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The Influence of Diluted Seawater and Ripening Stage on the Content of Antioxidants in Fruits of Different Tomato Genotypes

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The aim of this study was to investigate if the combined effect of diluted seawater and ripening can improve the beneficial nutritional properties of tomato fruits from an antioxidant point of view. To reach the goal, different tomato cultivars and breeding lines, genetically modified for ripening, were investigated, and analysis of NADPH and $NADP^+$ as well as of the main antioxidants such as ascorbic acid, lipoic acid, and tocopherols was performed at two ripening stages. The research was conducted on berries of the following genotypes of tomato: cv. Jama, Gimar wild type, Gimar *gf*, and Gimar *nor*. The mutant *gf* is a typical "stay green" mutant, characterized by an incomplete loss of chlorophyll; the *nor* mutation is characterized by a reduced biosynthesis of ethylene and carotenoids. Both ripening and salinity induced an oxidative stress, and the sensitivity to salt treatment was genotype-dependent. The genotypes cv. Jama and Gimar *gf* line showed increases in ascorbic acid, lipoic acid, and α-tocopherol during both ripening and salt treatment whereas total ascorbate and tocopherols decreased in the berries from salt-treated plants of Gimar wild type. Ripening also determined decreases in ascorbate and tocopherol amounts in the Gimar *nor* line where a positive effect of ripening and salinity was observed.

KEYWORDS: ***Solanum lycopersicum*** **L.; tomato; ripening;** ***nor*** **and** ***gf*** **breeding lines; salinity; ascorbic acid; lipoic acid; tocopherol; cell redox status**

INTRODUCTION

Tomato is a moderate salinity-tolerant species (*1*) that requires relatively high irradiance and temperature for optimum yield and quality. Tomato is cultivated all over the world, especially in Southern Europe where growers are increasingly facing both scarcity and deterioration of irrigation water. The controlled use of alternative water resources, such as diluted seawater (*2*), could be a valid tool to face drought in the Mediterranean regions.

The nutritional value of tomato fruits is affected by the presence of pigments and other antioxidant compounds such as vitamin C (ascorbic acid, AsA) and vitamin E (tocopherol), whose consumption is related to oxidative processes, which could be induced by natural physiological processes such as ripening or more by environmental changes such as salinity and drought. Indeed, a high salt level in the root zone stimulates the production of oxidizing agents in plant cells and results in redox reactions leading to metabolic alterations and reduced fruit yield (*2*, *3*). On the other hand, fruit ripening has also been described as an oxidative phenomenon that requires a turnover of active oxygen species (AOS) such as hydrogen peroxide and superoxide anion. There must be a balance between the production of AOS and their removal by antioxidant systems, which therefore play a crucial role in the ripening process (*4*). Indeed, fruit ripening is considered as a functionally modified form of senescence during which the cell membrane is deteriorated likewise in other senescing plant tissues (*4*). Tomato fruits exhibit a climacteric type of ripening physiology, since ethylene evolution and respiration increase with fruit ripening (*5*). In this species, however, various mutants affecting fruit ripening metabolism are known. Indeed, *rin* (ripening inhibitor), *nor* (non ripening), and *alc* (alcobaca) reduce ethylene synthesis whereas *Nr* (never ripe) and *Nr-2* (never ripe 2) are defective in the ethylene perception. Thus, the fruits from the corresponding homozygous plants are aclimacteric (mainly *rin* and *nor*).

Lipophilic and hydrophilic antioxidant activities increased in tomato berries at increasing salinity (*6*), and the levels of glutathione (GSH) and ascorbate increased during tomato ripening; these antioxidants are associated with significant changes in their redox status, becoming more reduced as ripening progressed (*4*). An increase in fruit antioxidants may enhance the shelf life and preserve the nutritional value, thus

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improving the overall quality of marketed fruits. Antioxidant compounds are universally recognized as beneficial for preventing widespread human diseases, including cancer and cardiovascular pathologies (*7*). Thus, the antioxidant activity is important for assessing the nutritional value of fruits and vegetables (*8*). Because it is well-recognized that the role of antioxidant molecules is critical in the detoxification of free radicals (*9*), mild salt treatment and fruit ripeness may significantly improve the beneficial nutritional properties of tomatoes and need to be investigated in order to cultivate cultivars with better nutritional characteristics under salinity conditions.

AsA is one of the most powerful antioxidants. It is involved in removing AOS and regenerating α-tocopherol (an important antioxidant in the lipid phase). Lipoic acid (LA), due to its solubility in both water and lipid phases, connects the activity of antioxidants in the cell membrane (α-tocopherol) with antioxidants in the cytoplasm (AsA and GSH), strengthening the antioxidant network (*10*). LA is unique, among antioxidant molecules, because it retains protective functions in both reduced and oxidized forms, although dihydrolipoic acid (DHLA) is the more effective one. Indeed, DHLA is able to donate an electron to the oxidized form of AsA and GSH, thus regenerating these compounds to their reduced powerful antioxidant forms. Eventually, AsA regenerates tocopherol from its oxidized form, the chromanoxyl radical, by means of a similar process of electron donation (*10*). This maintains a steady state of both vitamins C (AsA) and E (tocopherol). The LA/DHLA redox couple approaches the ideal, and it can be considered the "universal antioxidant" (*10*). LA has been used for detoxification of heavy metal poisoning, possible application in atherosclerosis, side effects of diabetes, and oxidative stress, and it may exert a protective effect against the formation of cataracts (*10*). LA has been listed among the main antioxidants in potatoes, and recently, it has been found also in wheat leaves and roots (*11*) and in tomato berries (*2*).

Vitamin E is often equated only with α-tocopherol; however, it is not a single compound and refers to at least four forms (α-, β-, γ-, and δ-tocopherol). α- and γ-Tocopherol are the two major forms of vitamin E found in human plasma and tissues. In experiments in vitro, α- and γ-tocopherol were found to inhibit the lipid peroxidation in large unilamellar liposomes (*12*). However, γ-tocopherol lacks one of the electron-donating methyl groups on its chromanol ring and, thus, is an antioxidant less effective than α-tocopherol. Anyway, γ-tocopherol is better able to trap nitrogen-based free radicals such as nitrogen dioxide and peroxynitrite to form 5-nitro-γ-tocopherol (*13*). γ-Tocopherol is abundant in many nuts and seed oils such as those derived from corn, soybean, and sesame (*14*). A synergistic effect in cell protection by β-carotene and vitamins E and C has been observed (*15*) and may be related to the fact that β-carotene does not only quench oxy-radicals but also repairs the α-tocopheroxyl radical.

The aim of the present research was to evaluate how a moderate level of salinity and the stage at which fruits are picked and put on the market (turning-red and red-ripe) affect antioxidative properties of berries due to the presence of vitamins and antioxidants. To better understand how the physiology of ripening influences the antioxidative response to salt, different tomato types, genetically modified for ripening, were employed. Jama and Gimar WT cultivars are typical "salad tomatoes", whereas Gimar *gf* and Gimar *nor* are near isogenic lines (NILs) of Gimar, respectively homozygous for either mutant genes. The mutant *gf* is a typical "stay green" mutant characterized by an incomplete loss of chlorophyll, due to an incomplete transformation of chloroplasts into chromoplasts. In the more advanced stages of maturity, although maintaining a residual proportion of chlorophyll, this fruit contains more carotenoids and lycopene with respect to the wild type (WT). The *nor* mutation (incompletely recessive) is characterized by a reduced biosynthesis of ethylene and, thus, by a reduced synthesis of carotenoids (lycopene and β-carotene) and does not have any respiratory climacteric during ripening (*16*).

MATERIALS AND METHODS

Chemicals. The standards LA, DHLA, α-, β-, γ-, and δ-tocopherol, glucose-6-phosphate, 2,6-dichlorophenol indophenol, phenazine methosulfate, NADPH, and $NADP^+$ were purchased from Sigma (Milan, Italy).

Plant Material. The different tomato (*Solanum lycopersicum* L.) genotypes studied in this work came from the Soressi germplasm collection at Tuscia University (Viterbo, Italy). Two "salad" tomato types (cv. Jama and cv. Gimar) and two NILs, obtained by backcrossing the mutant genes *gf* (green flesh; BC45S) and *nor* (non ripening; BC3S5) to the cv. Gimar, were analyzed. These two homozygous breeding lines are from now on reported as Gimar *gf* and Gimar *nor*.

Tomato plants were grown during the spring in a glasshouse with a minimum night temperature and a daytime ventilation temperature of 16 and 28 °C, respectively, and a mean value of daily radiation of 9.2 MJ/m^2 with a maximum photon flux density of 500−700 $\mu mol/m^2$ s. A closed-loop rockwool culture was used. Two salinity levels of the nutrient solutions were applied with an EC of 3.0 or 8.0 mS/cm, which corresponded to 0 and 10% of salt concentration of seawater. The concentration of nutrient solution was as reported by D'Amico et al. (*2*). Salinization was initiated 3 weeks after planting with 1.5 mS/cm daily increments to avoid osmotic shock. The nutrient solutions, renewed every 2 weeks, were checked daily for EC and pH (5.5−6.0).

The fruits were picked at two different ripening stages: turning-red (T) and red-ripe (R). The fruits considered for laboratory analysis were those picked from the second and third truss of separate plants. Fresh fruit materials were dried at 70 °C to determine the dry weight (DW).

AsA. Fresh tomato fruits were immediately homogenized at 4 °C in ice-cold 6% trichloroacetic acid (w/v) using first a Waring blender and then a cold mortar. The sample fresh weight (FW)/extraction solution proportion was 1:1. After centrifugation at 12000*g* for 30 min, the total and reduced ascorbate were determined in the supernatants. Determinations were carried out according to Sgherri and Navari-Izzo (*9*). Calibration curves for AsA and dehydroascorbic acid (DHA) in the range of 5−50 nmol were used.

LA. Both LA and DHLA were extracted from tomato berries by acidic hydrolysis according to Vianey-Liaud et al. (*17*). After hydrolysis, samples were extracted with chloroform following the procedure of Sgherri et al. (*11*). The resultant organic phases were collected, evaporated to dryness under vacuum, and stored at 4 °C under nitrogen. LA and DHLA contents in the extracted solutions were determined by isocratic reverse-phase high-performance liquid chromatography (RP-HPLC) using a Waters apparatus (model 515) with an electrochemical detector (Metrohom model 791) equipped with a glassy carbon electrode and a Millennium software (Waters) for integration of peaks. Detection was performed at +1.1 V at 25 °C with a Nova Pak C-18 4 μm column (3.9 mm × 150 mm). Extracts were eluted at 25 °C at a flow rate of 1 mL/min using 227.5 g of acetonitrile, 31.5 g of 2-propanol, and 674.5 g of 0.05 M KH_2PO_4 as the mobile phase (pH 2.5). Chromatographic peaks were identified by comparing both retention times and absorbance spectra with those of standards. Cochromatography of the standards with the samples was also used to identify peaks with close retention times. Mixtures of standards of LA and DHLA (Sigma) in the range of 4−100 ng were injected to calculate the calibration curve.

NADPH and $NADP^+$. Fresh tomato fruits were immediately homogenized at 4 °C first using a Waring blender and then a cold mortar with 0.1 N HCl for the determination of $NADP^+$ or 0.1 N NaOH for the determination of NADPH. Assay was performed spectrophotometrically (Spectrophotometer UV−Visible Varian Cary 1E) at 625

Table 1. Effect of Seawater Irrigation (10%, EC = 8 mS/cm) on Fruit Yield and Dry Matter in Different Genotypes of Tomato Berries[a]

EC (mS/cm)	fruit yield (kg/plant)	no. of fruits per plant	dry residue (% FW)	total soluble solids (°Brix)
		cv. Jama		
3	5.13 ± 0.29	22.3 ± 1.0	5.58 ± 0.23	4.23 ± 0.25
8	3.58 ± 0.15	21.5 ± 0.9	6.57 ± 0.31	5.68 ± 0.40
		Gimar WT		
3	5.47 ± 0.31	31.2 ± 0.7	5.63 ± 0.37	5.23 ± 0.33
8	3.30 ± 0.26	31.3 ± 1.1	8.08 ± 0.90	6.45 ± 0.64
		Gimar *gf*		
3	3.07 ± 0.31	19.3 ± 1.1	6.36 ± 0.67	4.98 ± 0.34
8	1.85 ± 0.23	18.9 ± 2.0	8.22 ± 0.52	6.13 ± 0.33
		Gimar *nor*		
3	3.97 ± 0.13	25.1 ± 3.1	6.26 ± 1.00	4.43 ± 0.31
8	2.50 ± 0.33	24.1 ± 4.0	7.44 ± 0.33	5.10 ± 0.47

[a] Results ± SE are the means from three replicates from three separate experiments ($n = 3$).

nm as previously described (*11*). The assay mixture contained extract (10 μL), 50 mM Tris-HCl buffer (pH 7.8), 6 mM glucose-6-phosphate, 7.5 mM 2,6-dichlorophenol indophenol, 3.7 mM phenazine methosulfate, and 5 U of glucose-6-phosphate dehydrogenase in the final volume of 1 mL. NADPH and $NADP^+$ levels were calculated referring to standard curves in the range of 5−75 pmol for NADPH and 5−50 pmol for $NADP^+$.

Tocopherol. Tocopherols were determined in lipid extracts from tomato berries. Extractions were performed in the dark according to Quartacci et al. (*18*) and under continuous flux of nitrogen. α-, β-, γ-, and δ-Tocopherol contents were determined by isocratic RP-HPLC using a Waters apparatus (model 515) with an electrochemical detector (Metrohom model 791) equipped with a glassy carbon electrode and Millennium software (Waters) for integration of peaks. Detection was performed according to Galatro et al. (*19*) at +0.6 V at 25 °C with a Nova Pak C-18 4 μm column (3.9 mm × 150 mm). The extracts were eluted with 95% methanol containing 20 mM $LiCLO_4$ at a flow rate of 1 mL/min. For identification of peaks, the retention times and maximum absorption spectra of tocopherols were compared with those of standards, which were also used for quantification. Standard mixtures of α-, β-, γ-, and δ-tocopherol (Sigma) in the range of 25−75 ng were injected to calculate the calibration curve.

Statistical Analysis. The results are the means from three replicates of three independent experiments ($n = 3$). All data are reported as mean values ± standard errors (SE). For each genotype, the data obtained were analyzed between the treatments and the two fruit-ripening stages considered. The data were subjected to two-way analysis of variance (ANOVA), and *, ** and *** indicated significant differences at confidence levels of $P \leq 0.05$, 0.01, and 0.001, respectively.

RESULTS

In all genotypes, the higher salinity did not affect the number of harvested fruits whereas it significantly reduced fruit yield and fruit weight (**Table 1**). Dry residue and total soluble solids increased or were not affected following salinity (**Table 1**).

All genotypes analyzed always showed a lower presence of NADPH than $NADP^+$ (**Table 2**). In cv. Jama, both NADPH and the NADPH/$NADP^+$ ratio increased during ripening whereas salt treatment and the interaction between ripening and salt had no significant effect on these parameters. In contrast with cv. Jama, all of the Gimar genotypes showed a significant effect of ripening and salt treatment, the latter determining a decrease in NADPH contents. The pattern of decrease of the NADPH/$NADP^+$ ratio with salt was similar to that of NADPH for both Gimar WT and Gimar *nor* whereas Gimar *gf* showed an increased ratio from 0.67 to 0.84 in the ripe fruits. Contrary to Gimar WT and Gimar *nor*, Gimar *gf* showed with ripening increases in NADPH by 73 and 82% at R3 (ripe fruit grown at 3 mS/cm EC) and R8 (ripe fruit grown at 8 mS/cm EC), respectively (**Table 2**).

Higher contents of AsA and AsA + DHA were present in Gimar *gf* in comparison with the other genotypes (**Table 3**). In cv. Jama, AsA + DHA levels increased with ripening by 4% whereas they did not change with salinity; salt treatment induced an increase of the AsA/DHA ratio. Ripening induced an increase by 12% in AsA contents at R3, but a reduction in the AsA/DHA ratio at R8 in comparison with T8 (turning fruit grown at 8 mS/cm EC) was observed (**Table 3**). In Gimar WT, the total ascorbate decreased with salt at both T8 and R8 whereas it increased during ripening at both salinity levels (R3, R8). AsA reached the highest value at R8. The AsA/DHA ratio increased with treatments, but from T3 (turning fruit grown at 3 mS/cm EC) to R3, it decreased from 1.19 to 1.01 (**Table 3**). In Gimar *gf*, an increase in the contents of AsA + DHA was observed from T3 to T8 and from T3 to R3. In contrast, a decrease by 7 and 16% was observed from T8 to R8 and from R3 to R8, respectively. Slight increases in AsA contents were observed with salt treatment where at the turning stage a decrease in the AsA/DHA ratio from 1.99 to 1.25 occurred, whereas at the ripe stage a double value of this ratio was observed (**Table 3**). In Gimar *nor*, both total ascorbate and AsA increased from R3 to R8 and an increase in the AsA/DHA ratio from 0.85 to 1.20 was observed as well (**Table 3**). In contrast, a decrease in all three parameters was observed from T3 to R3 (**Table 3**).

At T3, DHLA was three-fold higher in Gimar WT as compared with cv. Jama. DHLA increased with ripening in all genotypes, reaching almost similar values at R3 (**Table 4**). Increases in DHLA amounts were also observed with salt treatment with the exception of Gimar WT from T3 to T8 and Gimar *nor* from R3 to R8. LA values were always lower than those of DHLA, and the DHLA/LA ratio increased with both ripening and salinity in cvs. Jama and Gimar WT. The *gf* and *nor* Gimar NILs, instead, showed increases in the DHLA/LA ratio from T3 to T8 and from T3 to R3 whereas decreases were observed from T8 to R8 and from R3 to R8 (**Table 4**).

Major tocopherols determined were α- and γ-tocopherol; δ-tocopherol was found only in trace amounts, and no β-tocopherol was found. As compared with cv. Jama, Gimar genotypes presented at T3 the higher amount of α-tocopherol and in Gimar *nor* it reached a four-fold value (**Table 5**). Otherwise, at R3, α-tocopherol assumed in cv. Jama a value near to that of Gimar WT (106.39 and 132.7 μg/g DW, respectively). α-Tocopherol increased with ripening and salt treatment in cv. Jama and in Gimar *gf*, where it reached at R8 the maximum value (398.96 μg/g DW). In Gimar WT, instead, it decreased with salinity as in Gimar *nor* from T3 to T8. In this last genotype, α-tocopherol increased from R3 to R8 by 44% reaching an amount lower than the initial value (T3). In Gimar *gf*, γ-tocopherol increased with treatments, reaching values of 101.58 and 125.57 μg/g DW at R3 and R8, respectively, whereas in the other genotypes its amounts ranged from 10.97 (R3 of cv. Jama) to 32.61 μg/g DW (T3 of Gimar *nor*). Gimar *gf* was also the genotype where γ-tocopherol increased during both ripening and salinity determining a decrease in the α/γ tocopherol ratio (**Table 5**).

DISCUSSION

The present research confirms previous findings by D'Amico et al. (*2*) in which the application of diluted seawater to a soilless culture of tomato plants (cv. Jama) determined a reduced fruit yield but did not affect the number of harvested fruits, which at the level of salinity of 8 mS/cm EC were all marketable

Table 2. Contents of NADPH (nmol/g DW) and NADPH/NADP$^+$ Ratios in Berries of Different Tomato Genotypes Grown in Diluted Seawater till Red-Turning and Red-Ripe Stages[a]

	T3	T8	R3	R8	P
			cv. Jama		
NADPH	38.81 ± 1.10	41.91 ± 1.50	47.66 ± 1.30	46.79 ± 1.40	r = ***; s = ns; rxs = ns
NADPH/NADP$^+$	0.83 ± 0.02	0.86 ± 0.03	0.93 ± 0.02	0.89 ± 0.02	r = *; s = ns; rxs = ns
			Gimar WT		
NADPH	38.58 ± 1.10	14.92 ± 1.60	30.32 ± 1.20	9.58 ± 0.95	r = ***; s = ***; rxs = ns
NADPH/NADP$^+$	0.78 ± 0.03	0.60 ± 0.02	0.63 ± 0.02	0.36 ± 0.01	r = **; s = **; rxs = ns
			Gimar *gf*		
NADPH	21.25 ± 0.85	18.15 ± 0.87	36.83 ± 0.91	33.07 ± 0.98	r = ***; s = **; rxs = ns
NADPH/NADP$^+$	0.68 ± 0.02	0.63 ± 0.01	0.67 ± 0.04	0.84 ± 0.02	r = ns; s = **; rxs = ns
			Gimar *nor*		
NADPH	33.38 ± 0.99	21.00 ± 0.71	18.93 ± 0.85	12.83 ± 0.63	r = ***; s = ***; rxs = *
NADPH/NADP$^+$	0.81 ± 0.02	0.74 ± 0.03	0.96 ± 0.04	0.47 ± 0.01	r = ***; s = **; rxs = *

[a] Results are the means ± SE of three replicates that were each analyzed in triplicate. A two-way ANOVA was used to evaluate the ripening effect (r), the salt effect (s), and the interaction between ripening and salinity (r × s). Significance was as follows: NS, not significant; *, significant at the $P \leq 0.05$ level; **, significant at the $P \leq 0.01$ level; and ***, significant at the $P \leq 0.001$ level.

Table 3. AsA and Total Ascorbate (AsA + DHA, μmol/g DW) and AsA/DHA Ratio in Berries of Different Tomato Genotypes Grown in Diluted Seawater till Red-Turning and Red-Ripe Stages[a]

	T3	T8	R3	R8	P
			cv. Jama		
AsA	11.82 ± 0.56	13.58 ± 0.43	13.27 ± 0.55	13.76 ± 0.33	r = *; s = *; rxs = ns
AsA + DHA	22.78 ± 0.31	22.78 ± 0.33	23.76 ± 0.45	23.81 ± 0.41	r = *; s = ns; rxs = ns
AsA/DHA	1.17 ± 0.02	1.72 ± 0.03	1.31 ± 0.01	1.40 ± 0.02	r = *; s = **; rxs = ns
			Gimar WT		
AsA	12.53 ± 0.41	11.31 ± 0.37	12.65 ± 0.33	14.38 ± 0.51	r = *; s = **; rxs = ns
AsA + DHA	23.32 ± 0.32	20.58 ± 0.35	25.34 ± 0.41	24.71 ± 0.27	r = **; s = **; rxs = ns
AsA/DHA	1.19 ± 0.02	1.25 ± 0.02	1.01 ± 0.01	1.42 ± 0.03	r = *; s = **; rxs = ns
			Gimar *gf*		
AsA	19.76 ± 0.22	20.74 ± 0.32	19.39 ± 0.35	21.95 ± 0.43	r = ns; s = **; rxs = ns
AsA + DHA	32.39 ± 0.51	37.50 ± 0.39	41.52 ± 0.45	34.90 ± 0.45	r = **; s = **; rxs = ***
AsA/DHA	1.99 ± 0.03	1.25 ± 0.04	0.88 ± 0.01	1.75 ± 0.02	r = **; s = ***; rxs = *
			Gimar *nor*		
AsA	12.49 ± 0.15	12.79 ± 0.11	10.52 ± 0.75	15.39 ± 0.53	r = *; s = **; rxs = **
AsA + DHA	25.59 ± 0.33	26.52 ± 0.27	23.02 ± 0.51	28.33 ± 0.63	r = *; s = **; rxs = *
AsA/DHA	0.96 ± 0.02	0.93 ± 0.01	0.85 ± 0.01	1.20 ± 0.02	r = **; s = *; rxs = *

[a] For other details, see **Table 2**.

Table 4. DHLA (μg/g DW), LA (μg/g DW), and DHLA/LA Ratio in Berries of Different Tomato Genotypes Grown in Diluted Seawater till Red-Turning and Red-Ripe Stages[a]

	T3	T8	R3	R8	P
			cv. Jama		
DHLA	2.93 ± 0.23	3.88 ± 0.33	11.81 ± 0.35	12.66 ± 0.29	r = ***; s = *; rxs = ns
LA	2.81 ± 0.11	2.41 ± 0.13	2.42 ± 0.15	1.26 ± 0.12	r = ***; s = *; rxs = **
DHLA/LA	1.04 ± 0.02	1.60 ± 0.04	4.91 ± 0.05	10.04 ± 0.07	r = ***; s = ***; rxs = **
			Gimar WT		
DHLA	10.06 ± 0.81	8.51 ± 0.75	13.36 ± 0.53	17.14 ± 0.95	r = ***; s = ns; rxs = **
LA	4.26 ± 0.22	2.88 ± 0.15	2.17 ± 0.21	2.68 ± 0.17	r = ***; s = *; rxs = **
DHLA/LA	2.44 ± 0.02	2.96 ± 0.03	6.20 ± 0.08	6.41 ± 0.06	r = ***; s = *; rxs = **
			Gimar *gf*		
DHLA	7.66 ± 0.21	11.37 ± 0.30	10.26 ± 0.25	11.51 ± 0.33	r = *; s = ***; rxs = *
LA	2.30 ± 0.11	1.04 ± 0.19	1.79 ± 0.25	2.69 ± 0.35	r = **; s = *; rxs = ***
DHLA/LA	3.56 ± 0.05	11.11 ± 0.04	5.90 ± 0.07	4.30 ± 0.06	r = ***; s = **; rxs = **
			Gimar *nor*		
DHLA	5.41 ± 0.16	11.89 ± 0.08	12.92 ± 0.65	11.96 ± 0.03	r = ***; s = **; rxs = ***
LA	4.83 ± 0.53	2.46 ± 0.37	3.11 ± 0.11	3.04 ± 0.03	r = *; s = ***; rxs = **
DHLA/LA	1.12 ± 0.03	4.98 ± 0.05	4.14 ± 0.06	4.00 ± 0.02	r = **; s = **; rxs = *

[a] For other details, see **Table 2**.

(**Table 1**). Fruits of salinized and nonsalinized samples were picked at the same time since salinity did not affect ripening in any genotype analyzed.

Both salinity and ripening induce an oxidative process, which leads to the production of AOS (*4*, *20*). Activation of antioxidative defense mechanisms could be observed depending on stress intensity (*9*) and on cultivar tolerance to stress (*21*). Changes in the redox status of cells and in the presence of reduced forms of antioxidants should be explained taking into account the previous considerations.

Table 5. α-Tocopherol and γ-Tocopherol (μg/g DW) and α/γ Ratio in Berries of Different Tomato Genotypes Grown in Diluted Seawater till Red-Turning and Red-Ripe Stages[a]

	T3	T8	R3	R8	P
			cv. Jama		
α-tocopherol	73.65 ± 2.40	153.32 ± 3.50	106.39 ± 3.80	143.11 ± 2.90	r = ***; s = ***; rxs = ***
γ-tocopherol	21.87 ± 1.90	27.70 ± 0.90	10.97 ± 1.10	23.41 ± 0.88	r = ***; s = ***; rxs = **
α/γ	3.37 ± 0.05	5.53 ± 0.07	9.70 ± 0.06	6.11 ± 0.09	r = **; s = **; rxs = **
			Gimar WT		
α-tocopherol	112.66 ± 3.81	79.45 ± 2.75	132.70 ± 3.53	89.50 ± 2.95	r = ***; s = ***; rxs = ns
γ-tocopherol	26.00 ± 1.22	22.23 ± 2.15	30.75 ± 1.75	22.20 ± 1.07	r = *; s = ***; rxs = *
α/γ	4.33 ± 0.02	3.57 ± 0.03	4.31 ± 0.05	4.03 ± 0.06	r = ns; s = *; rxs = ns
			Gimar *gf*		
α-tocopherol	125.23 ± 9.30	279.23 ± 8.50	285.52 ± 9.50	398.96 ± 8.60	r = ***; s = ***; rxs = ***
γ-tocopherol	25.30 ± 2.12	72.87 ± 3.51	101.58 ± 3.75	125.57 ± 3.34	r = ***; s = ***; rxs = ***
α/γ	4.95 ± 0.05	3.83 ± 0.04	2.81 ± 0.02	3.18 ± 0.06	r = **; s = **; rxs = *
			Gimar *nor*		
α-tocopherol	301.55 ± 8.51	161.91 ± 6.83	172.27 ± 8.51	248.68 ± 7.63	r = ***; s = ***; rxs = ***
γ-tocopherol	32.61 ± 2.53	30.48 ± 1.37	13.57 ± 1.11	19.73 ± 1.03	r = ***; s = ns; rxs = *
α/γ	9.25 ± 0.08	5.31 ± 0.06	12.69 ± 0.09	12.60 ± 0.07	r = ***; s = ns; rxs = *

[a] For other details, see **Table 2**.

Very little is known about the values of the NADPH/$NADP^+$ ratio in plant tissue. This ratio in all tomato berries (**Table 2**) was similar to that found in wheat leaves in which it increased with time following copper treatment, whereas it decreased in roots, which were more likely subjected to a greater oxidative stress (*11*). In comparison with roots, berries as well as leaves are not in direct contact with salt solution; therefore, the impact of salinity on tomato fruits should not have been so strong. Tomato is in general moderately tolerant to salinity, but the fact that in cv. Jama there was no oxidation of NADPH with salt treatment whereas all Gimar genotypes showed a decrease in NADPH (**Table 2**) indicates that these genotypes can be differently tolerant to salt stress. Gimar genotypes were also more affected by ripening than cv. Jama with the exception of Gimar *gf* where the higher level of NADPH (**Table 2**) could be related to the presence of residual chloroplasts in the ripe fruits. Indeed, ripening, as well as senescence (*5*), determined a reduced photosynthetic activity and an unbalance between the photosynthesis and the pentose−phosphate cycle (*11, 22*).

The importance of NADPH is, besides in the photosynthetic process, as a reducing agent in the regeneration of antioxidants AsA and GSH through the AsA/GSH cycle (*9*). The increases in the AsA/DHA ratio (**Table 3**) and tocopherol contents (**Table 5)** with salt treatment in cv. Jama and the reduction in the amounts of total ascorbate and tocopherols in salinized Gimar WT suggest that cv. Jama can tolerate better high-salt conditions. Gossett et al. (*23*) and Hernández et al. (*24*) have found that under salinity α-tocopherol concentrations were significantly higher in the salt-tolerant cultivars than in the more salt-sensitive ones. It is the capacity to possess sufficiently elevated levels of antioxidants in the reduced form, to utilize and regenerate them, which is useful for the evaluation of the response of cells against AOS. In fact, reduced ascorbate pool represents an antioxidant reserve, thus assuming great importance in adaptive response to stress conditions (*25*). The increases in the AsA/DHA ratio with salinity (**Table 3**) found in Gimar WT suggest that this genotype reacted to salt conditions by increasing the relative proportion of the reduced, antioxidative active form of AsA. The increase in AsA constitutes a potential reserve, which could be used for a profitable shelf life. In addition, the AsA increase is relevant for the nutrition and the health of consumers; vitamin C (AsA) is one of the antioxidants able to protect animal cells against various diseases (*6*). The particularly high contents of AsA and AsA + DHA and the higher value of the AsA/DHA ratio in Gimar *gf* in comparison with the other genotypes at T3 (**Table 3**) make this mutant helpful for the above purpose. The results are in agreement with those of George et al. (*26*), who emphasize the variations in AsA contents among different tomato varieties as a function of genotype.

In the Gimar genotypes, the oxidation of NADPH with salinity (**Table 2**) could have been involved in the regeneration of AsA (**Table 3**) through the activation of the AsA/GSH cycle (*11*). The strong oxidation of AsA, as testified by the decrease in the AsA/DHA ratio, in Gimar *gf* during ripening in the control (from T3 to R3) could be explained with the fact that, besides climacteric respiration, photosynthesis is still present in the ripe fruit during which some electron leakage from the photosynthetic chain to oxygen gives rise to AOS (*27*). Besides this, DHLA was not used to reduce DHA to AsA (**Table 4**). Otherwise, in R8 of both Gimar *gf* and Gimar *nor*, a combined effect of ripening and salinity (from R3 to R8) could have been involved in the activation of antioxidative defenses, among which consumption of DHLA, as testified by the decrease in the DHLA/LA ratio (**Table 4**), which brought to the reduction of DHA to AsA (**Table 3**). A lower activity of ascorbate peroxidase at the end of ripening might have also been implicated allowing increased accumulation of AsA and reduced scavenging of H_2O_2, which could be important for the loosening of the cell wall leading to the fruit softening (*4*).

The decrease in AsA + DHA as well as in the AsA/DHA ratio in Gimar *nor* only during ripening (**Table 3**), from T3 to R3, could have been related to the absence of climacteric respiration, which is the primary source of superoxide radical (*28*). In fact, it has been seen (*5*) that tomato fruits respond to the increase in oxidative stress during development, where there is a substantial increase in respiration, by increasing AsA contents. However, the AsA increase also depends on the stage of ripening analyzed and it was demonstrated that at later stages a marginal decline in its content occurs (*5*). Indeed, during the last steps of ripening, the AOS scavenging system does not cope up with the production system, leading to the accumulation of AOS. Anomalous mitochondria functions (*16*) could have been involved in the incapacity to utilize DHLA, which accumulated in Gimar *nor* at R3 (**Table 4**), for regeneration of AsA (*11*). However, the presence in all samples of higher amounts of DHLA as compared with LA (**Table 4**) was particularly important since the reduced form is more effective in performing antioxidant functions (*29*), being only able to regenerate

endogenous antioxidants (*11*), thus maintaining the ascorbate redox status (**Table 3**).

The amounts of DHLA and LA in tomato berries were of the same magnitude of those recorded in leaves and roots of wheat (*11*) and varied depending on the genotype analyzed (**Table 4**). The presence of DHLA was very dissimilar in the different genotypes at T3. The fact that in all genotypes higher levels of this important antioxidant for human health (*10*) were present in the ripe stage strengthens the idea that ripe berries have more healthy properties than fruits picked at earlier stages of ripening (*4*, *5*). Contrary to the common belief that fruits harvested at full ripeness exhibit the highest levels of antioxidants (*30*), at full ripening (R3), γ-tocopherol reached the lowest value in cv. Jama as well as in the mutant *nor* of Gimar (**Table 5**). An increase in the DHLA content under salinity in almost all of the berries supports the contribution of this form to the formation of the antioxidant pool, ensuring protection against stress conditions.

In agreement with Seybold et al. (*31*), vitamin E in tomatoes was predominantly represented by α-tocopherol (**Table 5**). In contrast to the same authors, only trace amounts of δ-tocopherol could be detected. In addition to differences due to ripening and salinity, the genotypes analyzed here showed a marked variation with regard to tocopherol concentration. In particular, at T3, Gimar *nor* exhibited a value even three-fold higher than amounts generally reported for most tomato cultivars (*32*).

It is known that AsA recycles α-tocopherol from its α-tocopheroxyl radical and that a deficiency in AsA may affect antioxidative protection by α-tocopherol under stress (*33*); however, our findings do not indicate a clear relationship between AsA consumption and α-tocopherol formation, since in Jama and, to a larger extent, in Gimar *gf*, an active synthesis of α-tocopherol occurred during ripening or in response to salt stress (**Tables 3** and **5**). This marked synthesis upon ripening in Gimar *gf* could be explained with the maintenance of residual chloroplasts (*16*) where tocopherols are synthesized and accumulate (*34*). Increases in α-tocopherol contents were also reported in leaves of rosemary, sage, and lemon balm subjected to water deficit (*33*) whereas a decrease was observed in wheat roots exposed to a high concentration of copper (*35*).

Changes in the relative proportion between α- and γ-tocopherol affected the nutritional value of different genotypes of tomato berries upon both ripening and salt treatment (**Table 5**). Although γ-tocopherol has less antioxidant properties than α-tocopherol, it is considered the especially promising "other" vitamin E for human health (*35*), because it traps peroxynitrite formed during inflammation (*13*).

In conclusion, our results show that the antioxidant-related nutritional value of tomatoes is significantly improved when the fruits are picked at the red-ripe stage and when the plants are exposed to a moderate salinity stress conditions, such as those determined by the application of diluted seawater (10%). Fruit response to both ripening and salinity is affected by the genetic characteristics of the cultivated plants. In this sense, the mutant gene *gf* was found to confer a noticeable level of salt tolerance to the fruit in term of antioxidative response. Finally, in agreement with Raffo et al. (*30*), this work showed that not all biologically active compounds necessarily increase during fruit ripening.

ABBREVIATIONS USED

AOS, activated oxygen species; AsA, ascorbic acid; DHA, dehydroascorbic acid; DHLA, dihydrolipoic acid; DW, dry weight; FW, fresh weight; *gf*, green flesh; GSH, glutathione; LA, lipoic acid; *nor*, nonripening; r, ripening; R3, ripe fruit grown at 3 mS/cm EC; R8, ripe fruit grown at 8 mS/cm EC; s, salinity; T3, turning fruit grown at 3 mS/cm EC; T8, turning fruit grown at 8 mS/cm EC; WT, wild type.

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