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Effects of Salinity Stress on Carotenoids, Anthocyanins, and Color of Diverse Tomato Genotypes

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Supporting Information

ABSTRACT: One nonanthocyanin-accumulating (Ailsa Craig) and three anthocyanin-accumulating tomato genotypes (Anthocyanin fruit type, Atroviolaceum, and Sun Black) were analyzed to assess differences in their carotenoid and anthocyanin levels and color and to evaluate the effects of nutrient solutions with different salt concentrations on these parameters. The carotenoid content of control Atroviolaceum tomatoes was ca. 2−2.5-fold higher relative to the other two types, and the color of its puree could be visually distinguished from those of other genotypes. Salinity stress led in some cases to a 2−3-fold increase in the lycopene content. Saline treatment increased the accumulation of total anthocyanins in fruits of Sun Black (2-fold increase), while it reduced it in fruits of Anthocyanin (10-fold decrease). In general, the treatment increased the differences in color of different purees. These results indicate that salinity stress can lead to similar or higher increases in tomato carotenoids than those achieved by genetic engineering. In addition, these changes were accompanied by visually discernible color differences in tomato products. Our findings show the considerable potential of exploiting saline soils to obtain tomatoes with higher levels of secondary metabolites like carotenoids and anthocyanins.

KEYWORDS: Anthocyanin fruit type (Aft) tomato, Atroviolaceum (Atv) tomato, carotenoids, color, image analysis, lycopene, salinity stress, Sun Black tomato (SB)

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important crops in the world and a common component of the Mediterranean diet. It is the second most commonly consumed fruit and vegetable in Europe. Consumption of tomato along with that of its derived products has increased some 3-fold worldwide over the last 40 years. Its economic importance on a global scale is therefore beyond doubt, as is its nutritional importance, since tomato products are good sources of vitamins, carotenoids, and phenolic compounds,[1,2] which can be beneficial for the prevention and/or alleviation of oxidative stress and degenerative disorders.[3,4]

More specifically, tomato products are very good sources of the carotenoid lycopene, which is bioavailable and has been reported to accumulate in different organs in both laboratory animals and humans.[5,6] Lycopene, along with its metabolites, continues attracting much attention among scientists due to its capacity to scavenge radicals and the different biological functions it seems to be involved in.[7] In addition, lycopene is mainly responsible for the color of red tomatoes and is widely used as a colorant. The color of food is a very important factor in determining its acceptability; hence, the objective measurement of this attribute in different tomatoes and tomato products has been the subject of numerous studies.[8−11]

It is therefore not surprising that the enhancement of the carotenoid content of tomatoes has been an important research topic in recent decades. The typical accumulation of lycopene observed in ripe red tomatoes is known to be due to the downregulation of lycopene cyclases. It has also been reported that phytoene synthase-1 exerts the greatest control over the pathway flux. In addition, there is an alternative set of carotenoid biosynthetic genes that are induced during the onset of fruit ripening and ethylene, light and plastid biogenesis have also been reported as being related to the carotenogenesis in tomatoes.[12] This knowledge has been applied to studies on the development of carotenoid transgenic tomatoes with elevated carotenoid levels. Although there have been several successes,[13−15] these studies have been somewhat limited due to the consumers' concerns over the consumption of genetically modified foods.

Another strategy to increase the carotenoid levels of tomatoes is conventional plant breeding. The deposition of carotenoids in several genotypes of Andean wild relatives (*S. lycopersicum*, *S. chilense*, *S. peruvianum*, *S. pimpinellifolium*, *S. chmielewskii*) of the domesticated tomato has recently been evaluated in relation to the expression of the ripening-enhanced phytoene synthase (*Psy-1*) and lycopene-β-cyclase (*Cyc-b*).[16] In addition, introgression lines (IL) of *Solanum penellii* into the M82 tomato cultivar have been studied in order to pinpoint quantitative trait loci underlying high carotenoid phenotypes and ILs with high carotenoid bioaccessibility.[16,17] Interestingly, some exotic species (*S. chilense*, *S. cheesmaniae*, *S. lycopersicoides*) phylogenetically related to the cultivated tomato can also accumulate anthocyanin pigments on their epidermis. Some genes underlying this trait, such

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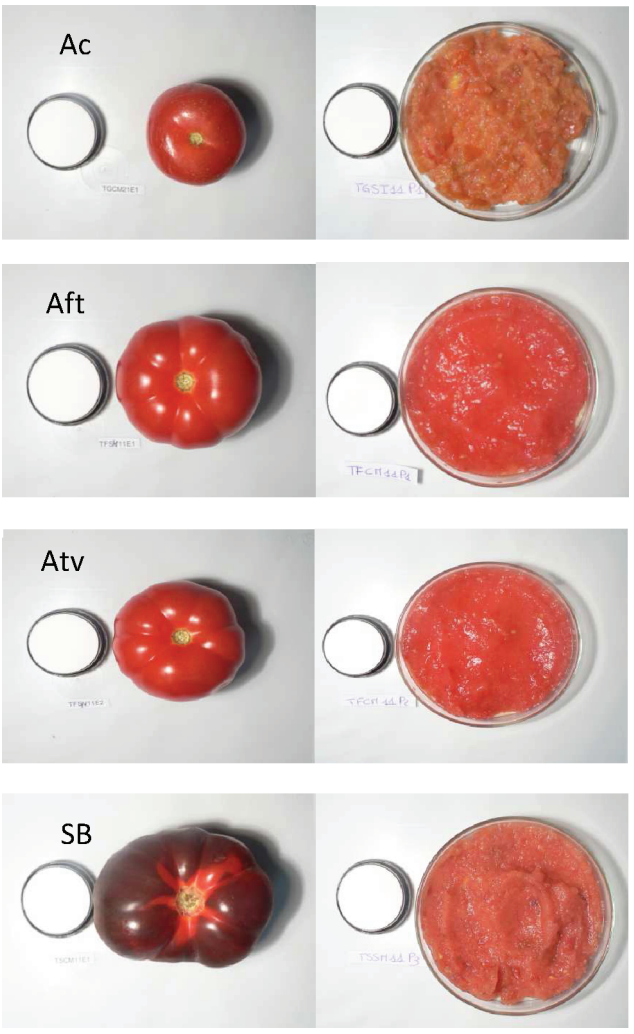

Figure 1. Tomato fruits and purees corresponding to control Ailsa Craig (Ac), Aft, Atv, and Sun Black (SB) samples.

as *Anthocyanin fruit* (*Aft*), *Aubergine* (*Abg*), and *atroviolaceum* (*atv*) have been transferred to the cultivated tomato through breeding.[18] Although the identity of the anthocyanins expressed in these genotypes has already been investigated,[19,20] little is known about their bioavailability in humans and the effects of these novel crossings on their carotenoid content and color.

Despite its undeniable importance, the genetic manipulation of crops is not the only alternative to increase the levels of compounds of interest as agronomical and environmental factors such as irrigation, mineral nutrition, light, and temperature can also be harnessed for that purpose.[21,22] In this study, we evaluated the effect of different salinity levels in the total anthocyanin and carotenoid levels and the color of one non-anthocyanin-accumulating (Ailsa Craig) and three anthocyanin-accumulating tomato genotypes (Anthocyanin fruit type, atroviolaceum, and Sun Black).

MATERIALS AND METHODS

Plant Material, Growing Technique, and Treatments. Tomato fruits from the cultivars Ailsa Craig (Ac), Anthocyanin fruit type (Aft), atroviolaceum (Atv), and Sun Black (SB) were studied (Figure 1). The fruits with *Aft* and *Atv* genes express anthocyanins in the epidermis, although not in the pericarp. The *Aft* dominant gene confers a purple coloration as a result of exposure to high light intensity and was introgressed into the domesticated tomato from *S. chilense*. *Atroviolaceum* (*Atv*) is a recessive gene introgressed into the domesticated tomato from *Solanum cheesmaniae*. The SB tomato, which is characterized by the strong purple pigmentation of its skin, was obtained as a result of crossing *Atv* × *Aft*.[18,19] Ailsa Craig (Ac) (accession number LA2838A), Aft/Aft (accession number LA1996), and atv/atv (accession number LA0797) seeds were provided by the Tomato Genetic Resource Center (TGRC, University of California, Davis). Seeds from the double mutant Aft/Aft atv/atv (SB) were obtained from G. P. Soressi (Department of Agrobiology and Agrochemistry, University of Tuscia, Viterbo, Italy) by crossing the single mutants Aft and atv.

The plants were hydroponically grown in a temperature-controlled glasshouse located in Pisa (latitude 43°43′N; longitude 10°23′E; Italy) during the autumn−winter season of 2008. The minimum temperature and ventilation air temperature inside the glasshouse were 13 and 27 °C, respectively; the maximum temperature reached 30−32 °C in the autumn sun. The maximum photosynthetic photon flux density (PPFD) ranged from 500 to 700 μmol m^{-2} s^{-1}; the mean value of daily global radiation (R) was 5.1 MJ m^{-2}.

Seedlings were transplanted 50 days after sowing into 1 m long rockwool slabs. The tomato plants were grown vertically with a single stem at a density of three plants m^{-2}, and pollination was favored by mechanical vibration of the flower clusters.

Drip irrigation was carried out using a nutrient solution with an electrical conductivity (EC) of 3.5 dS m^{-1} and pH 6.5. Exhaust nutrient solution was discharged after three weeks or whenever the EC was higher than 6 dS m^{-1}. The composition of the nutrient solution was as follows (concentrations are expressed in mol m^{-3}): 12 $N\text{-}NO_3$, 1.3 P-PO, 8 K^+, 4 Ca^{2+}, 1.2 Mg^{2+}, 9 Na^+, and 1.5 $S\text{-}SO_4^{2-}$. Micronutrients were added at Hoagland's concentration (in mmol m^{-3}: B 40, 40 Fe, 1 Cu, 5 Zn, and 10 Mn).

The experimental treatment consisted of two different salinity levels (EC) of the nutrient solution: 3.5 and 5.5 dS cm^{-1}. The solution with EC 5.5 dS cm^{-1} was prepared by the appropriate addition of 35 mol m^{-3} NaCl to the nutrient solution.

A complete randomized block experimental design was adopted, with three replicates for two treatments (C, control; S, salinity treatment). Each replicate consisted of 12 plants. Data were subjected to two-way analysis of variance (ANOVA). The means were separated using the least significant difference (LSD) test for $P = 0.05$.

The fruits were harvested at the commercial ripe stage when they showed a red color. The ripeness stage was characterized in accordance with the procedure reported elsewhere.[23]

The total soluble solids (TSS) were measured by a digital refractometer (MOD, 53011 Turoni, Italy) and expressed as °Brix at 20 °C. Titratable acidity (TA) was measured on samples titrated to pH 8.1 with 0.1 N NaOH and expressed as grams of citric acid per 100 g of fresh fruit weight. Fresh fruit materials were dried at 70 °C for 72 h to determine the dry weight (DW).

Carotenoid Analysis. Tomato carotenoids were determined as described elsewhere[16] with slight modifications; 10 mg of freeze-dried and homogenized tomato fruit material was vortexed with 250 μL of methanol and then with 500 μL of chloroform, sonicated, and subsequently spun at 18000*g* for 5 min at 4 °C. The lipophilic phase was removed with a Pasteur pipet, and the remainder was re-extracted with chloroform (500 μL). The pooled chloroform extracts were dried by centrifugal evaporation. Dried residues were stored under a nitrogen atmosphere at 20 °C prior to their HPLC analysis. For the chromatographic analyses, the samples were dissolved in 100 μL of HPLC grade ethyl acetate and centrifuged to pellet gross particles.

The HPLC analyses were carried out on an Agilent 1200 Series LC system (Agilent Technologies, Palo Alto, CA, USA), equipped with a

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quaternary pump, diode array detector, and autosampler. The data were acquired and analyzed using ChemStation software v.A.01.01. Throughout the chromatography, the eluate was monitored continuously from 220 to 780 nm. A reverse phase C_{30} column YMC-PackYMC (Wilmington, NC, USA) (5 μm 250 × 4.6 mm) was used, which was kept at 25 °C. The mobile phase consisted of methanol (A), 20% water/80% methanol/0.2% ammonium acetate (B), and *tert*-methyl butyl ether (C). The gradient elution was as follows: 95% A and 5% B for 6 min; 80% A and 5% B until 32 min; 30% A and 5% B until 56 min; and 95% A and 5% B until 62 min. The mobile phase was pumped at 1 mL min^{-1}, and the injection volume was 20 μL.

The colored carotenoids lycopene, β-carotene, and lutein were quantified by external calibration. The calibration curves were made with all-*E*-standards isolated in our laboratory in accordance with recommended procedures.[24] The colorless carotenoids phytoene and phytofluene were not determined.

Anthocyanin Determination. Anthocyanins were extracted in acidified methanol as described elsewhere.[19] Briefly, 100 mg of lyophilized tomato skins were ground into a fine powder and extracted overnight with 300 μL of 1% HCl/methanol at 5 °C. The extraction volume was adjusted to 500 μL with nanopure water, and 500 μL of chloroform was added to the tube. The tubes were centrifuged for 5 min at 18000*g*, and the aqueous phase was transferred to a new tube. The aqueous phase was dried under centrifugal evaporation. The sample was dissolved in 150 μL of HPLC grade methanol. The HPLC measurements were taken with the same equipment used for the carotenoid analysis, using a Prodigy ODS (5 μm, 250 × 4.6 mm) column fitted with a 4.0 × 3.0 mm i.d. guard column (Phenomex, Torrance, CA) that was kept at 35 °C. The injection volume was 20 μL. The HPLC protocol is reported elsewhere[25] to which we made a slight modification. A gradient of two solvents, acetonitrile (A) and a water solution containing 10% acetic acid and 1% phosphoric acid (B), was used. Chromatographic conditions were initially 100% B for 6 min, 98% B for 4 min, 95% B for 5 min, 90% B for 2 min, 88% B for 3 min, 85% B for 3 min, 82% B for 8 min, 80% B for 5 min, 60% B until the 40 min mark, and 98% B for 3 min before returning to the initial conditions at a flow rate of 1 mL min^{-1}. Simultaneous detection at 280, 320, and 520 nm was recorded, and UV−vis spectra were registered between 200 and 800 nm.

The anthocyans were quantified by external calibration. The quantification was made at 525 nm by comparing the areas and the retention times with a malvidin 3-glucoside standard isolated in our laboratory from skins of *V. Vinifera* red grapes of Tempranillo variety, by extraction with acidic methanol and further purification by semipressure liquid chromatography using a reversed-phase column, as described elsewhere.[26]

Anthocyanin Mass Spectrometry Determination. Samples were analyzed by HPLC-mass spectroscopy (MS), Ion Precursor positive, to determine the number of anthocyanin groups and their respective masses. Selected ions were m/z 303.0, 331.0, and 317.0, for the identification of delphinidin, malvidin, and petunidin, respectively. The parameters were as follows: energy ionization, +5500 V; curtain gas, 20 psi; gas1, 40 psi; gas2, 30 psi; declustering potential, 80 V; and collision energy, 25 V. Each spectrum was acquired in MCA mode, accumulating 33 scans.

The samples were dissolved in methanol/water (1:1) with 0.1% formic acid.

Color Determination. For the color measurements, three fruits of each cultivar were analyzed. The assessment of the external color of the tomatoes was made from three readings rotating the fruit by 120° between each reading. Because anthocyanins are only expressed in the peel of the fruits of the crosses and tomatoes are widely used to obtain puree-like tomato products, such as sauces, ketchup, soups, etc., the samples were homogenized to better ascertain the effects of the genotype and salinity stress on their color.

Table 1. Carotenoid Contents (μg g^{-1} DW) in the Different Tomato Cultivars Studied As a Function of Cultivation System[a]

cv.	treatment	lycopene	β-carotens	lutein
Ac[b]	C	322.7 d	149.8b	5.4bcd
	S	600.5 c	211.2 a	7.9 a
Aft[c]	C	252.6 d	70.3 d	4.0 de
	S	747.8 b	83.2 d	5.2 cde
Atv[d]	C	748.7 b	74.3 d	7.6 ab
	S	833.5 b	113.6 c	7.8 a
SB[e]	C	325.8 d	70.8 d	3.1 e
	S	989.4 a	87.5 cd	7.29 abc
cv.		*g*	*g*	*g*
treatments (t)		*g*	*g*	*g*
cv. × t		*g*	*f*	*f*

[a] C: control solution. S: high salt solution. Numbers followed by different letters in the same column differ significantly at the 5% level by the LSD test; significance level. [b] Ailsa Craig. [c] Anthocyanin fruit type. [d] Atroviolaceum. [e] Sun Black. [f] $P \leq 0.05$. [g] $P \leq 0.01$; n.s. not significant.

Table 2. Anthocyanin Contents (Expressed in Malvidin 3-Glucoside) in the Different Tomato Cultivar Skins Studied As a Function of Cultivation System[a]

	malvidin 3-glucoside (μg g^{-1} DW)	
	Sun Black	Aft
C	298.57 b	54.77 c
S	479.32 a	6.01 d

[a] C: control solution. S: high salt solution. Numbers followed by different letters differ significantly at the 5% level by the LSD test.

A DigiEye imaging system[27] was used to record digital images and assess the color of the samples. The system consisted of a Nikon D80 digital camera, a computer with dedicated software, and a box illuminated with a lamp emulating the Illuminant D_{65}. The digital images were downsized with a commercial photo editor software (Faststone image 6.2), a 150 pixel width × 150 pixel height, and were saved in bmp format. The CIELAB color parameters[28] were obtained from the images using CromaLab software,[29] considering the 10° Observer and the Illuminant D_{65} as references.

The color differences (denoted as ΔE^*_{ab}) between two colors in the CIELAB space are calculated as the Euclidean distance between their locations in the three-dimensional space defined by L^*, a^*, and b^*. Mathematically it is calculated by the formula $\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$.

RESULTS AND DISCUSSION

Pigment Content and Color of the Control Tomatoes. The levels of the main colored carotenoids (lycopene, β-carotene, and lutein) found in the hydroponically grown control tomatoes are summarized in Table 1. Considering the control samples, the genotypes with the highest levels of colored carotenoids were Atv (830.6 μg g^{-1} DW), followed by Ac (477.9 μg g^{-1} DW), SB (399.7 μg g^{-1} DW), and Aft (ca. 326.9 μg g^{-1} DW). The lycopene levels of Ac, Aft, and SB samples were quite similar (ranging from ca. 250 to 325 μg g^{-1} DW), whereas those found in the Atv tomato fruits were between 2- and 3-fold higher. The β-carotene levels were very similar in the Atv, Aft, and SB control

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Table 3. Anthocyanin Composition of Tomato Fruit Skin from Plants of Sun Black As Detected by HPLC-MS

functional groups	detected mass (m/z)		
anthocyanidin	delphinidin (303)	petunidin (317)	malvidin (331)
glycoside	465	nd[a]	493
unknown	483	497	511
rutinoside	611	625	639
p-coumaroyl- rutinoside	757	771	nd[a]
p-coumaroyl- rutinoside-glycoside	nd[a]	933	947
unknown	nd[a]	nd[a]	691

[a] nd = mass not detected.

Table 4. Color Parameters Corresponding to Control (C) and Treated (S) Tomato Fruits[a]

	genotypes			
color	Ac[b]	Aft[c]	Atv[d]	SB[e]
	Control Samples (C)			
L^*	39.28 a	40.79 b	41.68 c	36.93 d
a^*	43.08 a	44.85 b	42.72 c	40.18 d
b^*	40.06 a	36.80 b	40.77 c	33.48 d
C^*_{ab}	58.90 a	58.04 b	59.10 a	52.30 c
h_{ab}	42.88 a	39.27 b	43.63 c	39.72 d
	Treated Samples (S)			
L^*	43.62 ab	41.05 b	36.95 c	45.68 a
a^*	40.37 a	45.18 b	45.39 b	34.92 c
b^*	39.21 a	37.71 b	37.32 b	33.58 c
C^*_{ab}	56.37 a	58.90 b	58.80 b	48.49 c
h_{ab}	43.91 a	39.80 b	39.32 c	43.80 a

[a] Data were subjected to one-way ANOVA, and different letters within the same row mean that values are statistically different, $P < 0.05$. [b] Ailsa Craig. [c] Anthocianyn fruit type. [d] Atroviolaceum. [e] Sun Black.

samples, while those of Ac were around two times higher. The lutein content was negligible compared to that of β-carotene and lycopene. From these data, it can be concluded that the genetic differences between the plants surveyed accounted for large differences in the carotenoid content of their fruits.

Concerning the anthocyan content of the control samples, some differences were observed among the genotypes. Thus, the total anthocyan level in Aft was found to be six times lower than that in SB (ranging from ca. 54.8 to 298.6 μg g^{-1} DW) (Table 2). To determine the moieties attached to the anthocyanins, the samples were injected into an MS-electron scan. Table 3 represents the masses of the moieties present in cv. SB. These masses were compared with all combinations of known anthocyanidins and glycosyl and acyl moieties from the literature. The predominant acyled anthocyanin was the peak at 933 m/z, consistent with petunidin-3-(*p*-coumaryl)-rutinoside-5-glucoside, as found in the literature. This compound was also predominant in Aft.[19]

The main nonacyled anthocyanin was the peak at 611 m/z, consistent with delphinidin-3-rutinoside, which was found to be predominant in Aft.[19] Another discernible peak at 511 m/z was observed but not identified.

Table 5. Color Differences (In CIELAB Units) between Purees As a Function of the Genotype, for Each Cultivation System

	pairs of genotypes					
treatment	Atv-Aft[b]	Atv[c]-SB[d]	Atv-Ac[a]	SB-Aft	SB-Ac	Aft-Ac
C	4.59	9.06	2.53	6.91	7.56	4.01
S	4.12	14.14	8.56	11.99	8.10	5.66

[a] Ailsa Craig. [b] Anthocianyn fruit type. [c] Atroviolaceum. [d] Sun Black.

The CIELAB color parameters corresponding to the tomatoes and homogenates obtained from the fruits are summarized in Table 4. Considering the external color, the values of a^*, b^*, and C^*_{ab} of SB were the lowest. However, the highest values corresponded to the Aft genotype. Regarding the color of the homogenates, there were significant differences in virtually all the cases as a function of the genotype. The genotype with the highest levels of colored carotenoids (Atv), showed the highest values of L^*, b^*, C^*_{ab}, and h_{ab}. The puree from this genotype was the darkest, had the most vivid color, and had a more orange hue than the rest. The color difference (Table 5) between the Atv puree and the one corresponding to the genotype with the lowest content of colored carotenoids (Aft) was 4.59 CIELAB units. Because from an industrial point of view it is considered that color differences between 2.8 and 5.6 CIELAB can be discerned by individuals with normal color tolerances,[30,31] consumers should easily be able to see the differences between the purees corresponding to the Atv and Aft control tomatoes. The color difference between the purees obtained from the Atv and the Ac genotypes (2.53 CIELAB units) was below the lower limit of the range. This indicates that they would not be easily differentiated by all consumers, despite the fact that Atv accumulates anthocyanins in its epidermis. The purees from the nonanthocyanin accumulating Ac tomato and the anthocyanin-accumulating Aft tomato were visually discerned though (ΔE^*ab = 4.00 CIELAB units). However, the color difference between the purees from the Atv and the SB genotypes (9.06 CIELAB units) was clearly above the higher threshold. This suggests that the high amount of anthocyanins expressed in the peel of the SB tomatoes did contribute a great extent to the color of the purees. The color of the puree from control SB tomatoes was also easily distinguishable from those obtained from Aft (6.90 CIELAB units) and SB (7.56 CIELAB units) tomatoes.

Effect of Salinity Stress on the Yield, Anthocyanin and Carotenoid Content, and Color. Some studies indicate that salinity can improve the antioxidant content of tomato fruits.[32] Our results indicated that the yield of plants was significantly affected by salt treatment. There was little reduction in yield between the two treatments in the case of Aft (6%) to high yield losses in cv. Atv and SB, 56% and 43%, respectively. Furthermore, it was observed that the total soluble solids were higher in the plants subjected to the salinity treatment (Table 6).

The saline treatment affected the content of total anthocyanins differently in the Aft and SB cultivars (Table 2). The content of anthocyanins in Aft fruits grown with salinity stress decreased by about 10 times compared to that of the control (6.01 to 54.77 μg g^{-1} DW). In contrast, the total anthocyanins content of SB fruits grown with salinity stress was almost 2-fold higher (298.57 to 479.32 μg g^{-1} DW).

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Table 6. Yield (Gram Plant^{-1}), Dry Weight (DW), Total Soluble Solid (TSS, °Brix), and Titratable Acidity (TA, % Citric Acid) in the Different Tomato Cultivars Studied As a Function of Cultivation System (Standard Nutrition Solution (C) and Salinity Solution (S))[a]

		yield (g)	DW (%)	TSS (° Brix)	TA (% citric acid)
Ac[b]	C	1190 b	8.8 e	4.7 bc	0.8 n.s.
	S	999 b	12.45 a	5.2 b	0.8 n.s.
Aft[c]	C	4440 a	9.1 d	4.4 c	0.8 n.s.
	S	4195 a	11.1 b	4.9 bc	1.0 n.s.
Atv[d]	C	263 b	8.1 f	5.0 bc	0.8 n.s.
	S	116 b	10.6 c	6.4 a	1.0 n.s.
SB[e]	C	1436 b	8.7 e	4.4 c	0.8 n.s.
	S	816 b	12.5 a	5.2 b	0.8 n.s.
cv.	*g*	*f*	*f*	n.s.	
treatments (t)	n.s.	*g*	*f*	n.s.	
cv. × t	n.s.	*g*	*f*	n.s.	

[a] Numbers followed by different letters in the same column differ significantly at the 5% level by LSD test. Significance level. [b] Ailsa Craig. [c] Anthocianyn fruit type. [d] Atroviolaceum. [e] Sun Black. [f] $P \leq 0.05$. [g] $P \leq 0.01$; n.s. not significant.

Table 1 highlights that the increase in the salinity of the nutrient solution was accompanied by a clear rise in the levels of all the carotenoids determined. The lycopene content increased ca. 3-fold in the case of SB and Aft, ca. 2-fold in the case of Ac, and 1.1-fold in the case of Atv. The levels of β-carotene and lutein also increased as a consequence of the treatment by 1.16- and 1.53-fold and 1.02- and 2.35-fold, respectively. Taken together, it can be concluded that SB was the genotype that increased the carotenoid content to a greater extent as a result of the saline treatment. Indeed, the highest levels of carotenoids were found in these plants, independent of the treatment considered. The total carotenoid content of the fruits from Atv grown with salinity stress was the second highest, although this genotype was the least affected by the increase in salinity of the nutrient solution. This is explained by the fact that the control fruits of this genotype had a much higher carotenoid content (ca. 1.7−2.5-fold higher) than the others.

The increases in the carotenoid content of tomatoes were similar or even clearly higher than the increases accomplished by genetic engineering.[12] This is interesting for several reasons: on the one hand, developing GM crops is time-consuming and costly and is also unacceptable for many consumers, especially in Europe. On the other hand, the fact that high salinity can lead to a clear enhancement in the antioxidant levels of tomato could be harnessed to exploit saline soils. Although the accumulation of massive quantities of carotenes by the halotolerant microalga *Dunaliella* as a response of salt and other stresses has long been known,[33] the role of carotenoids in tomato plants subjected to salinity stress is still rather obscure. Nevertheless, there are several reports on the effects of high salinity on the antioxidant systems of the domesticated tomato and its wild relative *S. penellii*.[34,35] Therefore, further studies that help to unravel the mechanisms underlying the enhanced carotenoid deposition should be encouraged. In this regard, the study of the SB genotype seems especially interesting.

Concerning the external color of the treated tomatoes, salinity stress resulted in a clear decrease in the a^* and b^* values of the SB genotype, as a result of which its C^*_{ab} decreased considerably compared to that of the corresponding control (Table 4). Indeed, the highest color difference between control and treated tomatoes corresponded to SB (ΔE^*_{ab} = 20.49 CIELAB units), followed by those of the Atv genotype, which showed a much lower color difference (ΔE^*_{ab} = 7.61 CIELAB units).

Table 7. Color Differences (CIELAB Units) between Purees from the Same Genotype As a Result of the Increase in the Salt Concentration of the Nutrient Solution

genotype	ΔE^*_{ab}	ΔL^*	ΔC^*_{ab}	Δh_{ab}
Ac[a]	5.19	−4.32	2.53	1.03
Aft[b]	1.0	−0.26	−0.86	−0.53
Atv[c]	6.4	4.73	0.3	4.31
SB[d]	10.2	−8.75	3.81	−4.08

[a] Ailsa Craig. [b] Anthocianyn fruit type. [c] Atroviolaceum. [d] Sun Black.

With regard to the color parameters corresponding to the homogenates obtained from the tomatoes grown under salinity stress (Table 4), some significant differences among the genotypes were observed. The darkest puree corresponded to the Atv tomato (lowest value of L^*), whereas the brightest (highest value of L^*) corresponded to the SB genotype. The SB homogenate had the lowest values for both a^* and b^*, and thus, it had the lowest values of chroma and the highest value of hue. Apart from its higher carotenoid content, the intense accumulation of anthocyanins in the epidermis of this tomato may also account for the higher differences in the color parameters of its puree compared to that of the other genotypes. In terms of color differences (Table 5), all the purees taken in pairs could be visually distinguished (values of ΔE^*_{ab} over 2.8 CIELAB units). The highest color differences corresponded to the pairs that involved the SB purees and to the Atv-Ac pair (all with values of ΔE^*_{ab} over 8 CIELAB units).

The increase in salinity of the nutrient solution led to higher values of ΔE^*_{ab} except in one case (Atv-Aft). There was a considerable increase in color differences between the pairs Atv-Ac, Atv-SB, and SB-Aft (ca. 6.5 and 4 units). In terms of the color differences between purees from the same genotype as a result of the treatment, the highest values of ΔE^*_{ab} by far corresponded to the purees from SB. The lowest were observed in the purees obtained from the Aft fruits. In general, the changes in brightness of the homogenates were the major contributors to these color differences (Table 7).

In summary, it was concluded that the saline treatment increased the accumulation of total anthocyanins in the SB fruits, while it reduced it in the Aft fruits. The increase in salinity was accompanied by an enhancement of the levels of all the major colored tomato carotenoids. Two- to 3-fold increases in the levels of lycopene were observed in some cases. Overall, the high-salt nutrient solution led to higher values of ΔE^*_{ab} between purees from different genotypes. Furthermore, in most cases, there were clear color differences between purees from the same genotype as a result of the treatment. Changes in the external color of the tomatoes as a result of the treatment were especially noticeable in the SB and Atv genotypes. These results are interesting as the increments in tomato carotenoids achieved through salinity stress were similar to or higher than those accomplished by genetic engineering. This highlights how agronomic techniques can be used to design strategies for improving specific quality traits already present in the crop. Unlike the enhancement of

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these traits with transgenes, the resulting tomatoes do not raise as much controversy and suspicion as their genetically modified counterparts. The study of the SB genotype also sheds light on the molecular mechanisms involved in the noticeable increase in the levels of carotenoids caused by the salinity stress. This could be important in studying the viability of exploiting saline soils to obtain tomatoes with increased levels of these health-promoting compounds.

■ ASSOCIATED CONTENT

Supporting Information. HPLC chromatogram (detection wavelength: 450 nm) of carotenoids in mature tomatoes and HPLC chromatogram (detection wavelength: 525.5 nm) of anthocyans in SB tomatoes. This material is available free of charge via the Internet at http://pubs.acs.org.

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