same cultivation area; and (2) to identify superior landraces that, according to yield and quality parameters, may be suitable for processing.

## MATERIALS AND METHODS

**Plant Material.** Twenty-five landraces collected in the Sarno Valley (Campania region) were used in this study. Commercial cultivars and the San Marzano landrace Smec 20 from the same area were also included as checks. **Table 1** lists plant material used and some morphological characteristics of each genotype.

**$(GATA)_4$ DNA Fingerprints.** To evaluate the genetic variability and homogeneity of the Corbarino biotypes in comparison with eight round cherry tomato cultivars and the San Marzano landrace Smec 20, DNA digests were electrophoresed and blotted on nylon membrane to be hybridized to the oligo probe $(GATA)_4$. For this purpose young leaves were collected from 10 plants per landrace, deep frozen in liquid nitrogen, and then stored at −80 °C. DNA was isolated as described (*5*) and quantified on agarose gel electrophoresis in comparison with known amounts of $\lambda$ DNA; 2−4 $\mu$g of DNA was then digested with *Taq*I, separated on 0.8% agarose gel electrophoresis, and blotted onto Hybond N+ (Amersham). Blots were hybridized with the oligonucleotide $(GATA)_4$ end-labeled using $T_4$ polynucleotide kinase and [$\gamma$-$^{32}$P]-ATP according to standard protocols (*6*). Hybridization and stringent washing steps were performed at $T_m$ − 5 °C. Genetic uniformity of each landrace was monitored by comparing the hybridization patterns obtained by processing the DNA extracted by 7−10 plants of the same sample.

Fragments were evaluated for presence (1) or absence (0) and treated as a discrete character. Bands with same mobility were considered to be identical. The resulting matrix was analyzed with NT-SYS program version 2.1 (*7*). Variability in fragment intensity was not taken into consideration. The similarity matrix obtained with the Dice coefficient (*8*) was used to build a dendogram based on the UPGMA algoritm. The correlation coefficient, calculated with the MXCOMP program, allowed the comparison between the original similarity matrix and the cophenetic matrix.

**Agronomic, Morphological, and Qualitative Parameters.** Plant growth habit was determined for each genotype during field trials. These were carried out in 2000 at Angri (Sarno Valley), a traditional area of cultivation of Corbarino landraces, in a sandy soil. On the basis of morphological and molecular data, four Corbarino landraces and two hybrids were selected to be included in this trial. Treatments were arranged in a randomized complete block design with four replications. For each treatment plots were 14.4 m$^2$, consisting of 48 plants (100.2 cm between rows and 25 cm in the row). The plants were transplanted in mid-April and raised through the use of training stakes and galvanized wires. Before transplantation, 60 kg ha$^{-1}$ of nitrogen, 200 kg ha$^{-1}$ of phosphorus, and 120 kg ha$^{-1}$ of potassium were distributed. After transplantation, only nitrogen was applied, two times, at rates of 70 kg

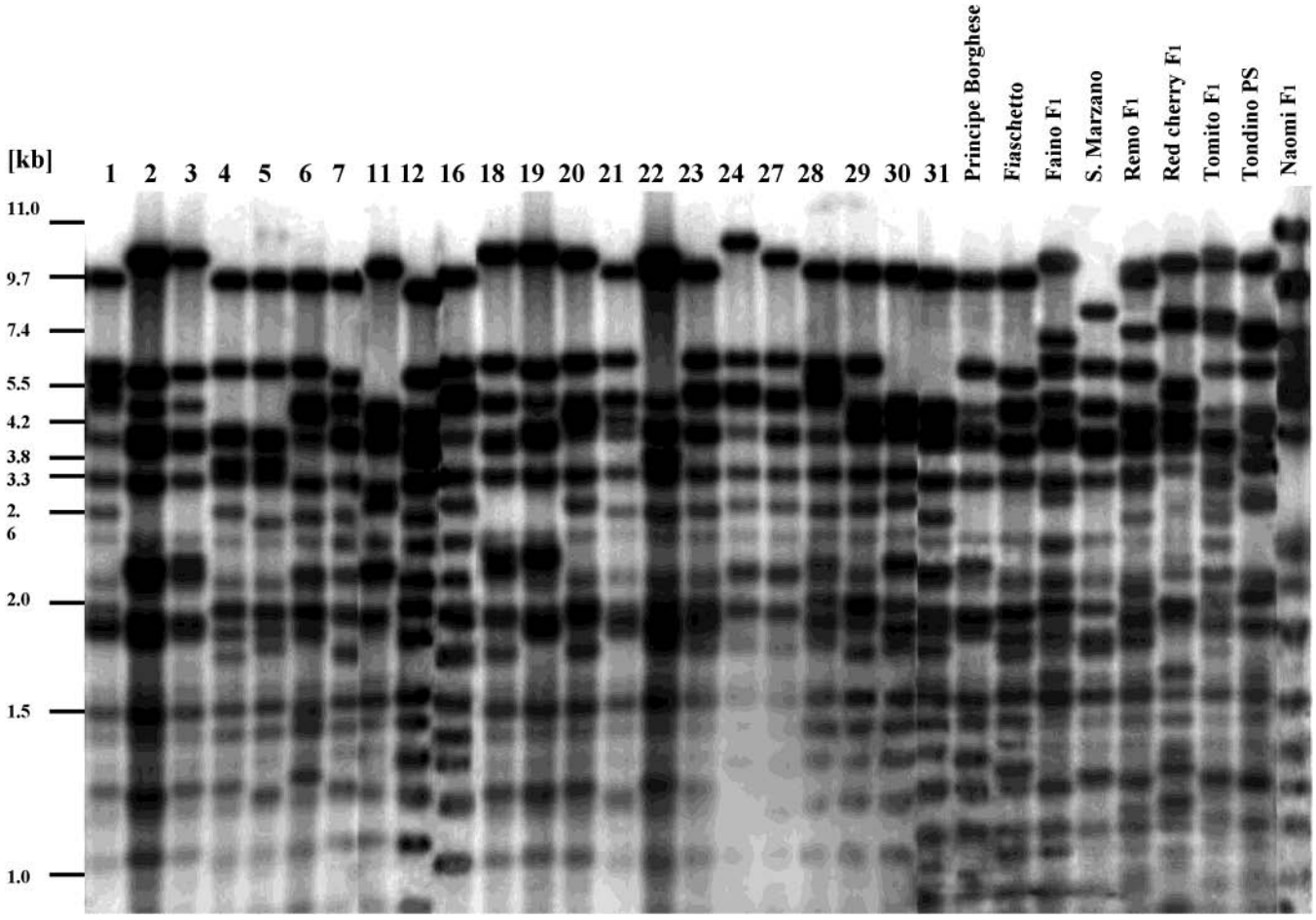


**Figure 1.** $(GATA)_4$ DNA fingerprint of 22 Corbarino landraces and other tomato cultivars. The hybridization pattern of DNA fragments is unique for each landrace or cultivar.

$ha^{-1}$ per application. Plants were irrigated four times (flush and furrow irrigation method) and fruits harvested twice: at the beginning of August and in mid-September. At each harvest fruit yield, average fruit fresh weight, and fruit length and width were evaluated. Carotenoid content and antioxidant activity were also estimated.

**Carotenoid Determination.** Carotenoid content was determined as described (*9*) with minor modifications (*10*). HPLC separation was carried out at a flow rate of 0.8 mL $min^{-1}$ using a Shimadzu HPLC with diode array detection and a Supelcosil $LC_{18}$ (250 × 4.6 mm i.d.). Carotenoid elution was achieved using the following linear gradient: starting condition, 82% A and 18% B, 20 min; 76% A and 24% B, 30 min; 58% A and 42% B, 40 min; 39% A and 61% B; where A was $CH_3CN$ and B was methanol/hexane/$CH_2Cl_2$ 1:1:1 v/v. Carotenoid quantification was achieved using standard curves based on commercial $\beta$-carotene purchased from Fluka or HPLC-purified lycopene. The concentrations of the standards were calculated using the extinction coefficient and corrected for the estimated recovery.

**Antioxidant Activity.** Two different radical cation assays were utilized to measure the antioxidant activity on lipophilic and hydrophilic fractions. The antioxidant activity was measured on the water-soluble fraction using the *N*,*N*-dimethyl-*p*-phenylenediamine (DMPD) method (*11*). Briefly, 20 $\mu$L of the tomato aqueous extract was added to 2 mL of a solution containing the DMPD radical cation in acetate buffer (0.1 M; pH 5.25). The quenching of absorbance of this solution was measured at 505 nm after 10 min, and then the value was compared with that obtained by a standard solution of ascorbic acid; antioxidant activity was expressed in millimoles of ascorbic acid on 100 g of fresh tomato.

The 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method performed as described in ref *12* was utilized to assess the antioxidant activity of water-insoluble fractions. For this analysis the same material obtained with the carotenoid extraction procedure was used. The antioxidant activity was expressed in millimoles of Trolox on 100 g of fresh tomato.

## RESULTS AND DISCUSSION

**Landraces Distinctness and Homogeneity.** The DNA fingerprinting of the genotypes included in this study was carried out through $(GATA)_4$ hybridization pattern (**Figure 1**). Several hypervariable and easily scorable number of DNA fragments were included in the size range of 11−1 kb. The combination of these fragments was unique for each sample. Our results provided evidence that the landraces are clearly distinct from the cherry-type tomato cultivars grown in the same area. For example, the $(GATA)_4$ hybridization pattern of ISCI 05, sample 20, includes eight fragments in the size range of 10−2.6 kb that allows its discrimination among all of the others. All of the Corbarino landraces were also clearly distinct from the San Marzano. The performed molecular analysis also allowed us to estimate intralandrace variability. Differences among individuals of the same landrace were observed in the case of samples coded 17, 25, and 26 (**Figure 2**). This can be attributed to seed mixture and/or the presence of segregating materials. Landraces that are not genetically homogeneous should not be considered for commercial purposes unless further "cleaning" is carried out. In contrast, the genetically homogeneous Corbarino landraces represent valuable genetic material in which the main morphological characteristics of fruits and plants are identical. This material can be easily discriminated through the DNA fingerprints carried out in this study.

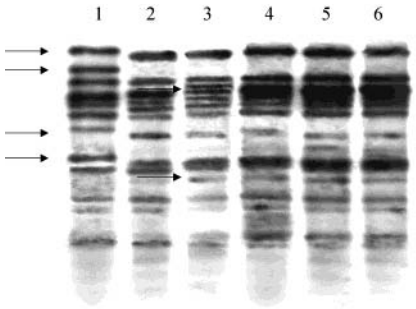


**Figure 2.** $(GATA)_4$ DNA fingerprint of six plants of landrace 25. Polymorphic DNA fragments present in plants 1–3 are indicated by arrows.

Unequivocal identification of plant cultivars and the assessment of genetic diversity within and between cultivars have been achieved with DNA fingerprinting in a large number of crops (*13*). $(GATA)_4$ DNA fingerprints specific for tomato cultivars and able to assess cultivar purity have been widely used (*5*, *14*, *15*). Moreover, the fingerprints of 60 individuals of two different breeding lines showed no deviation as a consequence of a high degree of homogeneity (*14*, *16*). Therefore, $(GATA)_4$ DNA fingerprints represent an excellent tool to protect and preserve this typical product from substitutions and seed mixing.

To estimate the relatedness of biotypes and cultivars, the band patterns were transformed into a binary matrix and analyzed through UPGMA. The dendrogram (**Figure 3**) contains a major cluster of quite closely related samples that includes 14 Corbarino landraces, cv. Fiaschetto, and San Marzano. These 14 landraces appear to have part of their genomes in common with San Marzano and Fiaschetto, from which they possibly originated and diversified due to selection carried out by farmers. This finding was not unexpected as these tomatoes routinely share the same area of cultivation. Thus, cross-pollinations may have well occurred. The other genotypes analyzed fall in other clusters, showing a much lower value of similarity coefficients. The most genetically distant group includes the varieties Faino $F_1$, Red Cherry $F_1$, Naomi $F_1$, and Tondino PS, which are very different from most of the Corbarino landraces. The landraces coded 2 and 3 are genetically very close and showed the highest similarity index (0.94). These landraces should be considered synonyms.

The morphological characterization carried out allowed us to identify four groups based on fruit shape: elongate/pear-shaped, oval, oval/pear-shaped, and round. It should be pointed out that fruit shape is of major importance for Corbarino cherry-like tomatoes (*4*). One sample for each group (ISCI 01, elongate/pear-shaped; ISCI 04, oval; ISCI 05, oval/pear-shaped; ISCI 07, round) was chosen and further characterized for yield and fruit quality components in comparison with Faino $F_1$ and Tomito $F_1$, two cherry-like tomato hybrids cultivated in the same area. The Corbarino landraces were all characterized by high yield (**Table 2**), with best performance (84 tons/ha) given by ISCI 05. Interestingly, this landrace was even better than the two commercial hybrids. The quality traits of the same genotypes are reported in **Table 3**. It is worth noting that all of the fruits were harvested at the same ripening degree. As for the other morphological traits the Corbarino landraces had soluble solids and carbohydrate contents higher than those of the two hybrids, and most of them had acceptable values of pH and acidity. However, the high average fruit weight and the high rate of fruits with adherent stems suggest that landraces ISCI 01 and ISCI 07 are unsuited for canning. From an analysis of all the data it can be suggested that ISCI 05 is the landrace most suitable for canning.

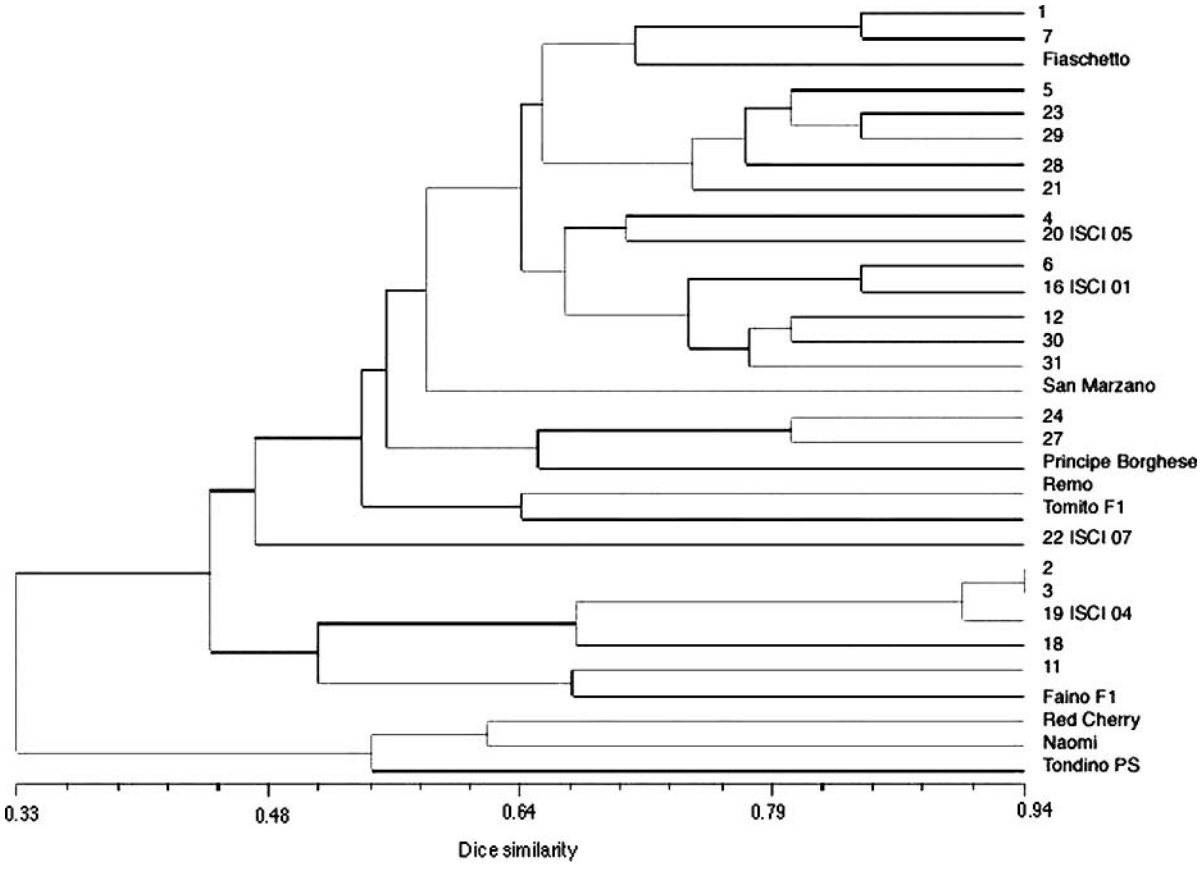


**Figure 3.** Dendrogram of 25 Corbarino landraces and cultivars based on GATA-containing fragments.

**Table 2.** Yield and Main Morphological Traits of Four Corbarino Landraces and Two Tomato Hybrids[a]

| genotype | marketable yield (tons ha$^{-1}$) | fruit wt (g) | fruit size length/ width (cm) | fruit consistency[b] | fruits with adherent stems (%) |
|---|---|---|---|---|---|
| ISCI 01 | 73.6 b | 22.6 a | 1.92 | 3.8 bc | 27.6 b |
| ISCI 04 | 62.2 c | 20.3 b | 1.31 | 4.3 a | 27.8 b |
| ISCI 05 | 84.3 a | 17.9 c | 1.52 | 3.7 c | 26.6 b |
| ISCI 07 | 75.6 b | 11.9 e | 1.17 | 3.8 c | 52.0 a |
| Faino $F_1$ | 67.9 bc | 22.2 ab | 1.22 | 4.4 a | 27.0 b |
| Tomito $F_1$ | 71.6 b | 14.8 d | 1.05 | 4.1 ab | 14.2 c |
| **mean** | **72.5** | **18.3** | **1.37** | **4.0** | **29.2** |

[a] Within each column, means followed by the same letter are not statistically different for $P < 0.05$ according to Duncan's multiple-range test. [b] Assessment based on a scale from 1 to 5 (1, worst; 5, best).

**Table 3.** Quality Traits of the Fresh Fruits of Four Corbarino Landraces and Two Tomato Hybrids[a]

| genotype | soluble solids (°Brix) | total solids (g%) | pH | acidity (g%) | carbohydrate (g%) | Hunter color *a*/*b* |
|---|---|---|---|---|---|---|
| ISCI 01 | 5.78 c | 6.48 c | 4.58 a | 0.34 a | 3.55 bc | 2.14 a |
| ISCI 04 | 6.19 b | 6.79 bc | 4.54 a | 0.37 a | 3.78 ab | 2.08 ab |
| ISCI 05 | 6.82 a | 7.21 a | 4.5 a | 0.45 a | 3.94 a | 2.13 a |
| ISCI 07 | 6.51 ab | 7.00 ab | 4.45 a | 0.45 a | 3.89 a | 1.91 c |
| Faino $F_1$ | 4.18 e | 4.66 e | 4.47 a | 0.46 a | 1.18 d | 1.96 bc |
| Tomito $F_1$ | 5.4 d | 6.08 d | 4.29 b | 0.52 a | 3.3 c | 2.04 ab |
| **mean** | **5.81** | **6.37** | **4.47** | **0.43** | **3.27** | **2.04** |

[a] Within each column, means followed by the same letter are not statistically different for $P < 0.05$ according to Duncan's multiple-range test.

**Table 4.** Hydrophilic and Lipophilic Antioxidant Activities of Four Corbarino Landraces and Two Tomato Hybrids[a]

| genotype | HAA (mmol of ascorbic acid/ 100 g of fresh wt) | LAA ($\mu$mol of Trolox/ 100 g of fresh wt) |
|---|---|---|
| ISCI 01 | 1.65 b | 40 b |
| ISCI 04 | 1.87 a | 35 bc |
| ISCI 05 | 2.05 a | 42 b |
| ISCI 07 | 2.15 a | 58 a |
| Faino $F_1$ | 1.72 b | 37 b |
| Tomito $F_1$ | 1.58 b | 31 c |

[a] Within each column, means followed by the same letter are not statistically different for $P < 0.05$ according to Duncan's multiple-range test.

**Nutritional Characteristics.** Data of antioxidant activity are reported in **Table 4**. Differences were observed both for hydrophilic (HAA) and for lipophilic (LAA) antioxidant activities. The landrace ISCI 07 displayed the highest value of HAA and LAA (2.15 nmol and 58 $\mu$mol, respectively) followed by ISCI 05 (2.05 nmol and 42 nmol, respectively). However, differences were not always statistically significant. It should be pointed out that correlation analysis provided evidence the LAA and HAA are positively correlated ($P < 0.05$).

To better characterize the selected tomato biotypes and to verify the relationship between LAA and carotenoid content, HPLC determination of carotenoids was carried out. Data are reported in **Table 5**. On average, the level of total carotenoids of the different Corbarino landraces was high, as expected for a cherry variety, due to the favorable ratio between surface and volume in small berries. Among the landraces the highest value (8.96 mg/100 g of fresh weight) was detected in ISCI 07 (round fruit shape). Data showed that differences between the landraces are related with those observed measuring the LAA, thus confirming the usefulness of the measure of antioxidant activity when an accurate HPLC detection is not possible. Differences in carotenoid concentration among the analyzed landraces are comparable to those reported in the literature (*17*). Carotenoid concentration in ISCI 05 and ISCI 07 berries was significantly higher than that of Faino $F_1$ and Tomito $F_1$. It should be emphasized that these results are of great interest as they were obtained from fruits harvested in the same field from plants that underwent identical growing conditions. The concentration of tomato berry antioxidants, as other qualitative parameters, is strongly influenced by environmental conditions and agronomic practices such as water regimen, covering shield, and position of the berry on the plant (*18*). Therefore, the comparison of antioxidant concentration values cannot be determined in berries grown in different experimental conditions as the variation caused by external factors can easily overcome the genetic factors.

**Table 5.** Carotenoid Content (Mean ± Standard Deviation) of Four Corbarino Landraces and Two Tomato Hybrids[a]

| sample | lycopene | $\beta$-carotene | phytoene | phytofluene | tota carotenoid |
|---|---|---|---|---|---|
| ISCI 01 | 5.7 ± 0.4 c | 0.42 ± 0.03 b | 0.44 ± 0.05 c | 0.25 ± 0.04 c | 6.85 |
| ISCI 04 | 4.9 ± 0.3 c | 0.37 ± 0.01 c | 0.61 ± 0.03 ab | 0.30 ± 0.05 c | 6.31 |
| ISCI 05 | 6.3 ± 0.1 b | 0.48 ± 0.02 b | 0.52 ± 0.02 b | 0.31 ± 0.01 c | 7.73 |
| ISCI 07 | 7.4 ± 0.5 a | 0.55 ± 0.03 a | 0.68 ± 0.06 a | 0.32 ± 0.03 c | 8.96 |
| Faino $F_1$ | 5.1 ± 0.2 c | 0.45 ± 0.02 b | 0.63 ± 0.02 ab | 0.58 ± 0.02 a | 6.78 |
| Tomito $F_1$ | 4.2 ± 0.1 d | 0.45 ± 0.02 b | 0.75 ± 0.01 a | 0.46 ± 0.01 b | 5.86 |

[a] Different letters, within each parameter, indicate significant differences for $P < 0.05$ (Duncan's multiple-range test). Values are in mg/100 g of fresh wt.

As far as the Corbarino landraces are concerned, our data, together with other agronomic and nutritional results (*2*, *4*, *18*), provided strong evidence that ISCI 05 can be indicated to local authorities for the institution of a Label of Origin, as also occurred for other species (*19*). In fact, ISCI 05 is genetically homogeneous and can be easily identified with a GATA probe. In addition, it gave good yield performances, low pest susceptibility, excellent fruit qualitative characteristics, and high processing ability over three years of field trials (authors' unpublished results). Moreover, it showed both carotenoid content and antioxidant activity above the average content of check varieties and other Corbarino landraces. Also interesting was the landrace indicated as ISCI 07 that showed the highest LAA and carotenoid contents. ISCI 07 fruits are morphologically similar to Tomito $F_1$ fruits, the most widely used commercial cherry tomato hybrid for processing. Its fruits are also similar to those of Naomi $F_1$, the so-called Pachino tomato, which has a very strong brand mark. ISCI 07 is genetically distinct from these two cultivars, as easily monitored through $(GATA)_4$ DNA fingerprints, and has valuable properties. Further agronomic trials and qualitative analyses are being carried out with this landrace, with the aim of increasing the typical and quality production of the Campania region.

Our data showed that although the use of molecular descriptors represents a powerful tool for the identification of tomato landraces and varieties, the measurement of parameters such as the antioxidant activity or the measurement of specific phytonutrients is a useful approach to describe nutritional characteristics, an appreciated added value for vegetables sold in European markets. The combination of both techniques allowed us to discriminate landraces that may be then recognized and "labeled" as a typical product of the Campania region. Consequently, selected landraces can be easily protected by substitutions.

## ABBREVIATIONS USED

DMPD, *N*,*N*-dimethyl-*p*-phenylenediamine; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); $T_m$, temperature of melting; [$\gamma$-$^{32}$P]ATP, adenosine triphosphate labeled in $\gamma$ with phosphorus 32.

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