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Tomato Fruit Antioxidants in Relation to Salinity and Greenhouse Climate

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ABSTRACT: A two-year study of antioxidants in greenhouse tomato was conducted. Plants were treated continuously with nutrient solution electrical conductivities (EC) of 2, 4, or 6 dS m^{-1}. Increasing EC reduced yield per plant and fruit size. Oxygen radical absorbance capacity (ORAC), lutein, β-carotene, lycopene, and vitamin C concentrations were evaluated in harvested fruit. ORAC and all antioxidants with the exception of lutein increased with EC. None of the 10 genes involved in antioxidant metabolism were affected by salinity in ripe fruit, but the expression of three of them (*ZDS*, *CrtR-b1*, and *NCED1*) varied with the stage of fruit development. Antioxidant concentrations were related to greenhouse climatic conditions. β-Carotene, lycopene, lutein, and vitamin C responded negatively to light and positively to temperature, whereas ORAC was unresponsive. Multiple regressions of antioxidants in relation to EC and climatic factors showed that antioxidants responded more strongly to light and temperature than to EC.

KEYWORDS: *lycopene, β-carotene, ascorbic acid, ORAC, salinity, lutein, tomato, greenhouse climate, hydroponics, vitamin C, temperature, light, electrical conductivity, antioxidant, gene expression*

INTRODUCTION

Tomato is listed in the top 20 commodities grown worldwide and in terms of vegetable production, is second only to that of potato.[1] As such, it constitutes an important component of the global diet. The nutritional value of food is an increasingly important consideration in the minds of consumers. Yet, in a meta-analysis of data taken from 1950 to 1999, 43 fruits and vegetables showed a statistical decrease in nutritional value over time.[2] The authors suggest that the changes are due largely to the development of new cultivars bred for increased yield at the expense of nutritional value. From a historical perspective, this may be true, but there is ample evidence to show that cultural practices also influence the nutritional value of many crops.[3−5]

Greenhouses offer unique opportunities to improve the human health benefits of fruits and vegetables because of the greater degree of control over growing conditions compared to a field situation. This is particularly true in hydroponic greenhouses, where virtually all aspects of the root-zone environment can be managed. For example, salinity or total mineral concentration in the feed nutrient solution measured as electrical conductivity (EC) is often adjusted depending on such factors as daily light levels, season, crop phenology, fruit load, or the EC in the substrate leachate. The easiest way to make those adjustments is simply to increase or decrease the concentrations of fertilizers in the feed, although NaCl has also been added to increase EC. In recent years, the tendency has been to grow greenhouse tomato crops at higher EC values, based on research showing improvements in a number of fruit quality attributes.[6] This is despite the well-documented salinity-induced reductions in yield. Although a short-term pulse of high EC nutrient solution applied at the fruit breaker stage has been shown to improve quality without compromising yield,[7] this is not practical for the indeterminant (continuously fruit-bearing) tomato cultivars commonly grown in greenhouses.

Effects of environmental conditions on nutritional components in tomato fruit have been well documented in recent years.[8] Reviews of these studies[3,4,6] indicate factors such as mulching, grafting, pruning, minerals, irrigation, salinity, light, temperature, and CO_2 may affect specific fruit components. For example, increasing EC has been shown to affect levels of important health components such as vitamins, total antioxidants, phenols, and pigments such as lycopene and β-carotene, but results may depend on the degree of salinity imposed, the basis for expression (fresh weight, dry weight, per fruit) of the compound of interest, fruit maturity, interaction with other environmental parameters, or cultivar.

The purpose of this two-year study was to evaluate the effects of nutrient solution EC on greenhouse tomato fruit antioxidants and on gene expression, and to also relate antioxidants to greenhouse environmental conditions. Values of EC were chosen which could typically be found in commercial hydroponic tomato greenhouses in North America or Europe.

MATERIALS AND METHODS

Plant Material and Experimental Design. Tomato (*Solanum lycopersicum* L. cv. Clarance, a cluster tomato) (De Ruiter Seeds, Monsanto, Oxnard, CA) plants grafted onto cv. Beaufort rootstock

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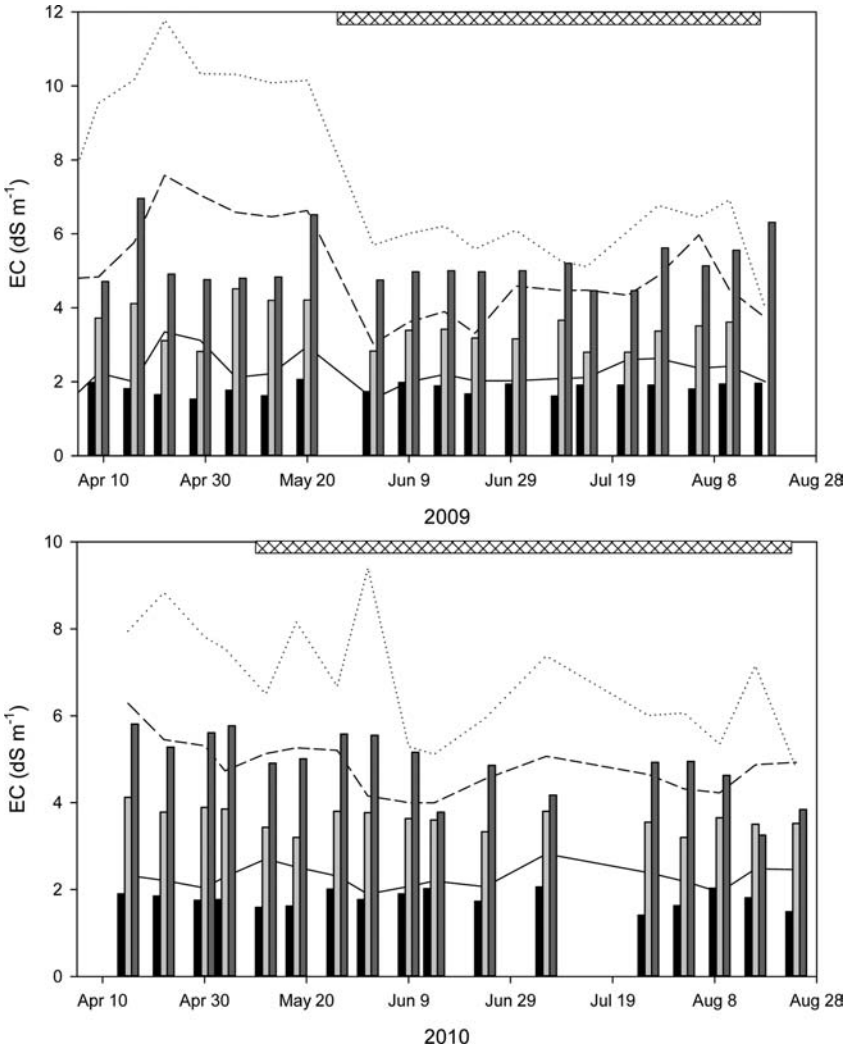

Figure 1. Values of EC for each treatment over time for 2009 and 2010. Columns represent EC measured in the supply tanks: 2 dS m^{-1}, black column; 4 dS m^{-1}, light gray column; and 6 dS m^{-1}, dark gray column. Lines represent EC measured in the substrate leachate: 2 dS m^{-1}, solid line; 4 dS m^{-1}, dashed line; and 6 dS m^{-1}, dotted line. The horizontal cross-hatched area represents the period over which fruits were sampled for antioxidant assessment.

(De Ruiter Seeds, Monsanto, Oxnard, CA) were grown in a 65 m^2 greenhouse compartment at the Pacific Agri-Food Research Centre (PARC), Agassiz, BC (lat. N49°14′33″, long. W121°45′35″) Canada. Plants were propagated in rockwool blocks and transplanted at 6 weeks old into rockwool slabs (Fibrgro, Sarnia, ON, Canada) in the greenhouse compartment. Each rockwool slab contained three plants. Greenhouse plant density was 2.6 plants m^{-2}.

The experimental design in the greenhouse consisted of a randomized complete block, with two blocks and six experimental units (rockwool slabs) per block for each experimental treatment. Blocks were positioned to account for experimental error due to minor light gradients in the greenhouse. Standard cultural practices for greenhouse tomatoes were followed.[9] Irrigation was based on light sum, whereby an irrigation event was triggered by the climate control computer (Argus Control Systems Ltd., White Rock, BC, Canada) when a specific amount of light had been received by a pyranometer (Li-200, LiCor, Lincoln, NE) on the greenhouse roof. Treatments consisted of low, moderate, and high nutrient solution ECs in the supply tank. The control nutrient solution contained $Ca(NO_3)_2$, $MgSO_4$, KNO_3, KH_2PO_4, and K_2SO_4 with 12.3 mM NO_3, 1.3 mM PO_4, 7.2 mM K, 3.2 mM Ca, 1.1 mM Mg, and 1.3 mM SO_4. The two higher EC treatments were achieved by increasing the concentration of these macronutrients to the desired level. All treatment solutions contained a commercial chelated micronutrient mix (Plant Products Co., Brampton, ON, Canada) consisting of 18.8 μM Fe, 5.5 μM Mn, 0.9 μM Zn, 0.2 μM Cu, 18.1 μM B, and 0.1 μM Mo. The pH of the solutions was adjusted to 5.5−6.0 with H_2SO_4. Supply tank EC values are presented in Figure 1. The EC values in the substrate leachate (Figure1) varied depending on climatic conditions and the rate of irrigation. Over the fruit sampling period in 2009, mean supply tank EC ± sd values were 1.89 ± 0.10, 3.32 ± 0.45, and 5.27 ± 0.64 dS m^{-1} in low, moderate, and high EC treatments, respectively; leachate EC ± sd values were 2.25 ± 0.43, 4.52 ± 1.06, and 6.66 ± 1.56 dS m^{-1} in low, moderate, and high EC treatments, respectively. In 2010, supply tank EC ± sd values over the fruit sampling period were 1.77 ± 0.17, 3.55 ± 0.20, and 4.75 ± 0.72 dS m^{-1} in the low, moderate, and high EC treatments, respectively. Leachate EC ± sd values over the same period were 2.29 ± 0.27, 4.60 ± 0.40, and 6.97 ± 1.28 dS m^{-1} in the low, moderate, and high EC treatments, respectively. Grand means (tank and leachate values) were 2, 4, and 6 in low, moderate, and high EC treatments, respectively, for each year.

Fruits were harvested as clusters once per week when most or all of the fruit on a truss were at breaker stage or later. Fruits were weighed individually and graded for defects such as blossom end rot, cat-facing, cuticle cracks, splitting, disease, insect damage, misshapenness, or immaturity based on a grade of 1, 2, or 3 (no, moderate, or severe defects, respectively). Fruits for antioxidant analysis were harvested on May 25, June 8 and 22, July 6 and 20, and August 4 and 17 in 2009

Table 1. Name, Function, and Primer Information for the Genes Evaluated in the Study

gene	enzyme	function	primer name and sequence (5′ to 3′)[a]
EF1	elongation factor	reference gene	LeEF1F1: TTGAGGCTCTTGACCAGATT′ LeEF1R1: AACATTGTCACCAGGGAGTG
CHS	chalcone synthase	synthesizes chalcone, an intermediate for many flavonoids	LeCHSF1: AAACTCTTGTCCCCGATAGC LeCHSR1: CCCTAGAGGTTGAAATGCTTC
PSY-1	fruit-specific phytoene synthase	synthesis of lycopene by condensation of two molecules of geranylgeranyl diphosphate to form the 15-*cis*-isomer of phytoene in fruit	Psy1-F-236: TGACGTCTCAAATGGGACAAG T′ Psy1-R-305: CCTCGATGAATCAAAAAAACG G
PSY-2	leaf-specific phytoene synthase	catalyzes the reaction from prephytoene diphosphate to phytoene	Psy2-F-231: AGGCAAGGCTGGAAGATATTTTT′ Psy2-R-303: GAAACAGTGTCGGATAAAGCTGC
PDS	phytoene desaturase	catalyze dehydrogenation reactions by introducing four double bonds to form lycopene	Pds-F-679: TGGGTGGTTTGTCTACAGCAAA Pds-R-749: ATCCCTTGCCTCCAGCAGTA′
ZDS	ζ-carotene desaturase	converts ζ-carotene to tetra-*cis* lycopene	Zds-F-1291: CAATGGCTGGGTTACAGAGTTG Zds-R-1357: CAATCCTGCAGCGCGC
Lcy-b	lycopene β-cyclase	converts lycopene to α-carotene and β-carotene	Lcy-b-F-828: TGCTTATGGCATTTTGGCTG Lcy-b-R-899: CGCCAATCCATGAAAACCA′
CrtR-b1	carotene β-hydroxylase 1	converts α-carotene to zeinoxanthin which is then converted to lutein	CrtR-b1-F-469: TGTTGGTGCTGCTGTAGGAATG CrtR-b1-R-537: AGTGAAGCATGCCACAGTGC
ZEP	zeaxanthin epoxydase	converts lycopene to zeaxanthin	Zepfor238: AAGGTTCCACAGAAGAAGTTGAAAG′ Zeprev309: TGCCAAAGCAAACACTAACCC′
NCED1	9-*cis*-epoxycarotenoid dioxygenase	production of abscisic acid	Nced1for477: TCGAAACGGAGCTAACCCTC Nced1rev547: GAACCATACCGTCGCCGTC
Cyc-b	lycopene cyclase analogue of neoxanthin synthase	carotenogenic enzyme	BetaGeneF988: GAGGAAGAGAAATGTGTGATCCCT BetaGeneR1330: TAGGATCAAGATCAAAGAAAGCG′

[a]Primer references for the *EF1* gene and the remaining genes are in refs 16 and 15, respectively.

and on May 10 and 25, June 7, 14, and 28, July 12 and 26, and August 9 and 23 in 2010. On each date, four uniformly ripe fruit, each at the red stage of ripening (according to the USDA color classification chart for United States Standards for grades of fresh tomatoes) were selected from each treatment (two from each block), and cubed, frozen, and freeze-dried at −50 °C. Samples were weighed before and after drying to determine moisture content and then ground to pass a 1 mm sieve using a centrifugal mill (ZM 200, Retsch, Haan, Germany). All analyses for antioxidant components were conducted on these samples.

Daily sums for global solar radiation measured with the roof-top pyranometer and greenhouse mean 24 h temperature and relative humidity (RH) obtained from sensors positioned at canopy midheight (1.7 m) in the center of the greenhouse compartment were computed from continuous 15 min records (Argus Control Systems).

Vitamin C Analysis. A 0.2 g dry fruit sample was homogenized in 10 mL of 0.4% oxalic acid and the aqueous supernatant partitioned against chloroform, then diethyl ether, and finally filtered.[10] Twenty five microliters of the resultant aqueous phase was analyzed with a high performance liquid chromatograph (Dionex model DX500, Thermo Scientific, Sunnyvale, CA, USA) with UV detection using a 5 μm, 250 × 4.6 mm, Zorbax C18RP ODS column (Rockland Technologies, Newport, DE, USA). The mobile phase was 2% ammonium phosphate (pH 2.8). Vitamin C (ascorbic acid) was determined at 245 nm and

quantified against a standard solution of L-ascorbic acid (Sigma-Aldrich Canada Ltd. Oakville, ON, Canada) in 0.4% (w/v) oxalic acid. There were four replicate fruits per treatment per harvest. Results are expressed on a fruit fresh weight (FW) basis.

Oxygen Radical Absorbance Capacity. Approximately 100 mg of freeze-dried sample was accurately weighed into 12 mL polypropylene test tubes, and 5.0 mL of pH 7.0 phosphate buffer was added immediately. Samples were homogenized for 30 s (VDI 12 homogenizer with a 7S head, VWR, Mississauga, ON, Canada) and then centrifuged at 4000 rpm (Jouan CR422, Canberra Packard, Vancouver, BC, Canada) and 4 °C for 30 min. Aliquots of 1.5−2 mL were pipetted into 2 mL microtubes and centrifuged for 20 min at 20,000*g* and 4 °C (Eppendorf 5804 R, VWR, Mississauga, ON, Canada). The supernatant was collected and used directly as a sample for the assay.

Oxygen radical absorbance capacity (ORAC) measurements were made according to the method of Cao et al.[11] with some modifications. Fluorescence was measured on a fluorescence spectrophotometer (Cary Eclipse, Agilent Technologies, Santa Clara, CA, USA) with an excitation wavelength of 490 nm and an emission wavelength of 513 nm. Fluorescein was used as the fluorophore, temperature was maintained at 37 °C, and run-time was 90 min. Free radicals were generated using 2,2′-azobis(2-aminopropane) dihydrochloride (AAPH), and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) was used as the standard. Each run included a blank, a 1 μM Trolox standard, and an appropriately diluted tomato sample, all in pH 7.0 phosphate buffer. After each run, the areas under the fluorescence curves (S) were calculated, and the ORAC was determined as dilution factor × $[(S_{sample} - S_{blank})/(S_{standard} - S_{blank})]$. ORAC results were given as μmol Trolox equivalent (TE) per g weight. Results are expressed on a FW basis. There were four replicate fruits per treatment per harvest. All chemicals were purchased from Sigma-Aldrich.

Pigment Analysis. The method for the extraction of carotenoids from freeze-dried tomatoes was based on previous studies.[12] All extractions were performed under reduced light conditions (yellow light). Initially, 0.75−1.5 g of freeze-dried tomato was added to a 250 mL sealable Erlenmeyer with 0.4g of $MgCO_3$ (VWR), 15 mL of distilled water, and 500 μL of 100 ppm β-apo-8′-carotenal (internal standard, Sigma). Ethanol−hexane (4:3 v/v, 80 mL) was added, and the sample was stirred for 5 min. The supernatant (hexane layer) was transferred to a 500 mL sealable Erlenmeyer flask, and the extraction of the aqueous tomato layer was repeated with 60 mL of ethanol−hexane. The tomato sample was then washed with 5 × 30 mL of hexane, and the supernatants were recovered after each extraction and added to the 500 mL Erlenmeyer flask. The hexane layer was filtered through a Buchner funnel with Whatman #4 filter paper, and the remaining aqueous tomato layer was added to the Buchner to remove all solids. The filtrate was combined with 400 mL of 5% NaCl (VWR) in a 1 L separatory funnel, shaken, and allowed to separate. The hexane layer was recovered in a 500 mL round-bottom flask. The aqueous layer was rewashed with another 15 mL of hexane in the separatory funnel, and the hexanes layer was added to the round-bottomed flask. The hexane layer was concentrated to dryness by rotovap, redissolved in a 2 mL wash (3×) of dichloromethane (VWR) and transferred to a 10 mL amber vial. The dichloromethane was removed under a stream of nitrogen, and the sample was redissolved in 6 mL of methyl *t*-butyl ether (MTBE, Caledon, Georgetown, ON, Canada) with sonication and transferred to a 2 mL HPLC sample vial.

A Varian HPLC (9000 Series, Varian Chromatography Inc., Walnut Creek, CA, USA) equipped with a 4.6 mm × 250 mm YMC Carotenoid 5.0 μm C30 column (Waters Corporation, Milford, MA, USA) and UV detection (λ = 450 nm) was used to qualify and quantify carotenoid levels in tomatoes based on standards of lutein, lycopene, and β-carotene (Sigma). Tomato extracts (15 μL injection) were eluted with a methanol/MTBE gradient over an 80 min run. Results are expressed on a FW basis.

Gene Expression. Ten genes involved in the production of antioxidant compounds were examined (Table 1). *NCED1* was included because it is involved in abscisic acid (ABA) production, and ABA is known to induce an antioxidant response in leaves.[13] The potential exists for similar relationships in the fruit. It is also a logical choice for inclusion because of the increase in tissue ABA levels induced by osmotic stress.[14] *CHS* was mostly found in the peels of tomato fruit and was used mainly to confirm that RNA was extracted from tomato skin.

RNA Isolation and Real-Time PCR Quantification (qPCR). Two separate experiments were conducted. The first examined gene expression in ripe fruit. Three fruits from each treatment, all at the red stage of maturity, were collected between August 24 and 27, 2009. In this experiment, all 10 genes were examined. The experiment was repeated in 2010, where 54 red tomatoes were collected on May 25 from the 3 EC treatments, with 6 plants per treatment and 3 fruits per plant. Here, 5 genes were examined (*ZDS*, *Lcy-b*, *CrtR-b1*, *NCED1*, and *CHS*). Intact fruits were cut into small pieces (no more than 5 mm in any dimension), and the seeds were removed. The pieces were immediately flash-frozen, which helps minimize the loss of total RNA. The pieces were placed in stomacher bags and homogenized for 40 s. The homogenates were then centrifuged, and the pellets were beaten for 1 min. RNA was obtained by precipitation with ethanol and stored in sterile ultrapure water at −80 °C after the determination of its concentration using a NanoDrop 2000c spectrophotometer (Fisher Scientific, Vancouver, BC, Canada). RNA was extracted from these fruits individually, but RNA from fruits from the same plant was pooled together after extraction, giving a total of 18 pooled tomato RNA samples. The second experiment studied gene expression at different stages of fruit development. A total of 9 fruits were collected on July 5, 2010 from the 3 EC treatments with 1 plant per treatment and 1 fruit at each development stage (green, breaker, or red) per plant. Fruit preparation was as previously described. In this experiment, *ZDS*, *Lcy-b*, *CrtR-b1*, *NCED1*, and *CHS* genes were examined.

The protocol involved a two-step method for determining relative gene expression in total RNA. The first step was cDNA synthesis. About 250 ng of total RNA extracted from the samples was reversely transcribed using Superscript II (Invitrogen, Carlsbad, CA, USA). In the second step, the cDNA samples were used to perform real-time PCR quantification (qPCR) using a fluorescent intercalating dye SYBGreen and a iQ5 iCycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA) according to the manufacturer's protocol in a final volume of 25 μL containing 5 μL of cDNA, 0.4 μM of each primer, and 12.5 μL of 2× iQSYBR Green Supermix (Bio-Rad Laboratories). The transcription levels of *CHS*, *PSY-1*, *PSY-2*, *PDS*, *ZDS*, *Lcy-b*, *CrtR-b1*, *ZEP*, *NCED1*, and *Cyc-b* genes in fruits were quantified with specific primers.[15] The EF1 (elongation factor) gene was used as a reference for normalization as previously described.[16] The qPCR conditions were 94 °C for 4 min, followed by 35 cycles of 4 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min. Relative quantification of the target RNA transcript level was performed by the comparative Ct (threshold cycle) method.[16]

Statistical Analysis. All statistical analyses were carried out with the GLM, REG, or STEPWISE procedures of SAS (SAS Institute, Cary, NC, USA). Significant effects of EC treatment on yield, fruit quality and antioxidants, and gene activity were evaluated using the GLM procedure in combination with Duncan's multiple range tests. In the evaluation of the effects of the stage of fruit development on gene activity, all EC treatment values were combined. Regressions (REG procedure) of antioxidants against light, temperature, or RH used combined EC treatment data. Values for light and temperature were averaged over the one or two weeks prior to each sampling date of fruit for antioxidant analysis. Multiple stepwise regressions (STEPWISE procedure) were conducted with values of EC (from substrate leachate), light, temperature, and RH averaged over the two weeks before each fruit sampling. A GLM procedure was used to determine interactions between EC and climate for that data.

RESULTS AND DISCUSSION

Effects of Salinity on Yield. Plant yield decreased considerably with increasing EC in both years, with average

Table 2. Effects of EC on Tomato Yield, Fruit Number, and Fruit Characteristics.[a]

year	treatment EC (dS m^{-1})	yield (kg $slab^{-1}$)	fruit number	fruit weight (g)	fruit dry matter (%)	grade 1 (%)	grade 2 (%)	grade 3 (%)
2009	2	35.7 a	266 a	133.7 a	5.2 c	96.5 a	2.7 b	0.7 b
	4	29.5 b	260 a	113.6 b	5.6 b	94.5 a	2.3 b	3.2 b
	6	21.4 c	229 b	93.6 c	6.1 a	83.4 b	4.2 a	12.3 a
2010	2	30.1 a	279 a	107.3 a	5.3 c	100.4 a	0.6 a	0.5 c
	4	26.0 b	283 a	91.7 b	5.9 b	95.8 b	0.8 a	3.4 b
	6	16.3 c	235 b	68.5 c	7.2 a	89.7 c	1.1 a	9.0 a

[a]Means within each column and year followed by different letters are significantly different ($P < 0.05$) according to Duncan's multiple range test.

Table 3. Effects of EC on Antioxidants Expressed on a Fruit Fresh Weight Basis[a]

year	treatment EC (dS m^{-1})	ORAC (μmol TE g^{-1} FW)	lutein ($\mu g\ g^{-1}$ FW)	β-carotene ($\mu g\ g^{-1}$ FW)	lycopene ($\mu g\ g^{-1}$ FW)	vitamin C ($\mu g\ g^{-1}$ FW)
2009	2	1.87 c	0.464 a	4.27 b	41.92 a	144.7 b
	4	2.05 b	0.464 a	4.71 a	46.03 a	154.0 a
	6	2.20 a	0.477 a	4.68 a	46.46 a	161.7 a
2010	2	2.00 c	0.162 ab	1.57 b	6.57 b	144.5 c
	4	2.33 b	0.150 b	1.72 a	7.10 b	163.9 b
	6	2.96 a	0.169 a	1.76 a	8.09 a	187.8 a

[a]Means within each column and year followed by different letters are significantly different ($P < 0.05$) according to Duncan's multiple range test.

reductions of 15% and 43% in the moderate and high EC treatments, respectively, compared to the low EC control (Table 2). This represents a linear yield decrease of approximately 10% per unit increase in EC from the control value of about 2 dS m^{-1}. A study with cherry tomato also showed a 10% decrease in yield per unit increase in EC (calculated from Serio et al).[17] Other studies have found a somewhat smaller response of yield to increasing EC. Tomato yield was not affected by increasing EC from 2 to 6 dS m^{-1}, but a 7.5% decrease per unit increase in EC was observed from 6 to 10 dS m^{-1} (calculated from Ehret and Ho).[18] In another study, only a minor effect of EC was observed from 0.5 to 8.5 dS m^{-1}, but there was a 6.5% decrease in yield per unit EC from 8.5 to 15.7 dS m^{-1} (calculated from De Pascale et al.)[19] Yet others have found no effect of increasing EC from 3 to 10 dS m^{-1} on tomato yield.[20] The differences in response may be due to differences in salt sensitivity of the cultivars, climate, or the length of time salt was applied. Fruit size (weight) also decreased with increasing EC (Table 2), averaging 15% and 33% in the moderate and high EC treatments, respectively. The decrease in yield at moderate EC is therefore entirely due to comparable reductions in fruit size; at high EC, the yield reduction is due in part to a decreased fruit size but also to a decrease in fruit number per plant (Table 2). Fruit dry matter content increased in both years with increasing EC (Table 2). In order to assess the effects of EC on the partitioning of dry matter to fruit, individual fruit fresh weights were recorded for samples taken in 2010. Despite a decrease in fruit size with increasing EC, dry matter per fruit was constant, with means of 7.2, 7.0, and 7.8 g $fruit^{-1}$ in low, moderate, and high EC fruit, respectively ($P > 0.05$). This indicates that the partitioning of dry matter to individual fruit was not affected by EC, supporting results of previous studies.[18] In 2009, high EC increased the percentage of fruit with moderate or severe visual quality issues, and in 2010, both moderate and high EC treatments increased those percentages (Table 2).

Effects of Salinity on Antioxidants. The effects of EC on antioxidant capacity and antioxidant components expressed per unit fruit fresh weight (FW) are given in Table 3. ORAC increased with increasing EC in both 2009 and 2010. Similarly, β-carotene increased with moderate EC in both years but showed no further change at high EC. Higher concentrations of β-carotene have also been found with increasing concentrations of NaCl[20,21] or major nutrients[21] up to 9 to 10 dS m^{-1}. However, total carotenoids have been observed to increase with increasing NaCl salinity to 4.4 dS m^{-1} but decrease at higher values.[19] Lycopene was not affected by EC in 2009, but levels increased at high EC in 2010. Lycopene has been found to increase with NaCl-induced salinity to 4.5 dS m^{-1} [22] and to 10 dS m^{-1} [20] but has also been found to increase with EC (to 4.4 dS m^{-1}) then decrease at higher values.[19] In other studies, lycopene was not affected by increasing EC from 3 to 6 dS m^{-1} [17] or from 2 to 9 dS m^{-1}.[23]

Lutein was not affected by EC in 2009 but was increased by high EC compared to moderate EC in 2010; neither moderate nor high EC fruits were different from the low EC control in that year. Vitamin C increased with moderate EC in 2009, but no further changes were observed at high EC. In 2010, vitamin C increased with increasing EC. Vitamin C has been observed to increase with salinity induced by NaCl concentrations up to 6 dS m^{-1},[17] 9 mS cm^{-1},[21] or 10 dS m^{-1},[20] or by increased concentrations of major nutrients up to 9 dS m^{-1}.[21] Overall, increasing EC in our study tended to increase antioxidant capacity as well as the concentrations of all antioxidant components, in support of a number of previous studies.

Effects of Salinity and Fruit Development on Gene Expression. EC treatment did not affect the expression of any of the genes examined ($P > 0.05$). Relative to the reference gene, EF1, grand mean values for combined EC treatments in 2009 were 24.39 (*PSY-1*), 0.101 (*PSY-2*), 0.408 (*PDS*), 0.046 (*ZEP*), 0.0004 (*CYC-B*), 0.625 (*ZDS*), 0.034 (*Lcy-b*), 0.252 (*CrtR-b1*), 0.2 (*NCED1*), and 0.0017 (*CHS*). Using a reduced gene set in 2010, grand mean values were 0.163, 0.008, 0.058, 0.126, and 0.034 for *ZDS*, Lcy-b, *CrtR-b1*, *NCED1*, and *CHS*, respectively. Either these genes were unresponsive to salinity or the fruits were sampled at a time when the genes were no longer as active, irrespective of EC treatment. To our knowledge, no other studies of these genes in response to

salt stress in tomato fruit have been conducted, although a few studies with tomato leaves have been reported. The expression of *ZDS* and *Lcy-b* in leaves of tomato has been shown to decrease under salt stress.[24] Conversely, the expression of *ZDS*, *Lyc-b*, *CrtR-b1*, and *NCED* increased with salt stress in leaves of a commercial tomato cultivar, but expression decreased (*ZDS*), showed no change (*Lcy-b*), or increased (*CrtR-b1*, *NCED*) in a relatively salt-tolerant tomato species (*S. pimpinellifolium*).[25] Despite the changes in gene expression, leaf carotenoid content did not change with salt stress in either species.

Changes in the expression of these genes over fruit development are shown in Table 4. The expression of *ZDS* was greater at the breaker stage than at the green stage of development. Similarly, *CrtR-b1* activity was highest at the breaker stage, while *NCED1* was highest at the green and breaker stages. The expression of *Lcy-b* and *CHS* was not related to the stage of fruit development. Overall, in those genes where expression changed with the stage of development, there was a tendency for the highest activity to occur at the breaker stage. Lycopene, β-carotene, and vitamin C appear to accumulate throughout the ripening process.[8] The relationship between gene expression and the accumulation of these antioxidants is unknown. Since factors such as plant-to-plant variation, genotype, and harvest time[16] could influence gene expression, our data indicate that more detailed investigations are warranted.

Table 4. Effects of Fruit Stage of Development on Relative Gene Expression[a]

stage	ZDS[b]	Lcy-b	CrtR-b1	NCED1	CHS
Relative Gene Expression					
green	0.043 b	0.008 a	0.018 c	0.140 a	0.021 a
breaker	0.156 a	0.004 a	0.045 a	0.172 a	0.154 a
ripe	0.104 ab	0.004 a	0.030 b	0.064 b	0.008 a

[a]Means within each column followed by different letters are significantly different ($P < 0.05$) according to Duncan's multiple range test. [b]All genes are compared to EF1, which had an expression value of 1.

Effects of Climate on Antioxidants. Studies have shown that concentrations of antioxidants in tomato fruit may vary through the season.[26−28] Much of this is likely due to changes in the crop environment. Indeed, antioxidant concentrations in fruit have been shown to be influenced by temperature, light quality and intensity, relative humidity, fertilizers, and soil pH, but the effects of a particular environmental feature are not often consistent from study to study.[3,4]

Two of the most likely environmental factors to affect antioxidants at harvest are light and temperature, with RH being a third possibility. Table 5 shows the relationship between levels of each antioxidant and mean light, temperature, and RH values recorded 1 and 2 weeks prior to the date of each fruit sampling. Generally, the relationships observed for each of the two years were remarkably similar, particularly with respect to light and temperature. Unless otherwise indicated, the relationship trends observed for the 1 and 2 week intervals within each year were the same.

ORAC was positively related to temperature in 2010, and there was no relationship between ORAC and light or RH in either year (Table 5). Lutein was positively related to light in 2009 (1 week interval) and was positively related to temperature in 2009 and negatively related to RH in 2010. To our knowledge, these are the first reports of the response of these constituents of tomato fruit to climate.

β-Carotene was negatively related to light in 2009 (2 week interval) and positively related to temperature in both years (Table 5). These results differ somewhat from previous studies. Carotenes in tomato have not been found to show any seasonal trends,[29] suggesting no effects of the environment. Even so, β-carotene in tomato has been negatively correlated with greenhouse temperature,[20] with a combination of greenhouse temperature and greenhouse light,[30] and with fruit temperature.[31] In the present study, β-carotene was negatively related to RH in 2010.

Lycopene was negatively related to light in both years (1 week interval only in 2010) and positively related to temperature in both years (Table 5). The relationship with temperature is similar to the results from other studies.[23] Further, lycopene increased with temperature in locally heated fruit under low fruit load but strongly decreased under high fruit load.[31] However, tomato lycopene can be lower in midsummer than at other times of year,[29] and in a shading experiment, lycopene was found to decrease with increasing fruit surface temperature.[32] Others have also found that

Table 5. Relationships between Antioxidants Expressed on a Fruit Fresh Weight Basis and Greenhouse Climate[a]

year	interval[b] (weeks)	variable	ORAC	lutein	β-carotene	lycopene	vitamin C
2009	1	light	ns	0.18***	ns	−0.11**	−0.12***
		temperature	ns	0.14***	0.10**	0.14***	0.11**
		RH	ns	ns	ns	0.08**	0.21***
	2	light	ns	ns	−0.12**	−0.23***	−0.41***
		temperature	ns	0.13***	0.06*	0.14***	0.11**
		RH	ns	ns	ns	0.08**	0.23***
2010	1	light	ns	ns	−0.08**	−0.28***	−0.12***
		temperature	0.13***	ns	0.39***	0.40***	0.34***
		RH	ns	−0.12***	−0.12***	−0.04*	−0.04*
	2	light	ns	ns	ns	ns	ns
		temperature	0.10**	ns	0.43***	0.63***	0.42***
		RH	ns	−0.05*	−0.21***	−0.22***	−0.13***

[a]ns, *, **, and *** indicate nonsignificance, or significance at $P < 0.05$, 0.01, or 0.001, respectively. The numeric value is the R^2 for a significant relationship. A negative relationship is indicated by a negative sign; a positive relationship has no sign. [b]Number of weeks over which environmental data were averaged prior to a fruit sampling for antioxidant determination.

increased temperature and increased light reduced lycopene.[30] This indicates that cultural factors may interact with the environment to affect fruit nutritional quality and could explain some of the inconsistent results with lycopene. Relationships with RH in the present study were positive in 2009 and negative in 2010.

Vitamin C was negatively related to light in both years (1 week interval only in 2010) and positively related to temperature in both years (Table 5). Others have found that a combination of increasing temperature and increasing light will increase total vitamin C (reduced ascorbate plus dehydroascorbate).[30] Conversely, vitamin C has been found to decrease in heated fruit.[29] Vitamin C has also been found to be unaffected by greenhouse temperature[20] and to not show any seasonal trends or correlations with radiation or temperature.[29] Relationships with RH in the present study were positive in 2009 and negative in 2010.

A ranking of the relative importance of each environmental parameter may be useful in making practical decisions about the best way to culturally increase specific antioxidants. The relative influence of EC and climatic conditions on the concentrations of each antioxidant is given in Table 6. Compared to climatic factors, EC was generally not a major contributing factor to antioxidant levels, being important only in the ORAC model. Temperature appeared to be the most important factor overall, being a significant part of all models in one or both years, with particularly high R^2 values in 2010 β-carotene, 2010 lycopene, and 2010 vitamin C. Light was also relatively important in a few models, particularly 2009 lycopene and 2009 vitamin C. RH appeared to be the least important, with high partial R^2 values only in the 2009 β-carotene analyses. Hence, compared to the more significant effects of climate, increasing EC may not be the most effective way for growers to improve antioxidant levels in tomato fruit, particularly since higher EC also reduces yield.

The effectiveness of EC in changing antioxidant concentrations was also found to be influenced in some cases by climate. Interactions between EC and light were found for ORAC (P = 0.02, 2009) and vitamin C (P = 0.001, 2009) and between EC and temperature for lutein (P = 0.04, 2009), ORAC (P = 0.01, 2010), and β-carotene (P = 0.0004, 2010). This suggests the possibility of matching specific climatic conditions to EC regimens in order to maximize antioxidant concentrations.

In summary, we have established, through multiple samplings over two years, that both salinity and greenhouse climate impact the fresh weight concentrations of lutein, β-carotene, lycopene, and vitamin C, as well as total antioxidant capacity in tomato fruit. Salinity does not influence the expression of several key genes involved in antioxidant production in ripe fruit.

Table 6. Antioxidants Expressed on a Fruit Fresh Weight Basis in Relation to EC and Climate Variables[a]

	2009		2010	
antioxidant	variable	relationship	variable	relationship
ORAC	EC	0.16***	EC	0.19***
	model	0.16***	temperature	0.12***
			RH	0.05**
			model	0.36***
lutein	temperature	0.13***	RH	−0.05*
	model	0.13***	model	0.05*
β-carotene	EC	0.07**	temperature	0.43***
	temperature	0.03*	model	0.43***
	light	−0.12**		
	RH	−0.19***		
	model	0.41***		
lycopene	temperature	0.20***	temperature	0.63***
	light	−0.23***	RH	0.02**
	model	0.43***	model	0.65***
vitamin C	temperature	0.18***	EC	0.05**
	light	−0.40***	temperature	0.42***
	model	0.58***	light	0.03**
			RH	0.04**
			model	0.54***

[a]ns, *, **, and *** indicate nonsignificance or significance at $P < 0.05$, 0.01, or 0.001, respectively. Relationships were determined with stepwise multiple regression. The numeric value is either the partial R^2 for the variable or the R^2 for the model. A negative relationship is indicated by a negative sign; a positive relationship has no sign. EC and climate variables were averaged over the two weeks prior to each fruit sampling for antioxidant determination.

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Notes

The authors declare no competing financial interest.

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