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Targeted secondary metabolic and physico-chemical traits analysis to assess genetic variability within a germplasm collection of "long storage" tomatoes

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Neochlorogenic acid (PubChem CID: 5280633)
Rutin (PubChem CID: 5280805)
Quercetin (PubChem CID: 5280343)
Naringenin (PubChem CID: 932)
Lycopene (PubChem CID: 446925)
β-Carotene (PubMed CID: 5280489)
trans-Lutein (PubChem CID: 5281243).

ABSTRACT

"Long storage" tomato is a crop traditionally cultivated in the Mediterranean area under no water supply, that recently has attracted the interest of breeders for its high tolerance to drought and as potential genetic source in breeding programs for water stress resistance. A collection of 28 genotypes of "long storage" tomato (*Solanum lycopersicum* L.) was studied for carotenoid and polyphenol profile and content, vitamin C, and other physico-chemical traits of fruits. Tomato carotenoids and polyphenols were identified and quantified using high-performance liquid chromatography coupled with diode array detection and electrospray-mass spectrometry (HPLC/DAD/ESI-MS); nineteen different phenolic compounds and six different carotenoids, for a total of 25 markers, have been detected, quantified and used to discriminate among the different landraces to find out which could be the best candidate for a medium-to-large scale cultivation. Different statistical approaches (ANOVA, Principal Components Analysis, Cluster Analysis) have been used for data analysis.

1. Introduction

Drought is one of the most significant environmental constraints in hot and dry climates (such as that of the Mediterranean area), affecting crop growth and yield. As a matter of fact, water stress can reduce crop yields up to 70 percent (Shamim, Saqlan, Habib-ur-Rehman, & Waheed, 2006). Considering that global water resources are continually decreasing and that climate change is expected to increase the amount of dry land, maintaining high crop yields in water stress conditions is a top priority in agricultural research (Petrozza et al., 2014). This is particularly true for those crops highly demanding in water, such as tomato, for which a poor water supply causes severe damages.

Tomato (*Solanum lycopersicum* L. syn. *Lycopersicon esculentum* Mill.) is the most widely consumed vegetable all over the world (Gómez-Romero, Arráez-Román, Segura-Carretero, & Fernández-Gutiérrez, 2007; Kavitha et al., 2014), present as key component in daily meals in many countries. Due to their high consumption, tomatoes and tomato products represent a significant source of natural antioxidants, including carotenoids and phenolic compounds, in human diet (Friedman, 2013; Shen, Chen, & Wang, 2007; Viuda-Martos et al., 2014). Lycopene and β-carotene are the main C_{40} carotenoids present in tomato (Hernandez, Rodriguez, & Diaz, 2007), whilst the chemical class of polyphenols is represented by rutin (quercetin 3-*O*-rutinoside), quercetin, narigenin, and chalconarigenin, which are just a few among the large number of different flavonoids found in this product (Slimestad & Verheul, 2009), together with organic acids (benzoic, protocatechuic) and cinnamic acids (Slimestad & Verheul, 2009; Vallverdú-Queralt, Jauregui, Medina-Remón, Andres-Lacueva, & Lamuela-Raventos, 2010).

Several approaches have been developed to improve tomato plant resistance to water stress, such as the screening and selection of genotypes/cultivars (Shamim et al., 2006) or wild relatives (Rigano et al., 2016), the use of grafting (Schwarz, Rouphael, Colla, & Venema, 2010), and the use of biostimulants (Petrozza et al., 2014). In this context, the rediscovery of traditional genetic resources with intrinsic water stress resistance might be an interesting approach.

"Long storage" tomato, so called for the textural properties of its

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small fruits which allow an extended shelf-life, provides a niche product that combines a good taste to excellent nutritional properties (Patanè et al., 2017; Siracusa, Avola, Patanè, Riggi, & Ruberto, 2013).

In our previous work (Siracusa, Patanè, Avola, & Ruberto, 2012) nine landraces of "long storage" tomato were studied for their morphological and nutritional characteristics, showing interesting and unique features. Recently, a research on long-storage tomato highlighted great physiological and yield differences among landraces in response to prolonged soil water deficit. In particular, those populations with greater tolerance to drought, therefore more suitable for growing in semi-arid regions, exhibited an osmotic adjustment through proline accumulation in leaves, which allows cells to retain turgor at low water potential (Patanè, Scordia, Testa, & Cosentino, 2016).

Compositionally speaking, "long storage" tomatoes exhibit a diagnostic polyphenol profile which can be related to their capability to grow under unfavourable climates or soil water deficit conditions (Siracusa et al., 2012). Indeed, long storage tomato is traditionally cultivated with no irrigation, and under such drought conditions, antioxidants (*e.g.* polyphenols) accumulate in plants in a greater extent as a defence mechanism to prevent stress damages (Bor, Özdemir, & Türkan, 2003; Reyes & Cisneros-Zevallos, 2003).

In the present research a phytochemical study on 28 landraces from the Southern Italy was performed by analyzing the polyphenol and the carotenoid profiles, with the aims to assess the genetic variability, to verify the most reliable chemotaxonomic markers in terms of traceability of the product, and finally to find out the best candidate(s) for a scale-up production.

2. Material and methods

2.1. Plant material and experimental design

A total of 28 landraces of "long storage" tomatoes, all belonging to the seed bank at the CNR-IVALSA (Catania, Italy), were investigated in this study. The landraces were recovered all throughout Southern Italy (Fig. 1), including Eolian islands (which represent an interesting example of microclimates), Apulia (samples 1, 2) Campania (samples 21, 23), and Sicily. The commercial cultivars "Febo" and "Faino" (Syngenta Seeds, The Netherlands), were adopted as controls, being similar to "long storage" tomatoes in terms of fruit shape. The tomatoes were open-field cultivated in a flat site of eastern coast of Sicily (10 m a.s.l., 37°35′ N Lat, 15°30′ E Long, asterisk in Fig. 1) during the summer season of 2011. The same agronomic techniques were applied to all of them, in order to obtain homogeneous material for assessment in the laboratory. A randomized complete blocks experimental design with three replicates was used in the field (Fig. S1 in Supplementary Material); a total volume of approximately 40 mm of water, split in two applications including transplanting, was distributed. After that, irrigation was interrupted. Harvest was performed when ripe fruit rate reached ~95% (late July). At harvest, fruits (approx. 2 kg per genotype per replicate) from the first two trusses were selected according to the level of ripeness.

2.2. Chemicals

All solvents and reagents used in this study were high purity laboratory products obtained from Carlo Erba (Milano, Italy); HPLC grade water, acetonitrile and ethyl acetate were purchased from VWR (Milano, Italy). The analytical standards used as references were in the purity range between 90% and 99% as declared by the corresponding vendors. Cynarin (1,3-dicaffeoylquinic acid) was provided by Extrasynthese (Lyon, France); chlorogenic acid, rutin, quercetin, narigenin and *all-trans*-lutein were obtained from Fluka, whilst all-*trans*-lycopene and β-carotene were provided by Sigma (Sigma-Aldrich s.r.l., Milano, Italy). A complete list of polyphenols and carotenoids identified in this study is given in Supplementary Material, Table S1.

2.3. Physico-chemical traits

All long storage tomato fruits were first evaluated for physico-

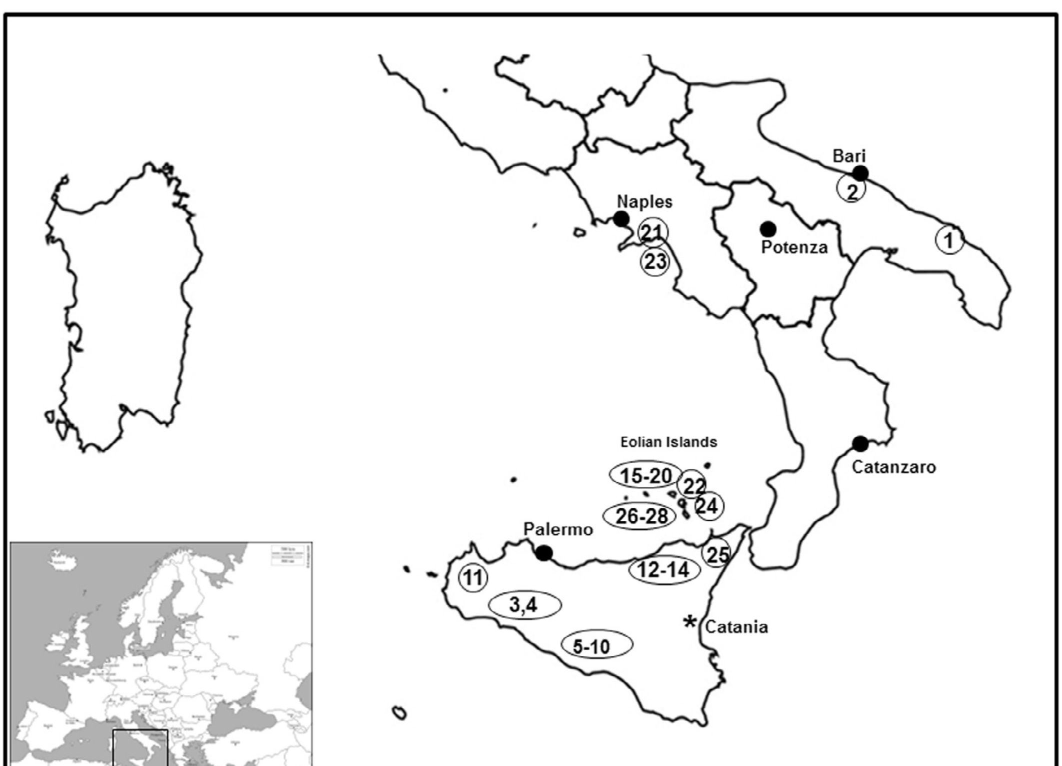

Fig. 1. Geographical origin of the 28 "long storage" tomato genotypes described in the text (see Table 1 for genotype list and origin). Cultivation area is marked with an asterisk.