




# Selection of tomato landraces with high fruit yield and nutritional quality under elevated temperatures

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## Abstract

**BACKGROUND:** Global warming and extreme or adverse events induced by climatic fluctuations are an important threat for plants growth and agricultural production. Adaptability to environmental changes prevalently derives from a large set of genetic traits affecting physiological and agronomic parameters. Therefore, the identification of genotypes that are good yield performer at high temperatures is becoming increasingly necessary for future breeding programs. Here, we analyzed the performances of different tomato landraces grown under elevated temperatures in terms of yield and nutritional quality of the fruit. Finally, we evaluated the antioxidant and anti-inflammatory activities of fruit extracts from the tomato landraces selected.

**RESULTS:** The tomato landraces analyzed here in a hot climate differed in terms of yield performance, physicochemical parameters of fruit (pH, titratable acidity, degrees Brix, firmness), bioactive compounds (ascorbic acid, carotenoids, and polyphenols), and anti-inflammatory potential. Three of these landraces (named E30, E94, and PDVIT) showed higher fruit quality and nutritional value. An estimated evaluation index allowed identification of PDVIT as the best performer in terms of yield and fruit quality under high temperatures.

**CONCLUSION:** The analyses performed here highlight the possibility to identify new landraces that can combine good yield performances and fruit nutritional quality at high temperatures, information that is useful for future breeding programs.

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**Keywords:** tomato; high temperature tolerance; yield performance; fruit quality; nutritional value

## INTRODUCTION

One of the most important challenges facing us today is dealing with global warming, which can greatly impact on crop production and food accessibility. Together with water stress and high salinity, temperatures and light fluctuations during plant growth are the most important abiotic stresses that plants have to face.<sup>1</sup>

Tomato is one of the major cultivated crops worldwide and a model system to study plant response to the changing environmental conditions. The optimal range of temperatures for tomato cultivation is between 25 and 30 °C during the day and 20 °C during the night.<sup>2</sup> However, tomato varieties or landraces can exhibit individual differences in terms of yield performances, depending on the genetic background and the adaptation to the environment in specific geographic areas. In this context, the exploration of the natural variation and the screening of genotypes and landraces that are good yield performers at high temperatures may help to understand the mechanisms underlying high-temperature tolerance and can provide agronomic traits and genetic diversity useful for breeding.<sup>3</sup>

Increases in the average temperatures and UV radiation can have a significant impact on plant growth and, consequently, on crop yield and fruit quality. Indeed, different biochemical mechanisms, including plastid biogenesis and pigments/secondary

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metabolites synthesis, can be activated in plant cells in response to abiotic stresses.<sup>1,4–7</sup> Since thermo-tolerance requires the modulation of biochemical pathways involved in reactive oxygen species (ROS) detoxification, antioxidant compounds, including carotenoids, ascorbic acid (AsA) and polyphenols, are accumulated in response to heat stress.<sup>8,9</sup> Carotenoids can also contribute to membranes fluidity and permeability in response to temperature fluctuations.<sup>5,6</sup> Indeed, these molecules can exert a protective role in photosynthetic membranes and play important roles in structural stabilization, light harvesting, and photoprotection.<sup>5</sup> The increase in the biosynthesis of polyphenolic compounds and flavonoids has also been reported following the exposure of many plant species to UV radiation, making their accumulation one of the most evolutionarily conserved responses to this type of abiotic stress.<sup>10,11</sup>

Polyphenolic compounds, AsA, and carotenoids are also relevant for human health, since antioxidant and anti-inflammatory properties have been described following their *in vitro* or *in vivo* administration.<sup>12–14</sup> In particular, phenolic compounds may have therapeutic roles in inflammation-based diseases and various type of cancers.<sup>15</sup> Furthermore, ascorbic acid shows significant antioxidant and electron donor capability and is able to protect DNA from oxidation-induced damage.<sup>15,16</sup> Finally, it has been demonstrated that carotenoids possess an apoptotic-inducing effect in cancer cells and reduce oxidized low-density lipoprotein cholesterol levels.<sup>17</sup> The health-promoting properties of these bioactive compounds contribute to the nutritional value of crops such as tomato and represent a parameter of preference in consumer choice of food products. Indeed, some of these bioactive compounds, such as AsA and carotenoids, cannot be synthesized by animals and thus have to be introduced with the diet.<sup>18</sup> For these reasons, the daily consumption of plant-derived food enriched in these compounds has been highly promoted in the last decade.

In this study, we analyzed the performances, in terms of final yield and fruit quality on a set of ten tomato landraces showing good performances when grown under elevated temperatures.

Their productivity and field performances under restrictive environmental conditions were estimated. Moreover, the bioactive compounds content and antioxidants/anti-inflammatory properties have been evaluated in extracts from ripe fruits from different tomato landraces, with the final aim of identifying the best ones in terms of both yield and nutritional fruit quality. Altogether, this study highlights the possibility to select tomato landraces combining desirable agronomic and nutritional traits, such as good yield performances and high nutritionally active phytonutrients content, for future applications in breeding programs.

## MATERIALS AND METHODS

### Plant materials

Plant material consisted of ten tomato indeterminate landraces (listed in Table 1) and the control variety 'Moneymaker'. In 2017 they were grown in Apulia (Pulsano, 40° 23' 03" N latitude, 17° 21' 17" E longitude), a region of southern Italy greatly devoted to tomato cultivation and usually characterized by high temperatures during the growing season. Seeds were sown in plateau under a plastic-house in April, and seedlings were then transplanted in open field in June. Plants were distributed following a complete randomized block design, with three replicate plots per landrace and ten plants per replicate. For fruit quality and nutritional traits analyses, ten fruits at the red ripe stage from at least three plants from each replicate plot were collected at the same time and pooled. Some traits were evaluated on fresh fruit, and others on fruit frozen in liquid nitrogen and stored at –80 °C until analysis. For yield determination, fruits at red ripe stage were collected on the same day from all the plants for each replicate plot. Total fruit number and fresh weight (FW) were measured to allow yield evaluation per plant.

### Quality traits evaluation

Physicochemical traits were evaluated on fresh fruit. The determination of pH was carried out by using a pH meter (Mettler-Toledo, Milan, Italy), and the total acidity was determined by titrating

**Table 1.** List of the plant materials tested for production, quality, and nutritional traits under high temperature

Genotype	Source	Accession no.	Common name	Country of origin	Collection site	Product destination	Fruit size	Fruit shape
E7	CRA-ORT <sup>a</sup>		Corbarino PC04	Italy	Nocera (Salerno)	Processing	Small (25–30 g)	Ovate
E8	CRA-ORT <sup>a</sup>		Corbarino PC05	Italy	Sant'Antonio Abate (Salerno)	Processing	Small (20–25 g)	Elliptic
E17	CRA-ORT <sup>a</sup>		Pantano Romanesco	Italy	Fondi (Latina)	Fresh market	Big (200–250 g)	Flattened
E30	CRA-ORT <sup>a</sup>		Sel PC07	Italy	Pagani (Salerno)	Processing	Small (15–20 g)	Ovate
E32	CRA-ORT <sup>a</sup>		Sel PC16	Italy	Nocera (Salerno)	Fresh market	Small (20–50 g)	Ovate
E36	Campania Region <sup>a</sup>		Vesuvio Foglia Riccia	Italy	S. Vito (Naples)	Fresh market/ processing	Small (25–30 g)	Ovate
E53	TGRC	LA0147	—	Honduras	Tegucigalpa mercado	Fresh market	Medium (80–100 g)	Oblate
E76	TGRC	LA4449	Black plum	URSS	—	Processing	Small (20–25 g)	Ovate
E94	NPGS	PI272890	1404	Guatemala	Quetzaltenango, Guatemala	nd	Small (40–50 g)	Irregular
PDVIT	ARCA2010 <sup>a</sup>		Caramella	Italy	Scafati (Salerno)	Fresh market/ processing	Small (10–15 g)	Elliptic
'Moneymaker'	TGRC	LA2706	Moneymaker	Great Britain	—	Fresh market	Medium (50–60 g)	Circular

<sup>a</sup> Germplasm collections maintained at Italian public institution.

10 mL of tomato juice with a solution of 0.1 mol L<sup>-1</sup> sodium hydroxide. A few drops of tomato juice were also used to estimate the total soluble solids (degrees Brix) by adding them to the prism plate of a refractometer (Hanna Instruments). Finally, the firmness was measured by a penetrometer (PCE-PTR200 penetrometer, Capannori, Italy) using an 8 mm tip. All extracts were from three biological replicates, and three technical assays were carried out on each biological repetition.

### Extraction and detection of polyphenols

Whole tomato fruits were cut and frozen in liquid nitrogen, freeze-dried, and finely ground. Samples of powder (200 mg) were extracted twice in methanol:water 80:20 (v/v). The extracts were centrifuged, and the supernatants were combined, filtered through a 0.22 µm filter, and stored at -20 °C until use. Polyphenols were detected at 320 nm by reversed-phase high-performance liquid chromatography with diode array detector (RP-HPLC DAD) (Agilent 1100 HPLC system). Separation was performed on a C18 column (5 UltraSphere, 80 Å pore, 25 mm), with a linear gradient from 20% to 60% acetonitrile, in 55 min, with a flow of 1 mL min<sup>-1</sup> at 25 °C. Concentrations were obtained by referring to calibration curves, and results were expressed in micrograms per gram or milligrams per gram (in the case of rutin and chlorogenic acid) of dried weight.

### Carotenoids content

Freeze-dried tomato powder (50 mg) was added to 2 mL of 60% potassium hydroxide, 2 mL of absolute ethanol, 1 mL of 1% sodium chloride (NaCl), 5 mL of 0.05% butylated hydroxytoluene in acetone. The mix was incubated at 60 °C for 30 min. A 1% solution of NaCl (15 mL) was added to the mix, and extractions were performed with 15 mL hexane:ethyl acetate 9:1 (v/v). Extracts were centrifuged, evaporated using a rotary evaporator, and collected in 1 mL of ethyl acetate. Analyses were performed using RP-HPLC DAD (Agilent 1100 HPLC system) according to the method previously described.<sup>19</sup>

### AsA determination

AsA determinations were carried out by a colorimetric method with modifications reported by Rigano *et al.*<sup>20</sup> Briefly, 500 mg of frozen powder from tomato fruits was extracted with 300 µL of 6% trichloroacetic acid (TCA). The mixture was vortexed, incubated on ice for 15 min, and centrifuged at 15 700×g for 20 min. To 20 µL of supernatant were added 20 µL of 0.4 mol L<sup>-1</sup> phosphate buffer (pH 7.4), 10 µL of double-distilled water, and 80 µL of color reagent solution. This last solution was prepared by mixing solution A (31% (w/v) phosphoric acid, 4.6% (w/v) TCA, and 0.6% (w/v) ferric chloride) with solution B (4% (w/v) 2,2'-dipyridyl). These mixtures were incubated at 37 °C for 40 min and measured at 525 nm using a UV-visible spectrophotometer (NanoPhotometer™; Implen). All extracts were from three biological replicates, and three technical assays were carried out on each biological repetition. The concentration was expressed in micrograms per gram FW.

### Determination of antioxidant activity

2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS; Sigma-Aldrich) radical cations were prepared by mixing an aqueous solution of 2.45 mmol L<sup>-1</sup> potassium persulfate (final concentration) with an aqueous solution of 7 mmol L<sup>-1</sup> ABTS (final concentration) and allowed to stand in the dark at room temperature for 12–16 h before use. The ABTS radical cation solution was diluted in phosphate-buffered saline (PBS; pH 7.4) to an absorbance of 0.40 at 734 nm. Trolox was used to prepare a

standard calibration curve (0–16 µmol L<sup>-1</sup>). After the addition of 200 µL of diluted ABTS to 10 µL of Trolox standard or extracts diluted in PBS, in each well of a 96-well plate (Costar), the absorbance was read at 734 nm 6 min after initial mixing using an Infinite200Pro plate reader (Tecan). All extracts were from three biological replicates, and three technical assays were carried out on each biological repetition. The percentage absorbance inhibition at 734 nm was calculated as a function of concentration of Trolox, and the Trolox equivalent antioxidant capacity (TEAC) value was expressed as Trolox equivalent (TE, µmol) using Magellan v7.2 software.

### Culture of dendritic cells and enzyme-linked immunosorbent assay

Bone-marrow-derived dendritic cells (BMDCs) were obtained from C57BL/6 mice, in agreement with national and international guidelines, approved by the authors' institutional review board (Organism for Animal Wellbeing – OPBA).

BMDCs were harvested as previously described<sup>19</sup> and plated in Roswell Park Memorial Institute 1640 medium supplemented with fetal bovine serum, antibiotics, recombinant mouse granulocyte macrophage stimulating factor and recombinant mouse interleukin (IL)-4 at 37 °C in a humidified 5% carbon dioxide atmosphere. BMDCs were treated with tomato methanol extracts (100 mg lyophilized powder per milliliter, 1:25 final dilution), after administration of lipopolysaccharide (LPS; 1 µg mL<sup>-1</sup>) at day 8 for 24 h. BMDCs culture media were analyzed for IL-6 and IL-12 in triplicate using an enzyme-linked immunosorbent assay (ELISA) kit as described by the manufacturer (R&D Systems).

### Data analysis

Values are expressed as mean plus/minus standard deviation (SD). Group differences were analyzed and compared by paired two-tailed Student's *t*-tests. Yield differences among the genotypes analyzed were determined using SPSS Package 6, version 15.0. Significant different yields were determined by comparing mean values through a factorial analysis of variance with Duncan *post hoc* test at a significance level of 0.05. Spearman correlations were calculated to analyze co-occurrence and associations among all traits measured. The *P*-values obtained for multiple tests were corrected using the Benjamini and Hochberg false discovery rate (FDR). In order to identify the landraces with a desirable combination of traits, an evaluation index (EI) was estimated by assigning a score to each trait, which was a maximum of 11 to a minimum of 1 descending from the highest to the lowest value for all traits except for pH, IL-6, and IL-12, where the maximum score (11) was assigned to the lower value and the minimum score (1) to the highest value.

## RESULTS AND DISCUSSION

### Agronomic performances of tomato landraces under harsh temperature conditions

A group of tomato landraces was previously selected for yield performances under high temperatures in two regions of southern Italy, Campania and Apulia, in 2016.<sup>21</sup> In the present study, we decided to test them again under high temperatures in an open field in the Apulia region in 2017. Plants were transplanted with a 1 month delay with respect to the usual agronomical practice of the area, exposing them to higher temperatures during the critical stages of flowering and fruit setting. Figure S1 reports the values of mean, maximum, and minimum temperatures recorded over the growing season, together with the average relative humidity of the whole day. As shown, more than 40 days (38.5%

**Table 2.** Yield (kg) per plant measured on ten landraces and the control cv Moneymaker during 2017

Genotype	Yield (kg)/plant	Production level
E7	3.43 ± 0.57 <sup>abc</sup>	Medium
E8	4.15 ± 1.12 <sup>c</sup>	High
E17	2.71 ± 0.29 <sup>ab</sup>	Medium
E30	1.70 ± 0.39 <sup>a</sup>	Low
E32	4.93 ± 0.83 <sup>c</sup>	High
E36	5.67 ± 0.58 <sup>d</sup>	High
E53	3.67 ± 0.25 <sup>bc</sup>	Medium
E76	2.59 ± 0.41 <sup>ab</sup>	Medium
E94	1.67 ± 0.71 <sup>a</sup>	Low
PDVIT	2.98 ± 0.51 <sup>abc</sup>	Medium
'Moneymaker'	4.85 ± 0.60 <sup>c</sup>	High

Following Duncan's *post hoc* test, the level of production under high temperature was also reported. Values followed by the same letters are not significantly different.

of the whole growing season, from the end of May to the beginning of September) reached temperatures over 32 °C, which is considered the critical temperature affecting the reproductive stage of tomato, as well as of other species.<sup>21</sup> On 16 days (15.4%), a temperature higher than 35 °C was even recorded. In addition, very high temperatures were also recorded in the night, and this is considered a critical point for pollen maturation.<sup>22</sup>

Based on the evaluation of production per plant (Table 2), four genotypes were classified as good (E8, E32, E36, 'Moneymaker'), five as medium (E7, E17, E53, E76, PDVIT), and two as low producers (E30, E94) under high-temperature conditions. Indeed, the first group exhibited a yield per plant higher than 4 kg, the second group yield ranged from 2 to 4 kg per plant, whereas the third group produced less than 2 kg per plant. As a whole, yield performances confirmed previous data and allowed a reliable classification of the genotypes in the aforementioned three groups.

### Assessment of main quality parameters

With the aim of identifying those landraces simultaneously exhibiting good productivity and good nutritional quality under high temperatures, and thus worth proposing as resilient varieties, we

evaluated the main quality parameters of the ten landraces. As already reported in a previous study,<sup>23</sup> we chose the indeterminate variety 'Moneymaker' as a control genotype, considering that it generally exhibits stable yields and fruit quality traits under different environmental conditions and in different years. Concerning the main fruit quality traits, the pH values ranged from 4.3 (landraces PDVIT and E76) to 4.6 (E17), with a mean value of 4.4, whereas the titratable acidity ranged from 0.38 g to 0.55 g of citric acid per 100 mL of tomato juice in E36 and E76 respectively (Table 3). The soluble solid content varied from 3.6 °Bx (E7) to 8.1 °Bx (E8). Finally, E94 showed the lowest level of firmness (8.7 kg cm<sup>-2</sup>) and PDVIT the maximum (18.9 kg cm<sup>-2</sup>). Noteworthy is that the three landraces E8, E30, and E76 showed high degrees Brix levels (>7), and two of these (E30 and E76) also had a titratable acidity value higher than 0.5. Altogether, these traits affect consumer taste, who generally prefer firm, sweet, and acid tomatoes,<sup>24</sup> and consequently the commercial value of tomatoes.

In recent years, more attention has been paid to the nutritional value of food products, focusing on the beneficial effects of fresh fruit and vegetables. To verify if the selected landraces could also provide a good nutritional value when grown under high temperatures, the main tomato phytonutrients contents, such as AsA, polyphenols, and carotenoids, were determined. These compounds have been associated to health benefits and the reduction of inflammatory and aging-related diseases.<sup>25–27</sup> Therefore, the dietary intake of these compounds is highly recommended.

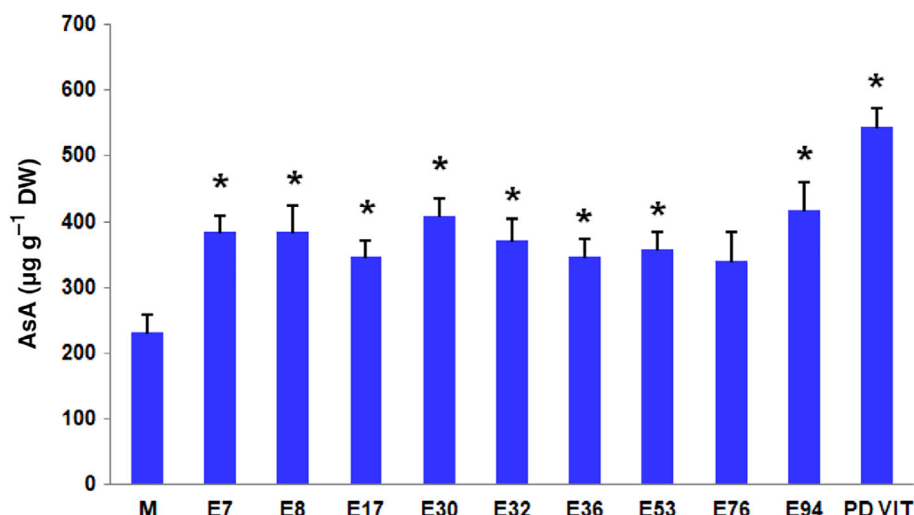
The AsA levels in the red ripe fruit of the ten tomato landraces and in the control cv Moneymaker are reported in Fig. 1. Most landraces exhibited values of approximately 350 µg g<sup>-1</sup> FW, which was statistically different from the value recorded in 'Moneymaker' (232 µg g<sup>-1</sup> FW), whereas two of them (E30 and E94) reached a mean value higher than 400 µg g<sup>-1</sup> FW. The landrace PDVIT was the best performer, with a mean value over 500 µg g<sup>-1</sup> FW. These values are in line with the values previously reported for tomato genotypes, where AsA ranged from 100 to 880 µg g<sup>-1</sup> FW, even though commercial cultivars are generally characterized by lower contents (from 100 to 400 µg g<sup>-1</sup> FW), probably due to the breeding process.<sup>28</sup> However, the AsA content in fresh tomato fruits is also dependent on genotype, climatic conditions, fruit development, and maturation.<sup>16</sup>

Carotenoids accumulation has been reported following abiotic stresses, such as high or low temperature and high light, upon which the homeostasis of ROS metabolism is challenged. For

**Table 3.** Qualitative traits (mean and standard error) parameters measured on red ripe fruit. Statistical analysis was carried out by comparing values with those recorded in the cv Moneymaker (M)

Genotype	pH	Soluble solid content (°Bx)	Titratable acidity (g citric acid/100 mL juice)	Firmness (kg cm <sup>-2</sup> )
E7	4.45 ± 0.05	3.62 + 0.26**	0.43 + 0.01	14.31 + 1.11
E8	4.41 ± 0.02	8.13 ± 0.01**	0.46 + 0.01	15.16 + 0.12
E17	4.65 + 0.01*	4.29 + 0.17**	0.49 + 0.02	14.02 + 2.10
E30	4.42 + 0.04	7.88 + 0.21**	0.53 + 0.04	13.21 + 1.34
E32	4.49 + 0.02	6.58 + 0.24	0.39 + 0.01	15.03 + 0.18
E36	4.40 + 0.05	3.60 + 0.45**	0.38 + 0.03	14.05 + 2.51
E53	4.40 + 0.04	5.92 + 0.20	0.47 + 0.02	11.17 + 1.45
E76	4.32 + 0.02*	7.74 + 0.26*	0.55 + 0.03*	10.91 + 0.98*
E94	4.36 + 0.04	6.78 + 0.12	0.48 + 0.03	8.68 + 0.01**
PDVIT	4.31 + 0.06	5.92 + 0.01	0.50 + 0.02	18.90 + 1.50
'Moneymaker'	4.47 + 0.04	6.17 + 0.27	0.43 + 0.02	15.18 + 1.05

\*P < 0.05, \*\*P < 0.01 (Student's t-test).



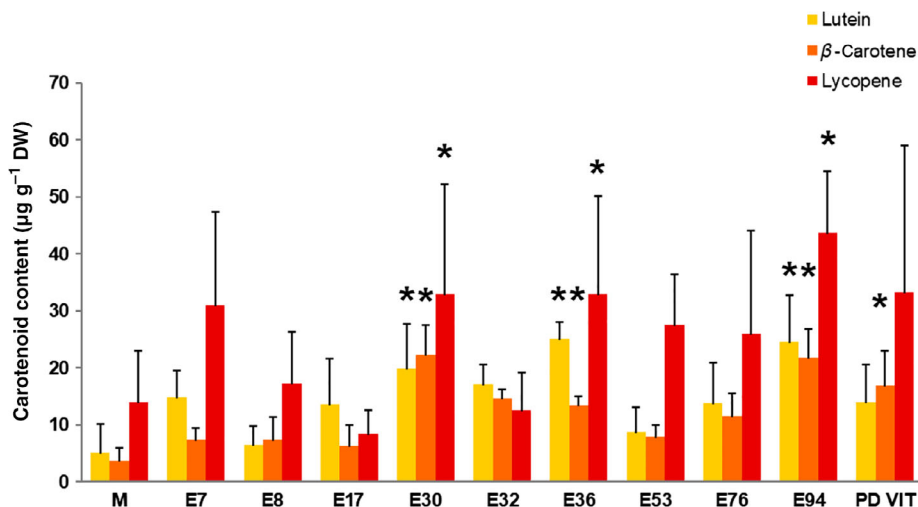
**Figure 1.** AsA content in the red ripe fruit of the ten tomato landraces and the control variety 'Moneymaker'. Data are shown as mean ± SD (n = 3). \*P < 0.05, \*\*P < 0.01 (Student's t-test).

these reasons, carotenoids are implicated in the thermo-tolerance of different plant species, including tomato.<sup>2,29</sup> Therefore, herein, we analyzed the carotenoids content in the fruits of the tomato landraces considered here. Concerning the main carotenoids (i.e. lycopene, β-carotene, and lutein), our data showed significantly higher levels for these compounds in E30, E36, and E94 compared with cv Moneymaker (Fig. 2). Noteworthy is that a higher β-carotene content compared with the control cultivar was also evidenced in PDVIT. This compound is considered a provitamin, which can be converted into retinol, a phytochemical essential for vision. β-Carotene is also known to act as a strong antioxidant and is the best quencher of singlet oxygen.<sup>16</sup>

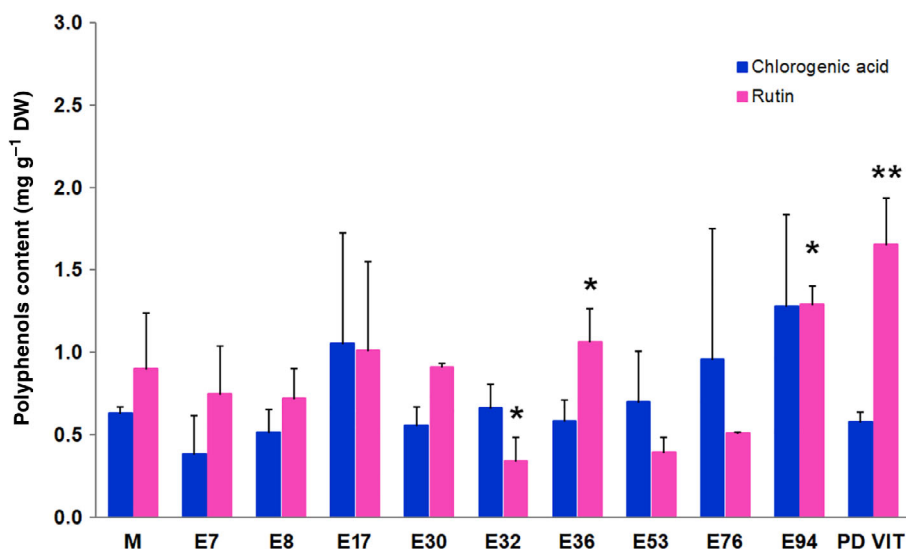
The β-carotene/lycopene ratio (Fig. S2) showed a different trend among the landraces tested, with higher levels recorded in E17, E30, and E32 (0.76, 0.68, and 1.16 respectively) compared with cv Moneymaker (0.27) and other landraces. The differences in the β-carotene/lycopene ratio can be attributable to a possible

different genetic background of the landraces tested, which might affect either the β-carotene or lycopene biosynthesis. Regarding this point, further molecular analyses are needed to better elucidate possible genetic variations, which could explain the differences in the amounts of these phytochemicals. In addition, harsh environmental conditions, such as high temperature, can also impact lycopene accumulation in tomato fruits.<sup>30–32</sup> Brandt *et al.*,<sup>30</sup> for example, reported decreased lycopene biosynthesis under high temperatures in the tomato F<sub>1</sub> variety 'Lemance'.

Flavonoids, such as quercetin-3-rutinoside (rutin), and hydroxycinnamic acids (such as chlorogenic, ferulic, and caffeic acids) are the most representative phenolic compounds of tomatoes.<sup>33,34</sup> These compounds are characterized by the presence of phenolics rings and hydroxyl groups in their structure that can scavenge free radicals,<sup>35,36</sup> thus inhibiting the generation of ROS. In this study, no significant differences were detected in the amount of



**Figure 2.** Content of the main carotenoids in tomato fruits of different landraces and the cv Moneymaker (M). Analyses were carried out by HPLC on fruit extracts. Statistical analysis was carried out by comparing the content of each compound with that recorded in the cv Moneymaker (M). Data are mean plus/minus SD (n = 3). \*P < 0.05 (Student's t-test).

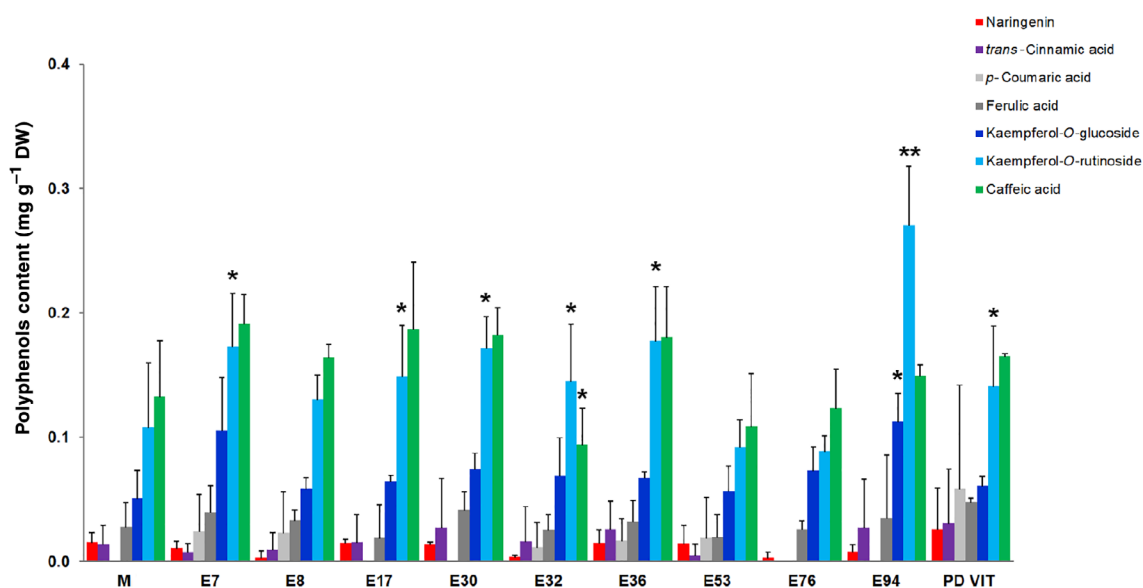


**Figure 3.** Chlorogenic acid and rutin content in fruits of different landraces and the cv Moneymaker (M). Quantification was carried out by HPLC using fruit methanolic extracts. Statistical analysis was carried out by comparing the content of each compound with that recorded in the cv Moneymaker (M). Data are mean plus/minus SD ( $n = 3$ ). \* $P < 0.05$ , \*\* $P < 0.01$  (Student's  $t$ -test).

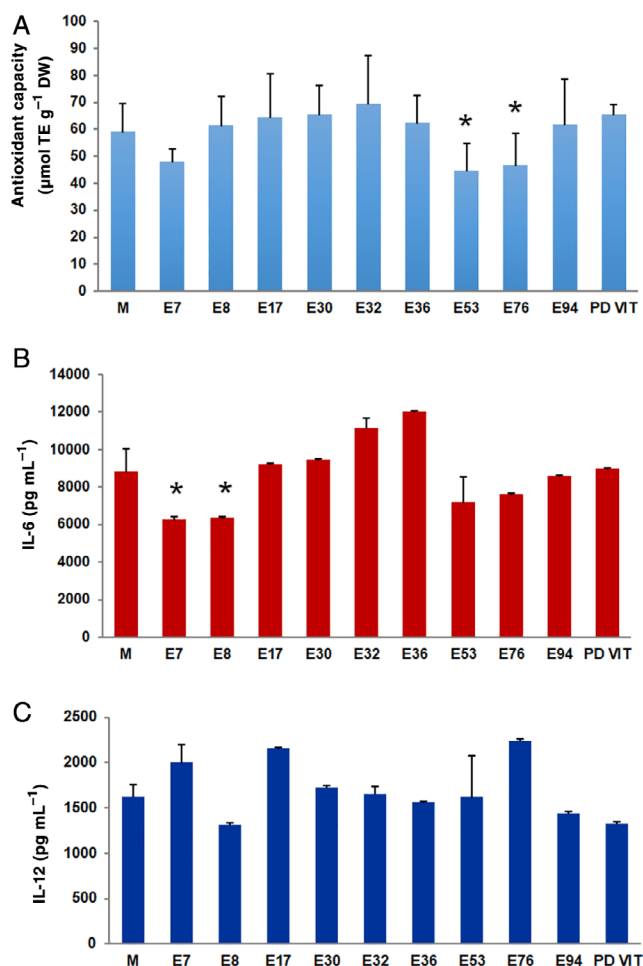
chlorogenic acid among the ten landraces and the commercial cv Moneymaker (Fig. 3). However, significantly higher rutin levels were detected in E36, E94, and PDVIT, compared with the control cultivar (Fig. 3). A lower rutin level was instead detected in E32. In the majority of the landraces analyzed in this study, higher levels of kaempferol-*O*-rutinoside, one of the most common derivatives of kaempferol in tomato fruit, were detected in E7, E17, E30, E32, E36, E94, and PDVIT in comparison with 'Moneymaker' (Fig. 4). A higher kaempferol-*O*-glucoside content was also detected in E94. Low levels of other phenolic compounds, such as naringenin, were overall detected in the majority of landraces (Fig. 4).

The higher polyphenols levels detected in some of the tomato landraces analyzed could also be explained as a

stronger response to abiotic stresses, including heat stress and exposure to UV radiation. Indeed, changes in the polyphenols content have already been reported in tomato following UV exposure.<sup>37</sup> In fact, genes implicated in polyphenol biosynthesis are activated by light exposure, and a 'sunscreen' function has been proposed for these phytochemicals to protect the tissues from possible damage generated by UV radiation.<sup>10</sup> Some authors reported that high temperatures and light exposure stimulate the production of phenolic acids and other flavonoids.<sup>38,39</sup> Indeed, heat stress positively modulates the activity of the enzyme phenylalanine ammonia-lyase and affects the total phenols content by activating their biosynthesis and inhibiting their oxidation in tomato plants.<sup>38,39</sup>



**Figure 4.** Polyphenols content in tomato fruits from different landraces and the cv Moneymaker (M). Quantification was carried out by HPLC using methanolic extracts of fruits. Statistical analysis was carried out by comparing the content of each compound with that recorded in the cv Moneymaker (M). Data are mean plus/minus SD ( $n = 3$ ). \* $P < 0.05$ , \*\* $P < 0.01$  (Student's  $t$ -test).



**Figure 5.** Antioxidant and anti-inflammatory activities of tomato extracts from different landraces. (a) Antioxidant capacity profiles measured by TEAC. Data are mean  $\mu\text{mol TE} \pm \text{SD}$  ( $n = 3$ ),  $*P < 0.05$  (Student's *t*-test). Levels of pro-inflammatory (b) IL-6 and (c) IL-12 in BMDCs stimulated with LPS and treated with methanolic tomato extracts from fruits of different tomato landraces. Concentrations of cytokines were determined by ELISA test. Data are expressed as mean plus/minus SD ( $n = 3$ );  $*P < 0.05$  (Student's *t*-test).

### Antioxidant/anti-inflammatory properties of tomato landraces

The antioxidant capacity of methanol extracts from fruits of the ten tomato landraces is reported in Fig. 5(a), using the TEAC assay. Previous studies reported that rutin and phenolic acids can contribute to the overall antioxidant capacity of tomato, neutralizing free radicals by acting as electron donors or inhibiting the activity of enzymes involved in the production of free radicals.<sup>39</sup> Our results indicated a slight, not significant, higher antioxidant activity in E32, followed by E17 and E30. Conversely, a significant lower antioxidant activity was evidenced in E53 and E76, which also showed a lower polyphenols content (see Fig. 4). E76 also showed a lower AsA content than in the other landraces. Indeed, it is well known that the antioxidant property of a food matrix is dependent on the presence and levels of different compounds comprising phenolic species and AsA.<sup>38</sup>

To assess the anti-inflammatory potential and the ability to trigger an *in vitro* immune-modulating response, tomato extracts were incubated with murine dendritic cells. LPS was used as an

inflammatory stimulant. Figure 5(b) shows the effects of the administration of tomato extracts on the production of pro-inflammatory IL-6 and IL-12. Significantly lower levels of IL-6 were detected in the presence of E7 and E8 extracts. Both these landraces showed increased levels of AsA, which could be related to the decreased levels of the pro-inflammatory activity of IL-6. E8, together with E94 and PDVIT extracts, was also able to decrease the secretion of the pro-inflammatory IL-12 (Fig. 5 (c)) even though the differences observed were not significant. Some studies have reported the reduction of pro-inflammatory interleukins in dendritic cells mediated by the aglycone quercetin, thus demonstrating an anti-inflammatory activity for this phytochemical.<sup>40–42</sup> In this context, rutin, which is the most abundant phenolic compound in our quantification, could be responsible, at least in part, for the anti-inflammatory activity detected in the tomato extracts. However, our data did not establish a clear correlation between the content in phenolic compounds and the anti-inflammatory activity in all the landraces tested. Indeed, the role of flavonoids has been generally described using chemically pure standards, only partially reflecting the real anti-inflammatory activity that can be exerted by a whole fruit or vegetable extracts.<sup>43</sup>

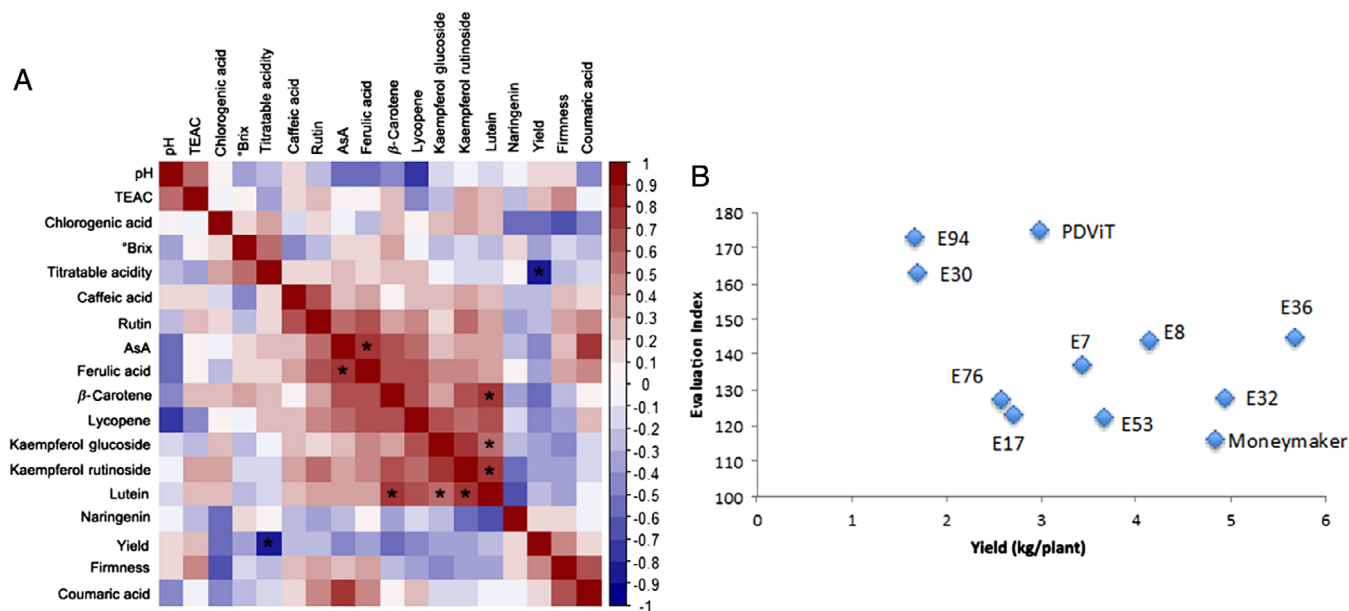
### Combining yield and quality parameters

To examine the possible co-occurrence of both yield and quality features in the landraces tested, we calculated Spearman correlations considering all the traits included in this study (Fig. 6(a)). Positive correlations, significant in some cases, were found among the secondary metabolites analyzed (AsA, carotenoids, phenolic acids, and flavonoids), indicating that the observed changes within the plant secondary metabolism involve the cross-talk among different classes of phytochemicals and thus different biochemical pathways. Furthermore, some of these metabolites positively correlated to physicochemical parameters such as acidity, degrees Brix, and fruit firmness, indicating an association between secondary metabolites and traits influencing fruit organoleptic properties.

An estimated EI was also calculated considering the quality and nutritional traits analyzed all together. EI varied from a minimum of 123 for E17 to a maximum of 175 for PDVIT, with a mean value of 141.2. The distribution of the ten landraces according their EI value and yield is shown in Fig. 6(b). From this analysis, it is evident that three landraces (E30, E94, PDVIT) exhibit better performances in terms of fruit quality ( $\text{EI} > 160$ ), though two of them (E30 and E94) showed low yield (less than 2 kg per plant) under high temperatures. All the better performing landraces in terms of yield (classified as high or medium producers), with the exception of PDVIT, exhibited lower EI levels, thus evidencing lower values of quality traits.

### CONCLUSION

In this paper, one landrace (PDVIT) was selected as a good compromise between yield performances and good fruit quality and nutritional traits when growing under high temperature. Additional molecular and physiological analyses in other environments are in progress in order to further characterize this selected landrace. Indeed, this landrace shows a level of adaptability that can be useful in adverse conditions, making it a suitable candidate for breeding programs, since it can be



**Figure 6.** Correlation analysis and EI. (a) Spearman correlation analysis of the measured traits in the tested landraces. The correlation values range from (–1.00) (blue) to 1.00 (red); \* FDR-adjusted  $P$ -values ( $q < 0.05$ ). (b) Scatter diagram of the ten landraces and the control genotype ‘Moneymaker’ according to their EI and yield per plant production.

considered as a ‘balanced landrace’<sup>44</sup> in terms of stress resilience and fruit nutritional quality.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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