The effect on colour of using salinity stress to increase carotenoid levels in different tomato genotypes

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Abstract

One non-anthocyanin-accumulating (Ailsa Craig) and three anthocyanin-accumulating tomato genotypes (Anthocyanin fruit type, Atroviolaceum and Sun Black) were analyzed to assess differences in carotenoid levels and colours 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 than the other two types, and the colour of its puree could be distinguished from those of other genotypes. Salinity stress led to a 2-3-fold increase in the lycopene content of several genotypes. Saline treatment increased the accumulation of total anthocyanins in fruits of SB (2-fold increase), while it reduced the accumulation in fruits of Aft (10 fold decrease). Overall, the treatment increased the differences in colour of different purees. These findings indicate that this type of 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 discernable colour differences in tomato products. We believe our findings show the considerable potential of exploiting saline soils for the cultivation of tomatoes.

Keywords: *Anthocyanin fruit type (Aft) tomato, Atroviolaceum (Atv) tomato, carotenoids, colour, image analysis, lycopene, salinity stress, Sun Black tomato*

Introduction

Tomato (*Solanum lycopersicum*) is one of the most important vegetables 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 the tomato along with that of its derived products has increased some three-fold worldwide over the last 40 years. Its economic importance on a global scale is therefore beyond doubt. So too is its nutritional importance, since tomato products are good sources of vitamins, carotenoids and phenolics [1-5], which can be beneficial for the prevention and/or alleviation of oxidative stress and degenerative disorders [6-9].

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 [10-13]. Lycopene along with its metabolites, is currently attracting much attention among scientists due to its capacity to scavenge radicals and the different biological functions these radicals seem to be involved in [14-16]. In addition, lycopene is mainly responsible for the colour of red tomatoes and is widely used as a colorant. The colour of food is a very important factor in determining acceptability, hence the objective measurement of this attribute in different tomatoes and tomato products has been the subject of a myriad of studies [17-26].

It is therefore not surprising that the enhancement of the carotenoid content of tomatoes has been an important research goal in recent decades. Although there are some missing links, several key mechanisms underlying the carotenoid deposition in tomatoes are well known. The accumulation of carotenoids in the tomato fruit is coordinated with other processes, such as fruit development and ripening, plastid formation, and flowering. The typical massive 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 [27]. This knowledge has been applied to studies on the development of carotenoid transgenic tomatoes aimed at increasing carotenoid levels. Although there have been several successes [28-30], studies have been limited due to consumer 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 key carotenogenic genes such as ripening-enhanced phytoene synthase (*Psy-1*) and lycopene-β-cyclase *Cyc-b* [31]. In addition, introgression lines (IL) of the stay-green tomato wild relative *Solanum penellii* into the M82 tomato cultivar have been studied in order to pinpoint quantitative traits loci underlying high carotenoid phenotypes and ILs with high carotenoid bioaccessibility [31-32]. 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 as *Anthocyanin fruit (Aft)*, *Aubergine (Abg)* and *atroviolaceum (atv)* have been transferred to the cultivated tomato through breeding [33]. Although the identity of the anthocyanins expressed in these genotypes has already been investigated [34-35], little is known about their bioavailability in humans (which is expected to be very low as they accumulate in the skin) and the effects of these novel crossings on their carotenoid content and colour.

Genetic engineering and traditional plant breeding are not the only strategies for enhancing the levels of carotenoids and other plant metabolites of a nutritional interest.

Agronomical and environmental factors such as light, temperature, irrigation and mineral nutrition can also be harnessed to increase the contents of these compounds [36-39], since the phenotype depends not only on the genotype, but also on its interaction with the environment.

In this study we analyzed one non-anthocyanin-accumulating (Ailsa Craig) and three anthocyanin-accumulating tomato genotypes (Anthocyanin fruit type, atroviolaceum and Sun Black). These were grown hydroponically to evaluate differences in their total anthocyanin content, carotenoid levels and colours, as well as to assess the effects of different salinity levels on these parameters.

Materials and methods

Plant material, growing technique and treatments

Tomato fruits cv, Ailsa Craig (Ac), Anthocyanin fruit type (Aft), atroviolaceum (Atv) and Sun Black (SB) were analyzed (Figure 1). The fruits with *Aft* and *Atv* genes express anthocyanins in different degrees in the epidermis, although not in the pericarp. The *Anthocyanin fruit (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 Sun Black tomato, which is characterized by the strong purple pigmentation of its skin, was obtained as a result of crossing *Atv* × *Aft* [33-34] .

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° C and 27°C, respectively; the maximum temperature reached 30-32°C in the autumn sun.

116 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}.

118 Seedlings were transplanted 50 days after sowing into 1-meter long rockwool slabs. The 119 tomato plants were grown vertically with a single stem at a density of three plants m^{-2} and 120 pollination was favoured by mechanical vibration of the flower clusters.

121 Drip irrigation was carried out using a nutrient solution with electrical conductivity (EC) 3.5 122 dS m^{-1} and pH 6.5. Exhaust nutrient solution was discharged after three weeks or 123 whenever the EC was higher than 6 dS m^{-1}. The composition of the nutrient solution was 124 as follows (concentration are expressed in $molm^{-3}$): 12 $N\text{-}NO_3^-$, 1.3 $P\text{-}PO^-$, 8 K^+, 4 Ca^{2+}, 125 1.2 Mg^{2+}, 9 Na^+, 1.5 $S\text{-}SO_4^{2-}$. Micronutrients were added at Hoagland's concentration (in 126 $mmolm^{-3}$: B 40, 40 Fe, 1 Cu, 5 Zn, 10 Mn).

127 The experimental treatment consisted of two different salinity levels (EC) of the nutrient 128 solution: 3.5 and 5.5 dS cm^{-1}. The solution with EC 5.5 dS cm^{-1} was prepared by the 129 appropriate addition of 35 mol m^{-3} NaCl to the nutrient solution.

130 A complete randomized block experimental design was adopted, with three replicates for 131 two treatments (C control and S salinity treatment). Each replicate consisted of 12 plants. 132 Data were subjected to two-way analysis of variance (ANOVA). The means were 133 separated using the least significant difference (LSD) test for $P = 0.05$.

134 The fruits were harvested at the commercial ripe stage when they showed a red colour. 135 The ripeness stage was characterized in accordance with the procedure reported 136 elsewhere [40].

137 **Carotenoid analysis**

138 Tomato carotenoids were determined as described elsewhere [31] with slight modifications, 139 10 mg of freeze-dried and homogenized tomato fruit material was vortexed with 250 μL of 140 methanol and then with 500 μL of chloroform, sonicated and subsequently spun at 18000

g for 5 min at 4℃. The lipophilic phase was removed with a Pasteur pipette and the aqueous phase was re-extracted with chloroform (500 µL). The pooled chloroform extracts were dried under a stream of nitrogen or by centrifugal evaporation. Dried residues were stored under a nitrogen atmosphere at 20℃ 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 quaternary pump, diode array detector and auto-sampler. The data were acquired and analysed 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℃. 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 minutes, 80% A and 5 % B until 32 min, 30% A and 5 % B until 56 min, 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 coloured carotenoids lycopene, β-carotene and lutein were quantified by external calibration. The calibration curves were made with all-*E*-standards obtained in our laboratory in accordance with recommended procedures [41]. The colourless carotenoids phytoene and phytofluene were not determined.

Anthocyanin determination

Anthocyanins were extracted in acidified methanol as described elsewhere Mes [34]. Briefly, 100 mg of lyophilized tomato skins were ground into a fine powder and extracted overnight in 300µL of 1% HCl methanol at 5 ℃. The extraction volume was taken to 500 µL with

nanopure water and 500 µL of chloroform were added to the tube. The tubes were centrifuged for 5 min at 18000 g and the aqueous phase was removed to a new tube. The aqueous phase was dried under centrifugal evaporation. The sample was dissolved in 150 µL HPLC grade methanol. The HPLC measurements were taken with the same equipment used for the carotenoid analysis, using a Prodigy 5 mm ODS (3 mm) 10 nm (250 x 4.6 mm) column fitted with a 4.0 x 3.0 mm i.d. guard column (Phenomex, Torrance, CA) that was kept at 35 ℃. The injection volume was 20 µL. The HPLC protocol is reported elsewhere [42] 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 minutes, 98% B for 4 minutes, 95% B for 5 minutes, and 90% B for 2 minutes, 88% B for 3 minutes, 85% B for 3 minutes, 82% B for 8 minutes, 80% B for 5 minutes, 60% B until the 40 minutes, 98% B for 3 minutes 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.

Anthocyanin mass spectrophotometry 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, Collision energy 25 V. Each spectrum was acquired in MCA mode, accumulating 33 scans.

The sample was dissolved in methanol: water (1:1) with 0.1% formic acid.

Colour determination

For the colour measurements, three fruits of each cultivar were analyzed. Assessment of the external colour of the tomatoes was taken from three readings rotating the fruit by 120° between each reading. Since 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 colour.

A DigiEye imaging system [43] was used to record digital images and assess the colour 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 x 150 pixel height, and were saved in bmp format. The CIELAB colour parameters [44] were obtained from the images using CromaLab software [45], considering the 10° Observer and the Illuminant D_{65} as references. A psychometric index of lightness, L^*, and the colour coordinates a^* and b^* are defined in this uniform colour space. L^* is related to the lightness of the samples, enabling any colour to be regarded as equivalent to a member of the grey scale. L^* takes values within the range 0 (for black) to 100 (for white). The colour coordinate a^* takes positive values for reddish colours and negative values for greenish colours, whereas the coordinate b^* takes positive for yellowish colours and negative values for bluish colours. From L*, a* and b*, other colour parameters, namely chroma and hue, are defined within this space. Hue (h_{ab}) is an angular parameter and is considered the qualitative attribute of colourfulness. In accordance with hue, colours have been traditionally regarded as greenish, bluish, yellowish, reddish, etc. Chroma (C^*_{ab}) is the quantitative attribute of colourfulness and can be used to assess the

degree of difference of any hue relative to a grey colour with the same lightness. The colour differences (denoted as ΔE^*_{ab}) between two colours 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

Anthocyanin and carotenoid content and colour of the control tomatoes

Greenhouse hydroponic cultivation could provide a valuable tool to improve fruit yield and phytochemical levels with a highly efficient use of resources such as water, energy and labour [46] .

The levels of the main coloured carotenoids, lycopene, β-carotene and lutein, found in the hydroponically grown control tomatoes are summarized in Table 1.

In the control samples, considering the three major coloured carotenoids, the genotype with the highest pigmentation due to total carotenoids was Atv (830.6 μg g^{-1}DW), followed by Ailsa Craig (477.9 μg g^{-1} DW), Sun Black (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. However, the β-carotene levels were very similar to the Aft and SB control samples, whilst the β-carotene levels of Ac were around two times higher. The amount of lutein was negligible compared to the β-carotene and lycopene carotenoids. From these data it can be easily concluded that the genetic differences between the plants surveyed accounted for large differences in the carotenoid pigmentation of their fruits.

Concerning the anthocyan content of the control samples, differences were observed among the genotypes; namely the total anthocyan level in Aft was found to be six times lower than in SB (ranging from ca.54.8 to 298.6 μg g^{-1}DW) (Table 2).

To determine the moieties attached to the anthocyanin, samples were injected into an MS-electron scan. Table 3 represents the masses of the moieties present in cv Sun Black. These masses were compared with all combinations of known anthocyanidins and glycosyl and acyl moieties. 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 [34].

The most predominant nonacyled anthocyanin was the peak at 611 m/z, consistent with delphinidin-3-rutinoside, which was found to be predominant in Aft [34]. Another discernable peak at 511 m/z was observed but not recognized.

The CIELAB colour parameters corresponding to the tomatoes and homogenates obtained from the fruits are summarized in Table 4. Considering the external colour, the values of a*, b* and C^*_{ab} of Sun Black were the lowest. On the other hand, the highest values corresponded to the Aft genotype. Regarding the colour of the homogenates there were significant differences in virtually all the cases as a function of the genotype. However, overall the differences were not very high. The genotype with the highest levels of coloured carotenoids (Atv), showed the highest values of L*, b*, C^*_{ab} and $h_{ab.}$ In other words, the puree from this genotype was the darkest, had the most vivid colour and a more orange hue than the rest.

The colour difference (Table 5) between this puree and the one corresponding to the genotype with the lowest content of coloured carotenoids (Aft) was 4.59 CIELAB units. Since from an industrial point of view it is considered that colour differences between 2.8 and 5.6 CIELAB can be discerned by individuals with normal colour tolerances [47-48], consumers should easily be able to see the differences between the purees corresponding

to the Atv and Aft control tomatoes. The colour difference between the purees obtained from the Atv and the Ailsa Craig 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 non-anthocyanin accumulating Ailsa Craig tomato and the anthocyanin-accumulating Aft tomato were visually discerned though (ΔE*ab = 4.00 CIELAB units). However the colour difference between the purees from the Atv and the Sun Black 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 to a great extent to the colour of the purees. The colour of the puree from control Sun Black tomatoes was also easily distinguishable from those obtained from Aft (6.90 CIELAB units) and Sun Black (7.56 CIELAB units) tomatoes.

Effect of salinity stress on the yield, anthocyanin and carotenoid content and colour

Tomato is moderately sensitive to salinity stress, which impairs nutrient uptake and reduces leaf expansion and crop yield [49]. According to some reports, an increase in irrigation water salinity is accompanied by reduced crop water consumption, plant growth, fruit and crop yield, and an increase in titratable acidity, osmotic pressure and sodium concentration, sugars and organic acids [50-54]. Furthermore, some studies indicate that salinity can also improve the antioxidant content of tomato fruits [55-57]. Table 6 shows the quantitative and qualitative parameters of hydroponically grown tomato cultures. 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.

Table 6 summarizes various qualitative parameters - total soluble solids were found to be higher in salinity treatment.

Saline treatment influenced the content of total anthocyanins differently in the two cultivars (Table 2). In Aft the content of anthocyanins in fruits grown with a saline nutrient solution decreased by about 10 times compared to the control (6.01 to 54.77μg g^{-1} dw). In contrast, the SB content of total anthocyanins almost doubled in the samples grown with a saline treatment (298.57 to 479.32 μg g^{-1}DW).

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 Ailsa Craig and 1.1-fold in the case of Atv. The levels of β-carotene and lutein also increased as a consequence of the treatment, more specifically between 1.16- and 1.53-fold and 1.02- and 2.35-fold, respectively.

Taken as a whole, these data are particularly interesting. On the one hand, it was clearly seen that Sun Black was the genotype that increased its carotenoid content to a greater degree as a result of the treatment. It reached the highest carotenoid content of all the genotypes studied, irrespectively of the nutrient solution considered. The genotype whose carotenoid content was least affected by the rise in salinity of the nutrient solution was Atv. However, the total carotenoid content of the fruits from this genotype grown with salinity stress was the second highest. In this sense, it is important to bear in mind 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 [27]. 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

316 carotenes by the halotolerant microalga *Dunaliella* as a response of salt and other
317 stresses has long been known [58] , the role of carotenoids in tomato plants subjected to
318 salinity stress is still rather obscure. Nevertheless there are several reports on the effects
319 of high salinity on the antioxidant systems of the domesticated tomato and its wild relative
320 *S. penellii* [59-61] . Therefore further studies that help to unravel the mechanisms underlying
321 the enhanced carotenoid deposition should be encouraged. In this regard the study of the
322 Sun Black genotype seems especially interesting.
323 Concerning the external colour of the treated tomatoes, salinity stress resulted in a clear
324 decrease in the a* and b* values of the Sun Black genotype, as a result of which its C^*_{ab}
325 decreased considerably compared to the corresponding control (Table 4). Indeed the
326 highest colour difference between control and treated tomatoes corresponded to Sun
327 Black (ΔE^*_{ab} = 20.49 CIELAB units), followed by those of the Atv genotype, which showed
328 a much lower colour difference (ΔE^*_{ab} = 7.61 CIELAB units).
329 With regard to the colour parameters corresponding to the homogenates obtained from the
330 tomatoes grown under salinity stress (Table 4), overall there were some significant
331 differences in them among the genotypes considered in pairs. The darkest puree
332 corresponded to the Atv tomato (lowest value of L*), whereas the brightest (highest value
333 of L*) corresponded to the Sun Black. The Sun Black homogenate had the lowest values
334 of both a* and b*, and thus had the lowest values of chroma and the highest value of hue.
335 Apart from its higher carotenoid content, the intense accumulation of anthocyanins in the
336 epidermis of this tomato may also account for the higher differences in the colour
337 parameters of its puree compared to the other genotypes. In terms of colour differences
338 (Table 5), all the purees taken in pairs could be visually distinguished (values of ΔE^*_{ab} over
339 2.8 CIELAB units). The highest colour differences corresponded to the pairs which
340 involved the Sun Black purees and to the Atv-Ac pair (all with values of ΔE^*_{ab} over 8
341 CIELAB units).

342 The effects of the treatment on the colour differences of the different purees led to an
343 increase in salinity of the nutrient solution led to higher values of ΔE^*_{ab}, except in one case
344 (Atv-Aft). There was a considerable increase in colour differences between the pairs Atv-
345 Ac, Atv-SB and SB-Aft (ca. 6. 5 and 4 units).
346 In terms of the colour differences between purees from the same genotype as a result of
347 the treatment, the highest values of ΔE^*_{ab} by far corresponded to the purees from Sun
348 Black. The lowest were observed in the purees obtained from the Aft fruits. In general, the
349 changes in brightness of the homogenates were the major contributors to these colour
350 differences (Table 7).
351 In conclusion, the carotenoid content of the anthocyanin-accumulating Atroviolaceum (Atv)
352 was ca. 2 and 2.5 times higher than the other genotypes when they all were hydroponically
353 grown with a control nutrient solution with electrical conductivity 3.5 dS m^{-1}. The puree
354 obtained from Atv fruits was easily visually distinguishable from SB and Aft tomatoes. The
355 purees from the control Sun Black tomatoes, with a dark purple peel, were especially
356 discernable. The anthocyan content in the fruit of genotype Sun Black was about six times
357 higher than the Anthocyanin Fruit Tomato (Aft).
358 The saline treatment influenced the accumulation of total anthocyanins in the SB fruits,
359 while it reduced accumulation in the Aft fruits.
360 Concerning the effects of a high-salt nutrient solution (EC=6 dS m^{-1}) in the carotenoid
361 content, an increase in salinity was accompanied by an increase in all the major coloured
362 tomato carotenoids. Two- to three-fold increases in the levels of lycopene were observed
363 in some cases. Sun Black was the genotype that increased its carotenoid content the most
364 as a result of the treatment, reaching the highest carotenoid content of all the genotypes
365 studied irrespectively of the nutrient solution used. On the other hand, Atv was the
366 genotype whose carotenoid content was least affected by the increase in salinity of the
367 nutrient solution. 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 colour differences between purees from the same genotype as a result of the treatment. Changes in the external colour of the tomatoes as a result of the treatment were especially noticeable in the Sun Black 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 these traits with transgenes, the resulting tomatoes do not raise as much controversy and suspicion as their genetically modified counterparts. The study of the Sun Black 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.

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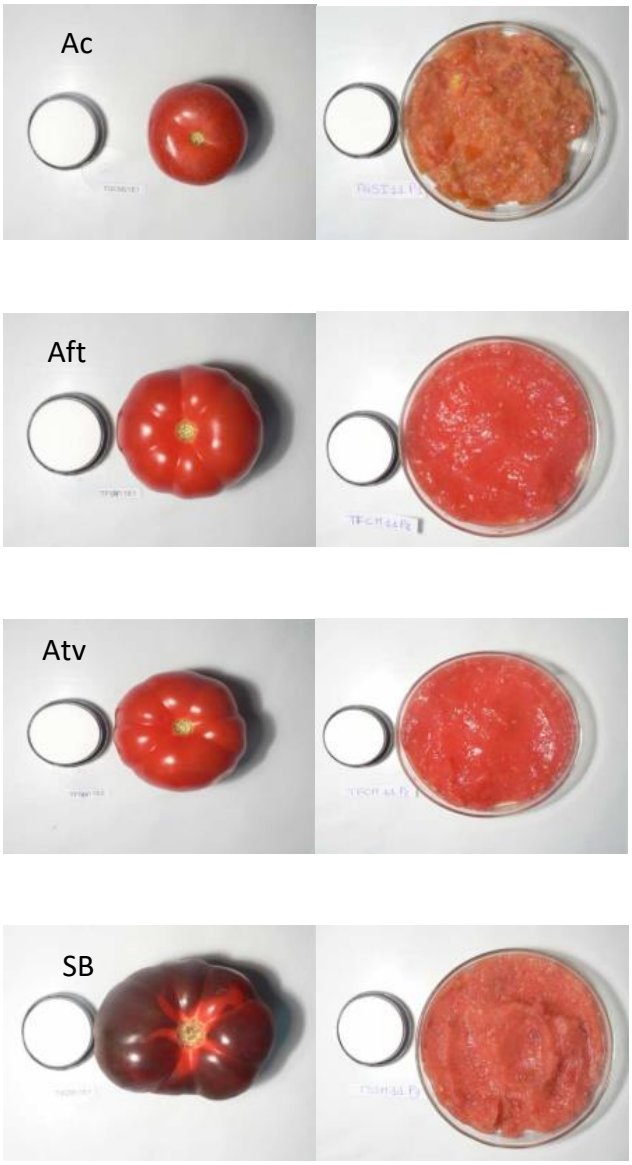

Figure 1. Tomato fruits and purees corresponding to control Ailsa Craig (Ac), and Aft, Atv and Sun Black (SB) samples.

	Treatment	Lycopene	β-carotens	Lutein
Ailsa Craig	C	322.7d	149.8b	5.4bcd
	S	600.5c	211.2a	7.9a
Aft	C	252.6d	70.3d	4.0de
	S	747.8b	83.2d	5.2cde
Atv	C	748.7b	74.3d	7.6ab
	S	833.5b	113.6c	7.8a
Sun Black	C	325.8d	70.8d	3.1e
	S	989.4a	87.5cd	7.29abc
cv		**	**	**
Treatments (t)		**	**	**
cv x t		**	*	*

*Table 1. Carotenoid contents (µg g^{-1} DW) in the different tomato cultivars studied as a function of cultivation system (C: control solution; S: high salt solution). Numbers followed by different letters in the same column differ significantly at the 5% level by LSD test; Significance level: *P≤ 0.05;**P≤0.01; n.s. not significant.*

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

Table 2. Anthocianin contents (expressed in malvidin 3-glucoside) in the different tomato cultivar skins studied as a function of cultivation system (C: control solution; S: high salt solution). Numbers followed by different letters differ significantly at the 5% level by LSD test.

Functional groups	Detected massa (m/z)		
Anthocyanidin	Delphinidin	Petunidin	Malvidin
	303	317	331
Glycoside	465	nd	493
Unknow	483	497	511
Rutinoside	611	625	639
p-coumaroyl+ rutinoside	757	771	Nd
p-coumaroyl+ rutinoside+glycoside	nd	933	947
Unknown	nd	nd	691

Table 3. Anthocyanin composition of tomato fruit skin from plants of Sun Black as detected by HPLC-MS. (nd = not detected mass)

Genotypes				
Colour	**Ailsa Craig**	**Aft**	**Atv**	**Sun Black**
Control samples (C)				
L*	39.28a	40.79b	41.68c	36.93d
a*	43.08a	44.85b	42.72c	40.18d
b*	40.06a	36.80b	40.77c	33.48d
C^*_{ab}	58.90a	58.04b	59.10a	52.30c
h_{ab}	42.88a	39.27b	43.63c	39.72d
Treated samples (S)				
L*	43.62ab	41.05b	36.95c	45.68a
a*	40.37a	45.18b	45.39b	34.92c
b*	39.21a	37.71b	37.32b	33.58c
C^*_{ab}	56.37a	58.90b	58.80b	48.49c
h_{ab}	43.91a	39.80b	39.32c	43.80a

Table 4. Colour parameters corresponding to control (C) and treated (S) tomato fruits. Data were subjected to one-way ANOVA and different letters within the same row mean that values are statistically different $P < 0.05$.

Treatment	Pairs of genotypes					
	Atv-Aft	Atv-SB	Atv-Ac	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

Table 5. Colour differences (in CIELAB units) between purees as a function of the genotype, for each cultivation system.

		Yield (g)	DW (%)	TSS (° Brix)	AT (%citric acid)
Ailsa Craig	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	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	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.
Sun Black	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		**	*	*	n.s.
Treatments (t)		n.s.	**	*	n.s.
cv x t		n.s.	**	*	n.s.

*Table 6. Yield (gram plant^{-1}), dry weight (DW), total soluble solid (TSS, °Brix) and titratable acidity (AT, %.citric acid) in the different tomato cultivars studied as a function of cultivation system (standard nutrition solution (C) and salinity solution (S). Numbers followed by different letters in the same column differ significantly at the 5% level by LSD test. Significance level: *P≤ 0.05;**P≤0.01; n.s. not significant.*

Genotype	ΔE^*_{ab}	ΔL^*	ΔC^*_{ab}	Δh_{ab}
Ac	5.19	-4.32	2.53	1.03
Aft	1.0	-0.26	-0.86	-0.53
Atv	6.4	4.73	0.3	4.31
SB	10.2	-8.75	3.81	-4.08

Table 7. Colour differences (CIELAB units) between purees from the same genotype as a result of the increase in the salt concentration of the nutrient solution.