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Higher Yield and Fruit Quality of a *Solanum pennellii* Introgression Line †

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Abstract: Cultivated tomato (*Solanum lycopersicum* L.) is an important source of antioxidants, such as ascorbic acid, carotenoids and phenolic compounds. Epidemiological results confirm that these antioxidant molecules are associated with a reduced risk of cancer, inflammation and cardiovascular diseases. Recently, one Introgression Line population deriving from *Solanum pennellii* has been exploited to identify favorable alleles that can improve fruit quality traits in commercial varieties, including antioxidants content. The aim of this work was to evaluate growth, final yield and content of nutraceutical compounds at the ripe red fruit stage in one subline coded R182 which carries only a small region (448 Kbp) of wild genome in the cultivated genetic background (M82). Analyses carried out on R182 and on the parental line M82, demonstrated that the subline showed better performances in terms of yield and fruit qualitative traits most considered for tomato processing. Indeed, higher yield (+28.96%), content of soluble sugars (+34.64%) and titratable acidity (+78.94%) were demonstrated for R182 compared to M82. Also, for the nutritional traits analyzed, an increase in the content of phenols (+ 69.96%), ascorbic acid (+ 48.55%), carotenoids (+ 29.66%), lycopene (+ 31.22%) and β-carotene (+31.67%) was observed. Therefore, it is possible to assert that the subline R182 may be considered as a good candidate to be used as parental genotype in breeding programs.

Keywords: wild species; *Solanum lycopersicum*; fruit quality; ascorbic acid; carotenoids; phenols; total yield

Introduction

Tomato (*Solanum lycopersicum* L.) belongs to the Solanaceae family, that is one of the most economically important family and that has a worldwide distribution, being present in all continents except Antarctica . Tomato plays an important role in human health. It is one of the main components of the Mediterranean diet and its consumption has been associated with a reduced risk of cancer, inflammation and cardiovascular diseases . These effects are attributed to the presence of antioxidant molecules such as ascorbic acid (vitamin C), carotenoids and phenols, in the fruit .

One of the most important antioxidants is ascorbic acid because humans are unable to synthesize it on their own but have to take it with the diet. Compared to wild species, cultivated tomatoes contain moderate amounts of vitamin C, as a consequence of several cycles of domestication and breeding activities.

In the last few years, there has been a growing interest in the Introgression Lines (IL) containing alleles from wild species that can be used to improve tomato productivity and nutritional quality. Previously, seventy-six Introgression Lines have been obtained cross-

ing *Solanum lycopersicum* cv. M82 with *Solanum pennellii* that are characterized by the presence in homozygosity of a portion of the genome of the wild species introduced into that of the cultivated species.

Sacco et al. studied one *Solanum pennellii* introgression line (IL7-3) and demonstrated that this line had an increased content of ascorbic acid and carotenoids in ripe fruit compared to the cultivated line M82. Additionally, a subline (coded R182) of IL7-3 was identified which showed a higher antioxidant content compared to the cultivated line M82 . In the present work, in order to further analyze the potential of the R182 subline, we evaluated titratable acidity, the content of soluble solids and of the main nutraceutical compounds, such as ascorbic acid, carotenoids and phenols, in the subline and in the cultivated line M82. Moreover, a phenotypic analysis was performed to evaluate the productivity of each genotype. Statistical analysis showed that the subline R182 has a higher antioxidants content and higher final yield compared to the parental line. Our findings indicate that this line could be used as breeding material to obtain new varieties with higher fruit quality.

Materials and Methods

Plant material

Plant material consisted of the cultivated genotype M82 (accession LA3475) and IL 7-3 subline (genotype coded R182). The Tomato Genetics Resource Center (TGRC) kindly provided the seeds (http://tgrc.ucdavis.edu/) of M82 and Dr. Dani Zamir (Hebrew University, Israel) those of the subline R182. The genotypes M82 and R182 were grown in open field in the Campania Region in Italy during the year 2020 in a completely randomized design with three replicates per genotype and 10 plants per each replicate and following the standard cultural practices of the area. Tomato fruits were collected at mature red (MR) stage. Subsequently, the seeds were removed, and fruits were ground in liquid nitrogen by a blender (FRI150, Fimar) to a fine powder, and stored at -80°C until further analyses.

Phenotypic analyses

At the end of the cultivation cycle, all plants of each genotype were harvested and plant height, fresh weight of biomass and yield *per* plant (YP) were recorded. YP was evaluated using the total weight of all collected fruits and dividing it for the number of total plants of the three replicates.

Qualitative analyses

For each genotype and biological replicate, fruits were collected at MR stage to evaluate soluble solids content (SSC), titratable acidity (TA) and firmness. The soluble solids content was measured as °Brix in the homogenized juice from ripe fruit by a digital refractometer (Hanna). Titratable acidity or total acidity was measured by titration to a pH end point with a standard base (NaOH 0.1 N). Firmness was measured by using a penetrometer PCE_PTR200 with a surface needle of 8mm.

Total carotenoids, lycopene and β-carotene determination

The evaluation of lipophilic fraction was carried out according to the method reported by Wellburn and Zouari et al. as modified by Rigano et al.. To extract the carotenoids, 500 mg of frozen sample powder was extracted with 8 mL of acetone/hexane (40/60, v/v), with the aid of a pestle. The mixture was stirred at 300 rpm for 20 min at room temperature and then centrifuged at 5000 rpm for 5 min at 4 °C. This procedure was repeated three consecutive times. Each time the supernatant was collected in a 50 mL Falcon tube and ultimately stored at −20 °C until analysis of total carotenoids, lycopene and β-carotene.

All biological replicates of samples were analyzed in triplicate. The absorbance was measured at 470 nm for carotenoids, 453, 505, 645, and 663 nm for lycopene and β-carotene, with a NanoPhotometerTM (Implen, Munich, Germany) using acetone/hexane as

reference. Total carotenoids, lycopene and β-carotene were determined by the equation reported by Wellburn . The concentration was expressed as mg/100 g of fresh weight (FW).

Ascorbic acid determination

Ascorbic acid determination, both reduced ascorbic acid (reduced AsA), and total ascorbic acid (AsA + DHA, total AsA), was carried out by a colorimetric method with modifications reported by Rigano et al. . Briefly, 500 mg of frozen tomato fruit powder were extracted with 300 µl of ice cold 6% TCA (Trichloacetic Acid). The mixture was vortexed for 10 s, incubated for 15 min on ice and centrifuged at 14000 rpm for 20 min at 4°C.

Reduced Ascorbic Assay. Twenty microliters of supernatant for each assay was transferred in a 1.5 ml Eppendorf tube with 20 µl of 0.4 M phosphate buffer (pH 7.4) and 10 µl of double distilled (dd) H2O. Then, 80 µl of color reagent solution were added. This solution was prepared by mixing solution A [31% H_3PO_4, 4.6% (w/v) TCA and 0.6% (w/v) $FeCl_3$] with solution B [4% 2,2'- dipyridil (w/v; made up in 70% ethanol)].

Total Ascorbic Assay. Twenty microliters of sample, 20 µl of 5 mM dithiotreitol in 0.4 M phosphate buffer (pH 7.4), were added to reduce the oxidized ascorbate, and the mixture was incubated for 20 min at 37°C. Ten microliters of N-ethyl maleimide (NEM; 0.5% (w/v) in water) were added, mixed, and left for 1 min at room temperature. Eighty microliters of color reagent were added as previously described for reduced AsA.

Both the final mixtures for the tests of reduced and total AsA were incubated at 37°C for 40 min and measured at 525 nm by a NanoPhotometerTM (Implen, Munich, Germany) using 6% TCA as reference. Three separated biological replicates for each sample and three technical assays for each biological repetition were measured. The concentration was expressed in nmol of AsA according to the standard curve, designed over a range of 0–70 nmol; then the values were converted into mg/100 g of fresh weight (FW).

Total phenolic determination

The extraction of hydrophilic fraction was carried out according to the Folin – Ciocalteu's test and Marinova et al. with minor changes. Briefly, frozen ground tissue (250 mg) was weighed, placed in a mortar and with the aid of a pestle the phenols were extracted with 1 mL of 60% methanol. The extracts were transferred in a 15 mL Falcon tube and volume was increased to 5 ml adding 60% methanol. The samples centrifuged at 14000 rpm for 15 min at 4 °C, and the supernatant was stored at −20 °C until analysis of total phenolic compounds.

Three separate biological replicates were measured for each sample and three technical analysis were performed for each biological replicate. In a 2 mL Eppendorf 62.5 µl of supernatant, 62.5 µl of Folin - Ciocalteu reagent (Sigma, St. Louis, MO, USA) and 250 µl of deionized water were mixed and incubated for 6 min in the dark; 625 µl of 7.5% sodium carbonate and 500 µl of deionized water were subsequently added and incubated for 90 minutes at room temperature in the dark. The absorbance was measured at 760 nm by a NanoPhotometerTM (Implen, Munich, Germany) using 60% methanol as reference. Total phenol concentration was expressed as mg GAE/100 g of fresh weight (FW).

Statistical analysis

Results were expressed as the mean value ± SD (n = 9). The comparison between the genotypes was carried out by a Student's t-test. Differences at $p < 0.05$ were considered to be significant. The percentage of variations of each parameter in subline R182 compared to M82 was calculated by using the following formula:

Increase or Decrease (%) = [(value of tested genotype – value of M82) x value of M82 $^{-1}$] x 100

Results and Discussion

The cultivated line M82 and subline R182, previously obtained and characterized , were analyzed in the year 2020 by measuring height, fresh weight of biomass and yield *per* plant (**Table1**). Moreover, fruit quality was evaluated by measuring nine different traits (**Table 2** and **Figure 1**).

Yield *per* plant and height were higher in R182 compared to M82, increasing respectively by 28.96% and 11.84%. On the other hand, no differences were evidenced in the fresh weight of the biomass between the two genotypes (**Table 1**). These results are interesting considering that previous analyses did not evidenced a higher productivity in R182 compared to M82. This could be due to the different environmental conditions of the experimental fields used in these different studies.

For all the qualitative traits analyzed R182 showed a better performance compared to M82 (**Table 2**). An increase in °Brix of 34.64% was evidenced, as already previously demonstrated , and also titratable acidity increased by 78.94% in R182 compared to the cultivated genotype. No differences in terms of fruit firmness were instead evidenced herein, in contrast with results obtained by *Calafiore et al.* (2019) .

The nutraceutical compounds were evaluated at the stage of ripe red fruits in the cultivated line M82 and in the subline R182. Assessing the content of lipophilic antioxidant, the R182 genotype showed a 30% increase in the content of carotenoids, lycopene and β-carotene compared to the M82. This result is in contrast with previous results that showed no different in terms of carotenoids content between the two lines, and should be further verified .

The content of hydrophilic antioxidants was higher in the subline R182 compared to the cultivated line M82. Results obtained are reported in **Figure 1**. Genotype R182 showed an increase of 48.55% in total ascorbic acid content and 40.09% in reduced ascorbic acid content compare to M82 plants, confirming data previously obtained in our laboratories. The average of total phenolic compounds of M82 was 39.71 mg GAE/100g FW. The subline R182 showed an increase of 70% compared to the cultivated line. It is the first time that also an increase in the amount of these compounds was evidenced in this subline. The increased performances demonstrated for R182 in this study could be due to the presence of wild *S.pennellii* alleles introgressed in the cultivated genome and transcriptomic and functional analyses are currently under way to identify and characterize these wild genes.

Table 1. Biometric parameters of two tomato genotypes grown in open field. The values are means ±SD (n = 9). The differences between the two genotypes (M82 vs. R182) were evaluated by the Student's t-test (*P < 0.05; **P < 0.01; ***P < 0.001).

Genotype	Height (cm)	Fresh weight (g)	Yield (Kg/pt)
M82	64.20 ± 7.07	316.00 ± 57.97	0.37 ± 0.06
R182	71.80 ± 5.96 *	314.00 ± 65.35	0.48 ± 0.02 *

Table 2. Fruit quality traits of two tomato genotypes grown in open field. The values are means ±SD (n = 9). The differences between the two genotypes (M82 vs. R182) were evaluated by the Student's t-test (*P < 0.05; **P < 0.01; ***P < 0.001).

Genotype	Soluble solids (°Brix)	Titratable acidity (g/100 g FW)	Firmness (Kg/cm²)	Total carotenoids (mg/100g FW)	Lycopene (mg/100g FW)	β-carotene (mg/100g FW)
M82	5.60 ± 0.39	0.33 ± 0.01	5.73 ± 0.68	16.44 ± 1.83	1.01 ± 0.13	0.14 ± 0.01
R182	7.54 ± 0.38 ***	0.59 ± 0.06 ***	6.15 ± 0.59	21.32 ± 1.94 ***	1.32 ± 0.13 ***	0.18 ± 0.01 ***

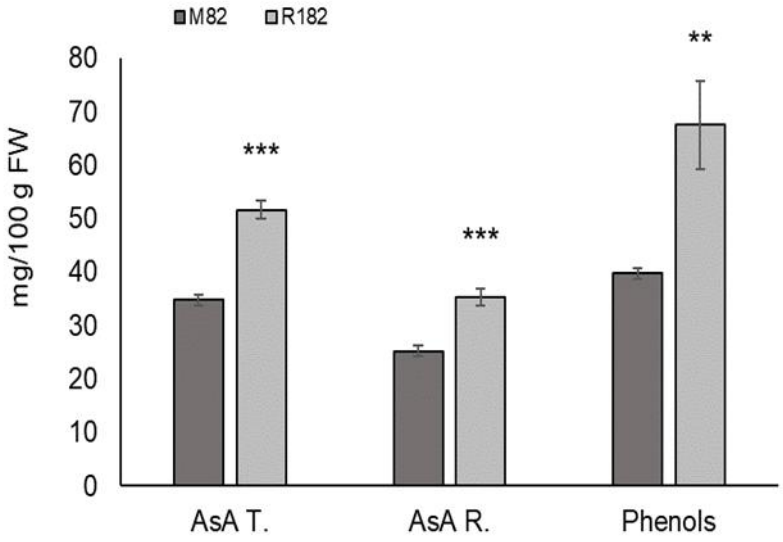

Figure 1. Content of total ascorbic acid (AsA T.), reduced ascorbic acid (AsA R.) and total phenols in two tomato genotypes analyzed. All are expressed as mg 100 g-1 FW. Values are means ± SD (n = 9). Asterisks indicate statistically differences of R182 compared to M82 by Student's t-test (*P < 0.005; **P < 0.01; ***P < 0.001).

Conclusion

Tomato fruit is one major source of antioxidants for the benefit of nutrition and human health, so it is very useful to select genotypes with high content of antioxidant molecules. From an agronomic point of view, it is important that these genotypes also have a good productivity. The results of this study demonstrated a better performance of one *S.pennellii* subline named R182 compared to that of the control line M82. Our results suggested that it could be possible to use this introgression line as breeding material to obtain new varieties. Now, additional studies are necessary to identify the genes controlling the quality traits evidenced in this line.

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