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Effects of Salt Stress on Fruit Antioxidant Capacity of Wild (*Solanum chilense*) and Domesticated (*Solanum lycopersicum* var. *cerasiforme*) Tomatoes

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Abstract: The effects of salt on the quality of fruits were investigated in order to compare the impact of salt on key fruit properties of the cultivated domesticated tomato species (*Solanum lycopersicum*) and its wild halophyte relative *Solanum chilense*. To this end, cherry tomato plants (*S. lycopersicum* var. *cerasiforme*) and from accession LA4107 (*S. chilense*) were maintained for 112 days in the absence or presence of NaCl (40 and 80 mM) in nutrient solution. Among others, salinity decreased fruit weight and increased total soluble solid (TSS) in *S. lycopersicum* but not in *S. chilense*. The fruit antioxidant capacity estimated by ferric reducing antioxidant power (FRAP) analysis was higher in *S. chilense* than in *S. lycopersicum* and increased in the former while it decreased in the latter in response to NaCl. Salinity increased the lycopene (LYC) content but decreased ß-carotene (b-CAR) concentration in the fruits of *S. lycopersicum*, while these compounds were not detected in the wild halophyte *S. chilense*. The oxidative status of salt-treated fruits was more tightly regulated in *S. chilense* than in *S. lycopersicum*. The two considered species, however, possess complementary properties and interspecific crosses may therefore be considered as a promising option for the improvement of salt-stress resistance in tomatoes.

Keywords: halophyte; lycopene; NaCl; salinity; tomato

1. Introduction

The tomato (*Solanum lycopersicum* L.) is cultivated throughout the world as a major horticultural crop and assumes key functions in human nutrition. Tomato fruit contains a wide range of health-promoting bioactive compounds, such as lycopene (LYC), β-carotene (b-CAR), total polyphenols (TP) or ascorbic acid (vitamin C) [1]. *Solanum lycopersicum* contains several carotenoids (CARs) in its

fruits and is the main source of LYC in the human diet. Antioxidant properties of LYC and β-carotene in relation to reactive oxygen species (ROS) quenching are likely the mechanism of action by which tomatoes prevent and reduce the incidence of chronic degenerative diseases. Besides CARs, numerous phenolic compounds also contribute to the health value of tomatoes, as well as improving attributes of functional and sensorial qualities [2–4].

Plant production has to face numerous environmental abiotic and biotic constraints in the world. Salinity is extremely toxic for most cultivated plant species and strongly limits crop production [5–7]. *Solanum lycopersicum* is considered to be highly salt-sensitive [8–10]. Numerous studies already demonstrated that high salinity reduces fruit yield in tomatoes in terms of fruit weight and number [10–14]. The mean fruit size is negatively correlated with the level of salinity, while fruit fresh to dry weight ratio increased in salt-treated plants [14]. High salinities cause broad modifications in fruit metabolism and physiology [15] and also lead to structural modifications [16]. Paradoxically, a moderate NaCl dose was reported to improve several fruit qualities, including dry matter percentage, total soluble sugars, titratable acidity, antioxidant capacity and volatile flavors in tomato fruit [3,17,18]. Farmers are thus prone to irrigate tomatoes with water containing low amounts of salt, which may lead to detrimental environmental effects on a long-term basis [6,19]. However, the effect of salinity on fruit quality in Cherry tomatoes showed that salinized fruits encountered an increase in ethylene synthesis, leading to higher sensitivity to mechanical stress, which limits their storage [20–22].

In the perspective of sustainable agriculture development, there is an urgent need to increase tomato resistance to salt stress and to reduce water use and fertilizers for agricultural production.

Most of the commercial tomato cultivars were selected considering yield-related parameters under optimal conditions, and important properties allowing the plant to cope with stress conditions were lost during the selection process. Landraces adapted to harsh environmental conditions or closely related wild relatives may be used as a valuable source of genes for breeding purposes [8,23]. It might also be hypothesized that wild relative halophyte may constitutively express fruit qualities that could be transferred to cultivated domesticated plants without the need to expose the plant to environmental disastrous salt treatment [7].

Chile comprises the main centers of origin of the tomato. In these areas, plant material exhibiting a high level of tolerance to various environmental constraints and a high water use efficiency may still be available. This is especially the case for halophyte plant species that could be crossed with the cultivated glycophyte *Solanum lycopersicum* [24]. *Solanum chilense* is a halophyte wild tomato spontaneously growing in the Northern part of Chile. It is present in the salt desert of the Atacama, one of the driest areas in the world. Populations of this species present in this area indeed exhibit a natural resistance to high level of salinity in the soil but also to drought and extreme temperatures occurring concomitantly [25,26]. Most studies dealing with salt-resistance in *S. chilense* consider short-term exposure at the seedling stage in order to identify the main physiological properties involved in the plant response [27–31]. Although Martínez et al. [12] reported that salt stress had no impact on the number of fruits per plant in *S. chilense*, data regarding the impact of NaCl on fruit qualitative attributes are still lacking for this species. The present study aims to analyze the long-term impact of moderate salinity on fruit quality and their antioxidant properties in the halophyte *S. chilense* and to compare its behavior with cultivated glycophyte *S. lycopersicum*.

2. Materials and Methods

2.1. Plant Growth Conditions and Stress Treatments

Seeds of *Solanum chilense* (accession LA4107) issued from Catarata-Taltal (Northern Chile) kindly provided by the INIA-Intihuasi (Experimental Center Vicuña) seed bank and seeds from the domesticated cultivated species (*Solanum lycopersicum* L. var. *cerasiforme* (Alef) Fosberg, cultivar Rubino Top) were sown on a peat substrate for initial germination. Seedlings were then grown in portable greenhouses in containers filled with a vermiculite–perlite substrate (3:1 *v/v*), watered with

half-strength modified Hoagland nutrient solution containing: 1 mM $NH_4H_2PO_4$, 5 mM KNO_3, 5.5 mM $Ca(NO_3)_2$, 0.5 mM $MgSO_4$, 10 μM H_3BO_3, 25 μM KCl, 1 μM $MnSO_4$, 1 μM $ZnSO_4$, 0.25μM $CuSO_4$, 10 μM Na_2MoO_4, and 1.87 mg L^{-1} Fe-EDDHA.

Three weeks after germination, the plants were transplanted to a greenhouse at the Regional Centre of INIA-La Cruz, Valparaíso Region (32°59′ LS; 71°10′ LW, 105 m.a.s.l.) in September (spring in the Southern hemisphere) setting 2.6 plants m^{-2}. Plants were grown in rockwool irrigated by nutrient solution with a pH and an electrical conductivity (EC) of 6.65 and 0.88 dS.m^{-1}, respectively. Seven days after transplanting (DAT), NaCl was added in order to obtain a final concentration of 0 mM (control treatment, CK) (0.88 dS m^{-1}), 40 mM (6 dS m^{-1}) or 80 mM (9 dS m^{-1}) NaCl. The EC and pH were recorded periodically with a handheld pH-EC meter (Hanna® Instruments, HI98130) and adjusted when required. The natural light intensity at the top of the canopy was estimated to be at least 300 μmol m^{-2} s^{-1} (photosynthetic photon flux density, PPFD) at 11 a.m. The air temperature ranged from 25 °C to 28 °C during the day, with an average of 18 °C during the night. The relative humidity was set at 60 ± 5% during the day and maintained at 80 ± 5% during the night. Clusters of fruits were collected at the ripening stage (stage "5–6" at the color scale, USA for *S. lycopersicum*, corresponding to 112 days of culture). Two independent experiments were performed during two consecutive years under similar environmental conditions.

2.2. Fruit Weight and Quality

Single fruit fresh weight (FFW), dry weight (FDW) and water content (WC) were estimated on 10 fruits per replicate according to Martínez et al. [31]. Fruit quality parameters were estimated from firm red-ripe fruits on centrifuged tomato juice extracted from three fruits per replicate per species and per treatment: pH was estimated with a laboratory pH-meter and total soluble solids (TSS) (refractometric index °Brix at 20 °C) using hand refractometer; titratable acidity (TA) was quantified by titration against 0.1 mM NaOH, according to Almasoum [32]. Another set of fruits was harvested, immediately frozen in liquid nitrogen and stored at −80 °C until use to measure antioxidant contents, antioxidant capacity and enzymatic activities (six replications per each treatment) as described below.

2.3. Proline and Malondialdehyde Content

For proline (PROL) quantification, fresh matter (c.a. 0.25 g) frozen in liquid nitrogen was homogenized in 10 mL (3%) sulfosalicylic acid and centrifuged at 10,000× *g* for 10 min. Then, 1 mL of glacial acetic acid was added to 500 μL of the supernatant. Acid ninhydrin reagent was prepared by dissolving 2.5 g ninhydrin (Merck KGaA, Darmstadt, Germany) in a mixture of 40 mL 6 M phosphoric acid and 60 mL glacial acetic acid. One mL of this ninhyrin reagent was added to the sample which was then incubated at 100 °C for 1 h. Samples were then transferred to an ice bath in order to stop the reaction. The chromophore was extracted with 2 mL of toluene and warmed at room temperature. Absorbance was read at 520 nm using toluene as a blank with a Shimadzu UV-1800 spectrophotometer and converted using a standard curve performed with L-proline (Merck KGaA, Darmstadt, Germany) [33].

Malondialdehyde (MDA) is a by-product of lipid peroxidation and is frequently used as an indicator of oxidative stress. Samples (c.a. 0.25 g of frozen fresh matter) were ground in 5 mL of trichloroacetic acid (5% *w/v*). Samples were centrifuged at 12,000× *g* for 15 min at 4 °C and two mL of supernatant were collected and reacted with two mL of thiobarbituric acid (0.67% *w/v*) at 100 °C for 30 min. After cooling, the absorbance was read at 532 nm and the results were corrected by subtracting the non-specific absorbance read at 600 nm [34]. The concentration of MDA (nmol g^{-1} DW) was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}.

2.4. Antioxidant Capacity

The antioxidant capacity of fruit was measured by two methods: (i) ferric reducing antioxidant power (FRAP) and (ii) 2,2-diphenyl-1-picrylhydrazyl (DPPH) procedures.

The FRAP assay was performed according to Benzie and Strain [35]. Two grams of sample stored at −80 °C were powdered in liquid nitrogen in the presence of 12 mL of 80% methanol and centrifuged at 300× g during 60 min. After filtration, 100 μL of the test sample were added to 300 μL of distilled water and to 3 mL of FRAP reagent (10 mM 2,4,6-tripyridyl triazine (TPTZ) and 20 mM ferric chloride in 0.25 M acetate buffer, pH 3.6). After 5 min incubation at room temperature, absorbance was read at 593 nm with a UV/Vis spectrophotometer (Perkin Elmer, Lambda 25, Beaconsfield, Bucks, UK). Standard curve was established using vitamin C; FRAP values were expressed as mmole $FeSO_4$ per g of fresh weight (FW).

The DPPH assay was performed using a modified colorimetric method proposed by Brand-Williams et al. [36]. Briefly, 100 μL of 80% methanol extract were mixed with 3.9 mL of 103.5 μM solution of DPPH radical in methanol and incubated for 60 min at room temperature in the dark. The reduction of the DPPH radical was estimated by absorbance measurement at 517 nm. The scavenging activity of the DPPH radical was determined using Trolox as a standard. The results were expressed as IC50, mg mL^{-1}. The IC50 was determined by dilution curve of each sample.

2.5. Non-Enzymatic Antioxidant Content

Total phenolic compounds (TP) were determined according to Singleton and Rossi [37] with slight modifications [38]: 100 μL of 80% methanol extract was mixed with 50 μL Folin-Ciocalteu reagent. After 5 min. incubation, 700 μL distilled water and 150 μL of sodium carbonate (20% *w/v*) were added. Samples were then incubated in the dark for 30 min at room temperature and 4.5 mL distilled water were added to 0.5 mL mixture. The absorbance was read at 760 nm. Standard curve was established with a gallic acid solution (Merck KGaA, Darmstadt, Germany). The total phenolics content was reported as μmoles of gallic acid equivalents per g FW. All analyses were run in triplicate.

Lycopene (LYC) and β-carotene (b-CAR) were extracted with ethanol 96% at −20 °C. Samples were centrifuged at 5000× g for 5 min at 4 °C. LYC and b-CAR contents were determined by high-performance liquid chromatography (HPLC) stationary reversed phase [39]. Samples were injected in a C18 column (μBondapack C18 (300 mm × 2 mm), 10 μm of pore size with methanol/acetonitrile (90:10 *v/v*) + triethylamine 9 μM as a mobile phase); flux was fixed at 0.9 mL min^{-1}; the column temperature was fixed at 30 °C and the absorbance was read at 475 nm by a UV-vis detector (Themo Separation Spectra Series) [40].

Glutathione (GSH) was quantified as indicated by Griffith [41] through oxidation by 5,50-dithiobis 2-nitrobenzoic acid (DTNB) and reduction by NADPH in the presence of glutathione reductase. Tissue (c.a. 1 g FW) were ground in a prechilled mortar and pestle in the presence 4 mL 5% sulphosalicylic acid and centrifuged at 10,000× g for 10 min. A 330 μL aliquot was removed and neutralized by addition of 18 μL 7.5 M triethanolamine. Two aliquots were considered, one to determine concentrations of total glutathione (GSH + GSSG) and another to quantify GSSG alone after pre-treatment with 3 mL 2-vinylpyridine for 60 min at 20 °C to mask the GSH by derivatization. In both cases, 50 μL aliquots were mixed with 100 μL DTNB, 700 μL 0.3 mM NADPH and 150 μL buffer containing 6.3 mM ethylenediaminetetraacetic acid (EDTA) in 125 mM sodium phosphate at pH 6.5. A 10 μL aliquot of glutathione reductase (GR) (EC 1.6.4.2; 5 U. mL^{-1}; 1 unit reduces 1 mmol oxidized GSH min^{-1} at pH 7.6 at 25 °C) was then added, and the change in absorbance at 412 nm was monitored at 30 °C.

Ascorbic acid (vitamin C) was extracted from frozen tissues in cold 5% metaphosphoric acid solution (1:5, *w/v*) and then centrifuged at 20,000× g and 4 °C for 10 min. Total reduced (AsA) and oxidized ascorbate (DHA) contents were determined according to Wang et al. [42] considering Fe^{3+}–Fe^{2+} reduction by ascorbate in acid solution. Ascorbate assay mixture contained 100 μL of the extract, 0.6 M trichloroacetic acid, 500 μL of absolute ethanol, 8 mM H_3PO_4, 3 mM bathophenantroline and 0.17 mM $FeCl_3$. The samples were then allowed to stand at 30 °C for 90 min. The absorbance was read at 534 nm. The total ascorbate assay mixture contained 100 μL of the sample, 150 μL of 3.89 mM dithiothreitol, and 350 μL of absolute ethanol in a total volume of 0.6 mL. During 10 min incubation,

dehydroascorbate was reduced to ascorbate. Then, 0.15 mL of 20% trichloroacetic acid was added. Addition of 300 μL of 0.5% (*w/v*) bathophenantroline–ethanol, 150 μL of 0.4% (*v/v*) H$_3$PO$_4$–ethanol, and 150 μL of 0.03% (*w/v*) FeCl$_3$–ethanol was used to develop the color. The difference of total ascorbic acid (AsAT) and AsA concentration allowed us to quantify DHA concentrations. Standard curve in the range 0–10 mmol AsA or DHA was used.

2.6. Ascorbate Peroxidase and Superoxide Dismutase Activities

Fruit tissue samples were ground on ice in the presence of 50 mM potassium phosphate buffer (pH 7; buffer volume 8:1) with 1 M NaCl, 1 mM EDTA, 1% polyvynilpyrrolidone (*w/v*), 1 mM ascorbate and 5% glycerol (*v/v*). After centrifugation at 10,000× *g* for 15 min, supernatant was used for estimation of the ascorbate peroxidase activity (APX; EC 1.11.1.11) considering ascorbate oxidation at 265 nm (ε = 13.7 mM^{-1} cm^{-1}). The reaction mixture contained 60 mM potassium phosphate buffer (pH 7), 100 mM ascorbate, 100 mM H$_2$O$_2$ and 50 mL of the enzyme extract in a total volume of 750 μL. The absorbance decrease was recorded 10–60 s after addition of H$_2$O$_2$. Corrections were afforded for the oxidation of ascorbate in the absence of H$_2$O$_2$ and the non-enzymatic oxidation of ascorbate by H$_2$O$_2$. Enzyme activity was expressed in mmol AsA min^{-1} mg^{-1} protein.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed according to Gianno-Politis and Ries [43]. The method follows the inhibition of photochemical reduction of nitroblue tetrazolium (NBT). Tissues (c.a. 0.5 g) were homogenized in a pre-chilled mortar and pestle in 5 mL of an ice-cold phosphate buffer (50 mM, pH 7) containing 1% PVP. The homogenates were centrifuged at 15,000× *g* for 30 min at 4 °C. The supernatants were used for the quantification of SOD activity in a reaction medium consisting in 50 mM phosphate buffer (pH 7.8), 75 mM NBT, 0.1 mM EDTA, 2 mM riboflavin 13 mM methionine. Reactions with increasing volume of extract (from 0 to 100 μL) were carried out at 25 °C during 20 min under a light intensity of 90 μmol m^{-2} s^{-1}. The reduction of NBT was monitored at 560 nm. One unit of SOD is defined according to Asada et al. [44]: *v/v* = 1 + K0 [SOD], where *V* and *v* represent the rate of the assay reaction in the absence and the presence of SOD, respectively. One unit of SOD is defined as the amount of enzyme for which K0 [SOD] equals unity and thus *v/v* = 2.

Protein (PROT) concentrations were determined according to Bradford [45].

2.7. Statistical Analysis

Data were analyzed using a two-way analysis of variance (ANOVA) at a significance level of $p \leq 0.05$. The model was defined as a split-plot design with fixed effects and hierarchical classification criterion. The main factors are the species and NaCl doses, and their interaction was also considered. When the ANOVA was significant at $p \leq 0.05$, Duncan Multiple Range Test was used for comparison of means. All of the analyses were conducted in R version 3.0.2 (RDevelopment, 2012). We also performed principal component analysis (PCA) using the 'FactoMineR' package, to compare the antioxidant activities of the cherry and wild tomatoes, as well as the fruit parameters. Visitation rates were added as a supplemental variable in the PCA, to illustrate the relationships with the antioxidant activities and fruit quality parameters. The experiment was performed twice, and similar results were obtained. Data presented hereafter are therefore from one representative experiment.

3. Results

3.1. Fruit Weight and Quality

The mean individual fresh (FFW) and dry weight (FDW) of the fruits of the first bunch of *S. lycopersicum* were significantly affected by salt stress (Figure 1A,B). Although mean values for these parameters were lower in *S. chilense* than in *S. lycopersicum*, they remained unaffected by salt stress in the wild species. Fruit numbers were not affected by salt stress (detailed data not shown).
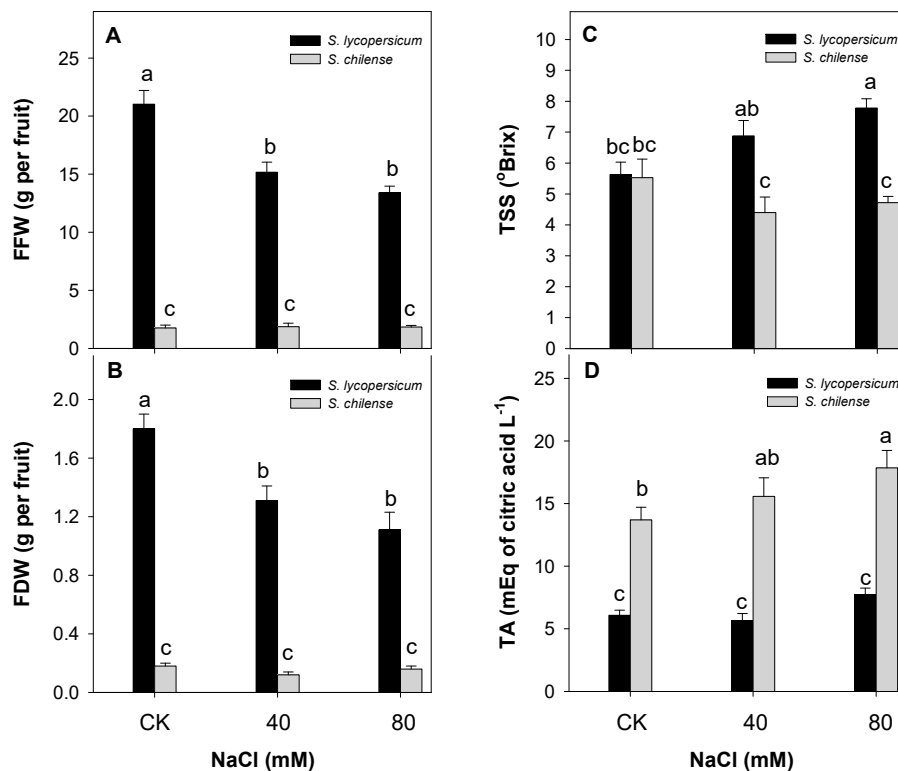
Figure 1. Fruit fresh weight (FFW; (**A**)), fruit dry weight (FDW; (**B**)) (g per fruit), total soluble solid (TSS, °Brix; (**C**)) and titratable acidity (TA, mEq of citric acid L^{-1}; (**D**)) in two species of tomato (domesticated *Solanum lycopersicum* and wild-relative *Solanum chilense*) cultivated for 112 days in nutrient solution containing 0 (control treatment, CK), 40 or 80 mM NaCl. Each value represents the mean ± SE (*n* = 6). Lower-case letters indicate differences (*p* ≤ 0.05, Duncan test) between treatments and species.

The fruits of *S. lycopersicum* showed a significant increase in TSS (Figure 1C) in response to NaCl; however, no impact was recorded in *S. chilense* in response to salt. Titratable acidity values (Figure 1D) were higher in *S. chilense* than in *S. lycopersicum* and increased in response to salinity in the wild species. As a consequence, the TSS/TA ratio was higher in *S. lycopersicum* than in *S. chilense*.

In control (0 mM NaCl) and 40 mM NaCl-treated plant, the fruit protein content was slightly higher in *S. chilense* than in *S. lycopersicum* (Table 1), although the difference was not significant. Proline concentration strongly increased in the fruits of *S. lycopersicum* in response to NaCl while the recorded increase in *S. chilense* was significantly lower. The MDA concentration was higher in *S. lycopersicum* than in *S. chilense*, although the difference was significant for control plants only. MDA did not increase in response to salinity in the domesticated species while it was significantly higher in the presence of NaCl than in controls in *S. chilense*.

Table 1. Protein (PROT; mg/g FW), proline (PROL; μmol/g FW) and malondialdehyde (MDA; in nmol/g FW) contents in fruits of two tomato species (cultivated Cherry tomato *Solanum lycopersicum*, and halophyte wild-relative *S. chilense*) cultivated in the presence of 0 (CK), 40 or 80 mM NaCl. Each value represents mean ± SE (*n* = 6).

| Parameter | *Solanum lycopersicum* | | | *Solanum chilense* | | |
| | NaCl (mM) | | | NaCl (mM) | | |
	CK	40	80	0	40	80
PROT (mg g^{-1} FW)	0.75 ± 0.04 a [1]	0.92 ± 0.21 a	1.15 ± 0.12 a	1.16 ± 0.07 a	1.25 ± 0.13 a	0.96 ± 0.21 a
PROL (μmol g^{-1} FW)	16.9 ± 2.93 bc	34.98 ± 4.90 a	34.46 ± 4.00 a	12.57 ± 3.74 c	20.85 ± 3.92 b	14.73 ± 4.50 c
MDA (nmol g^{-1} FW)	13.25 ± 1.48 a	13.78 ± 1.00 a	12.91 ± 2.31 a	8.57 ± 1.11 b	12.69 ± 1.95 a	11.36 ± 1.85 a

[1] For a given parameter, different lower case letters indicate differences (*p* ≤ 0.05, Duncan test) between treatments.

3.2. Antioxidant Capacity of Fruit

Solanum chilense showed higher FRAP values than *S. lycopersicum* in the presence of NaCl (Figure 2A). Salinity had a detrimental impact on FRAP index in *S. lycopersicum* ($p \leq 0.05$) while plants of *S. chilense* showed an obvious increase at the intermediate NaCl dose of 40 mM ($p \leq 0.05$). Salinity had no impact on DPPH, whatever the considered plant species and it remained similar in the two plant species (Figure 2B).
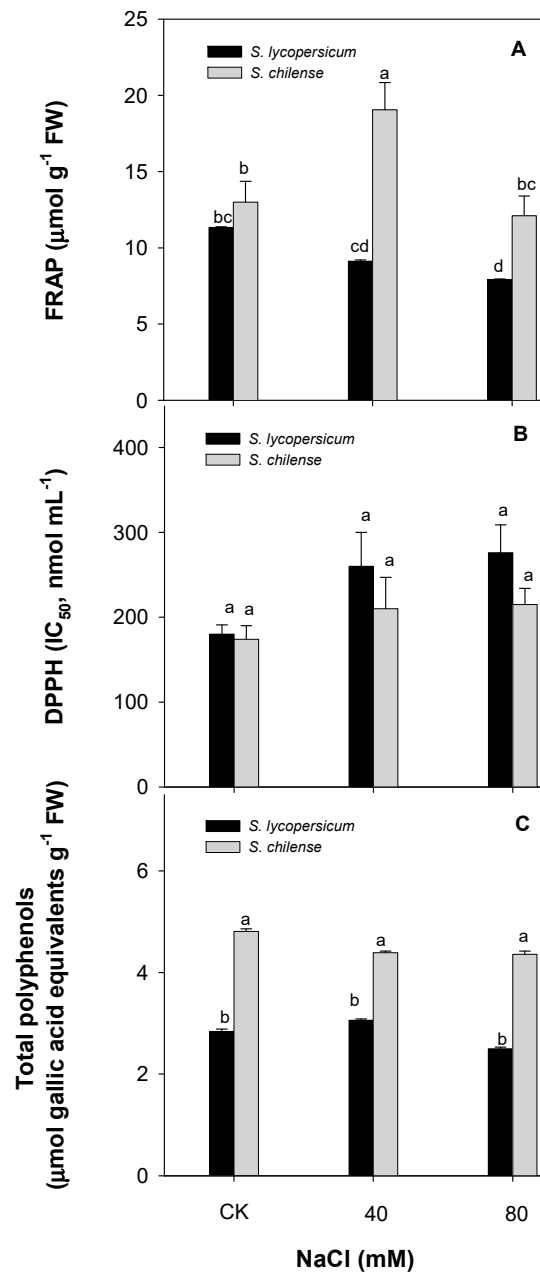

Figure 2. Fruit ferric reducing antioxidant power (FRAP) ($\mu mol\ g^{-1}$ FW; (**A**)) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (nmol g^{-1} FW; (**B**)) indexes and fruit total polyphenol content (TP) (μmol gallic acid equivalents g^{-1} FW; (**C**)) in two species of tomato (domesticated *Solanum lycopersicum* and wild-relative *S. chilense*) cultivated for 112 days in nutrient solution containing 0 (control treatment, CK), 40 or 80 mM NaCl. Each value represents the mean ± SE ($n = 6$). Lower-case letters indicate differences ($p \leq 0.05$, Duncan test) between treatments and species.

As shown in Figure 2C, the TP values were always higher in the fruits of the wild species than in *S. lycopersicum* and this parameter remained unaffected by salt stress in the two species. Salinity significantly increased LYC content in *S. lycopersicum* ($p \leq 0.05$) (Figure 3A) while it decreased b-CAR (Figure 3B). These two pigments were not detected in *S. chilense*, whatever the salt treatment.
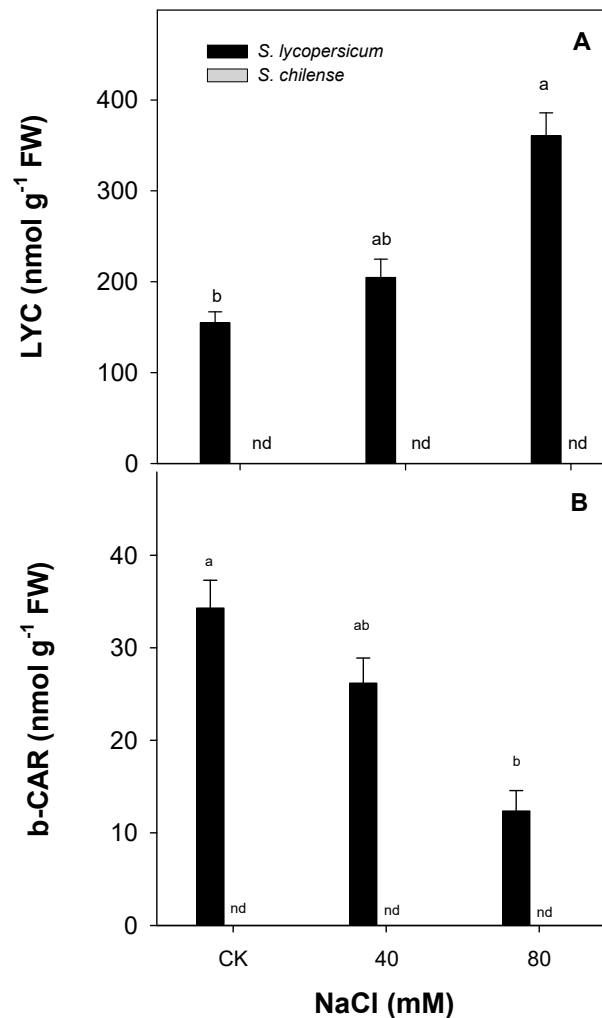

Figure 3. Fruit lycopene (LYC) (nmol g^{-1} FW; (**A**)) and β-carotene (b-CAR) content (nmol g^{-1} FW; (**B**)) in two species of tomato (domesticated *Solanum lycopersicum* and wild-relative *S. chilense*) cultivated for 112 days in nutrient solution containing 0 (control treatment, CK), 40 or 80 mM NaCl. Each value represents the mean ± SE ($n = 6$). Lower-case letters indicate differences ($p \leq 0.05$, Duncan test) between treatments and species.

The GSH and GSSG contents were lower in *S. lycopersicum* than in the wild genotype under control treatment (0 mM NaCl) (Table 2). Salinity had no impact on GSH contents in *S. lycopersicum*, while it induced a decrease in the wild species *S. chilense* ($p \leq 0.05$; Table 2). The oxidized form GSSG was not affected by salinity, and it was higher in *S. chilense* than in *S. lycopersicum* in the absence of salt; it however decreased at the highest dose (80 mM NaCl) in *S. chilense*. As an overall consequence, total glutathione (GSH + 2 × GSSG) was more than two times higher in control plants of the wild species *S. chilense* than in the domesticated plant *S. lycopersicum* (2886 nmol g^{-1} FW versus 1341 nmol g^{-1} FW, respectively), but the difference between the two species were reduced in salt-treated plants and was not significant for both doses of salinity. The ratio of GSSG to (GSH + GSSG) was not significantly affected by NaCl stress in both tomato species (Table 2).

Table 2. Antioxidants (reduced (GSH) and oxidized glutathione (GSSG)) in nmol g^{-1} FW; ascorbate (AsA) and dehydroascorbate (DHA) in nmol g^{-1} FW in fruits of two tomato species (cultivated Cherry tomato *Solanum lycopersicum*, and halophyte wild-relative *S. chilense*) under saline stress conditions at the end of experimental period (112 days). Each value represents mean ± SE (*n* = 6).

Treatment (NaCl, mM)	Specie	GSH	GSSG	GSSG/(GSH + GSSG)	AsA	DHA	DHA/(DHA + AsA)
CK	*S. lycopersicum*	675 ± 111 b	333 ± 38 b	0.34 ± 0.03 a	190 ± 32 e	609 ± 44 a	0.76 ± 0.04 a
	S. chilense	1520 ± 421 a [1]	683 ± 173 a	0.35 ± 0.05 a	680± 83 ab	729 ± 169 a	0.50 ± 0.07 c
40	*S. lycopersicum*	586 ± 62 b	322 ± 66 b	0.33± 0.05 a	343 ± 39 d	863 ± 86 a	0.72 ± 0.01 a
	S. chilense	892 ± 217 ab	543 ± 110 ab	0.38 ± 0.05 a	506 ± 24 c	1085 ± 158 a	0.67 ± 0.03 ab
80	*S. lycopersicum*	601 ± 145 b	360 ± 103 ab	0.30 ± 0.04 a	638 ± 83 bc	795 ± 193 a	0.54 ± 0.05 bc
	S. chilense	702 ± 143 b	259 ± 41 b	0.28 ± 0.01 a	799 ± 42 a	649 ± 197 a	0.42 ± 0.06 c

[1] Lower case letters indicate differences (*p* ≤ 0.05, Duncan test) between treatments for a given compound or a given ratio.

AsA contents were significantly higher in the wild genotype *S. chilense* than in *S. lycopersicum* (Table 2). The highest AsA value was recorded for *S. chilense* exposed to the highest salinity (80 mM NaCl). In *S. lycopersicum*, AsA contents increased in response to salt stress, while in *S. chilense* AsA contents decreased up to 40 mM NaCl and then strongly increased at the highest dose ($p \leq 0.05$; Table 2). Dehydroascorbate (DHA) remained constant and similar in the two species, except for *S. chilense*, which exhibited a higher DHA content in response to 40 mM NaCl. The ratio DHA/(DHA + AsA) decreased in both species at the highest NaCl dose.

Salt stress strongly decreased APX activity at the highest dose in *S. lycopersicum* ($p \leq 0.05$; Figure 3A) but had no impact on this parameter in *S. chilense* (Figure 4A). Salinity had no impact on total SOD activity in both species which remained similar in *S. lycopersicum* and *S. chilense* (Figure 4B).
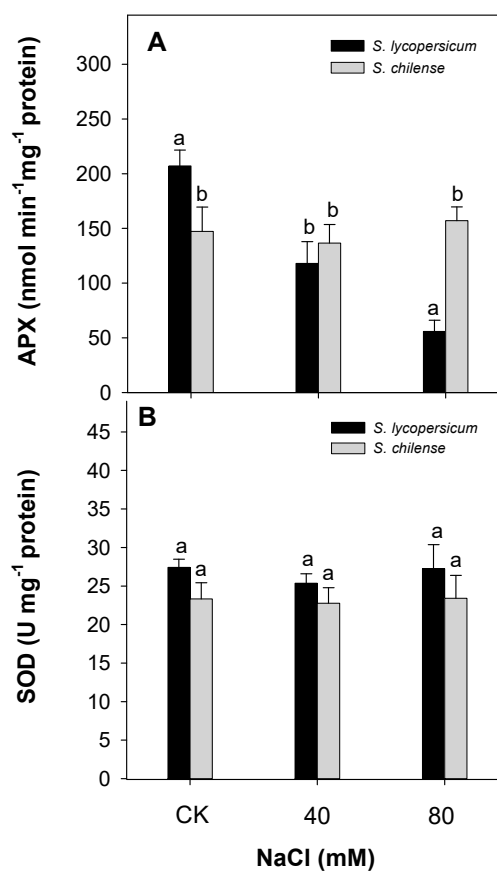

Figure 4. Ascorbate peroxidase activity (APX) (nmol min^{-1} mg^{-1} protein; (**A**)) and superoxide dismutase (SOD) (U mg^{-1} protein; (**B**)) enzymatic activities in two species of tomato (cultivated *Solanum lycopersicum* and wild-relative *S. chilense*) cultivated for 112 days in nutrient solution containing 0 (control treatment, CK), 40 or 80 mM NaCl. Each value represents the mean ± SE ($n = 6$). Lower-case letters indicate differences ($p \leq 0.05$, Duncan test) between treatments and species.

3.3. PCA Analysis of the Antioxidant Capacity and Quality Fruit at the Harvest

PCA was performed with the aim to investigate whether the bioactive compounds can explain the antioxidant capacity and fruit quality at harvest in the two studied species. Principal component **1** (PC1) represented 29.68% of the variance (Figure 5A). This was mainly explained by the fruit quality (FFW, FDW, TSS/TA, TSS, PROL and TA) and the antioxidant capacity (LYC, b-CAR, FRAP and PT). PC2 accounted for 13.62% of the variance, and this showed a higher variability of the GSHT and AsAT contents in *S. chilense* than *S. lycopersicum* (Figure 5A). PCA analysis showed that the bioactive compounds, antioxidant capacity, fruit quality at the harvest and tomato species clustered separately

(Figure 5B). This strengthens the hypothesis that *S. chilense* may interestingly be used in interspecific crosses with *S. lycopersicum* in order to improve salt stress resistance at the fruit maturation stage.
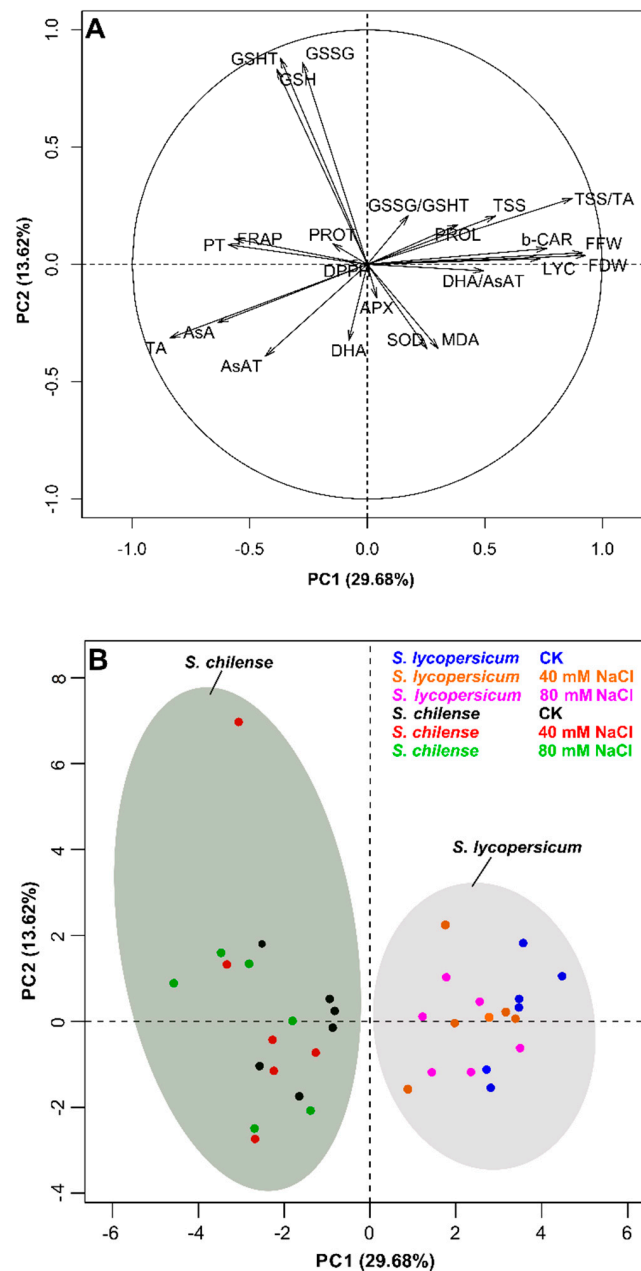

Figure 5. Principal components analysis (PCA) of the bioactive compounds, capacity antioxidant and fruit quality at the harvest for cultivated *Solanum lycopersicum* and wild-relative *S. chilense* under control (CK) and saline conditions (**A**,**B**). Individual graphs showing the mean values per each species. Abbreviations are explained as follows: APX: Ascorbate peroxidase; AsA: Total reduced ascorbate content; AsAT: Total ascorbic acid content; b-CAR: β-carotene; DHA: Total oxidized ascorbate content; DPPH: 2,2-diphenyl-1-picrylhydrazyl antioxidant power; FRAP: Ferric reducing antioxidant power; FDW: Fruit dry weight; FFW: Fruit fresh weight; GSH: Total reduced glutathione content; GSHT: Total glutation content; GSSG: Total oxidized glutathione content; LYC: Lycopene; MDA: Malondialdehyde; PROL: proline content; PROT: protein content; SOD: Superoxide dismutase; TA: Titratable acidity; TP: Total phenolic compounds; TSS: Total soluble solids; DHA/AsaT: Arcorbate oxidation state; GSSG/GSHT: Glutation oxidation state; and TSS/TA: Sweetness and acidity ratio).

4. Discussion

Taken together, our data provide evidence that *S. chilense* has a higher capacity to cope with salt stress at the fruit maturation stage, compared to the cultivated tomato *S. lycopersicum*. Indeed, numerous fruit-related parameters remained unaffected in the wild species but were clearly modified by NaCl in *S. lycopersicum*. This supports the hypothesis that *S. chilense* exhibit constitutive properties allowing the plant to ensure fruit maturation in the presence of NaCl, and that it might consequently behave as a true halophyte. This is confirmed by the fact that the wild species exhibit a higher FRAP capacity and total polyphenol (TP) in both the presence and absence of NaCl. The constitutive presence of a high antioxidant status should indeed allow the plant to quickly react to a rise of salinity in halophytes, while triggering similar process will require time for signal transduction and gene expression and will require investing metabolic energy in glycophytes [5,7].

The high FRAP antioxidant capacity of *S. chilense* was associated with a higher TP concentration and titratable acidity, AsA and GSH contents compared to *S. lycopersicum*. Acting as major antioxidants, phenolic compounds (including polyphenols) are valuable health-promoting molecules and their high and stable concentration in *S. chilense* could be regarded as an interesting property. Sumalan et al. [46] recently characterized twenty halotolerant tomato landraces and confirmed that the highest values for total phenolics, AsA and total antioxidant activity were found in the most salt-stress resistant landraces. The values we recorded for total phenolics and AsA concentrations were clearly higher in *S. chilense* than in the salt-stress resistant material identified by Sumalan et al. [46].

In addition, it is well known that LYC is also a valuable antioxidant, and tomato fruit is the primary source of LYC in the human diet [47]. According to our results, the LYC content was not associated with evaluated antioxidant capacities (FRAP and DPPH) if we consider the two species, mainly because LYC was undetected in *S. chilense*. However, as far as *S. lycopersicum* is concerned, salt-induced increase in LYC content should be considered as a positive impact of NaCl on fruit quality. LYC is the precursor of b-CAR, which was sharply reduced by salt stress in *S. lycopersicum*, suggesting that NaCl inhibited lycopene-b-cyclase, thus explaining a concomitant increase in LYC and decrease in b-CAR. From a quantitative point of view, however, LYC accumulation was higher than b-CAR decrease, and a salt-induced increase in the precursor phytoene could thus not be ruled out. In contrast, neither LYC nor b-CAR were detected in *S. chilense*, suggesting that modalities of ripening clearly differ in the two species. ß-carotene is a precursor of zeaxanthin which is itself a precursor of abscisic acid. Our previous study nevertheless demonstrated that *S. chilense* produces normal amounts of this phytohormone, which could assume important functions in response to abiotic stress [28,29]. Specifically, Gharbi et al. [29] showed that NaCl-induced ethylene accumulation in *S. chilense* occurred concomitantly with an increase in stomatal conductance, osmotic adjustment and the maintenance of carbon isotope discrimination value (Δ13C). These physiological responses suggest that ethylene plays a key role in salt tolerance of this species [28,29]. As a consequence, there is no reason to claim that LYC and b-CAR are not synthesized in *S. chilense*, but the absence of accumulation probably results from specific modalities of their catabolism in this species.

Ascorbate (vitamin C) is also of paramount importance in the human diet, not only because of its role as an antioxidant but also through its positive effect on nutritional iron availability [1,11,42,48]. Since the human body is unable to synthesize AsA, it must be provided by edible fruit. A constitutively higher amount of AsA in *S. chilense* comparatively to *S. lycopersicum* is of particular interest. It may partly explain the higher antioxidant capacity of the wild species indicated by the FRAP index. Ascorbate accumulation in fruits is frequently recorded under high irradiance treatment [48]. This finding suggests that ascorbate accumulation may be one of the protecting mechanisms developed by *S. chilense* against light-induced oxidative damages under extremely high sun intensities occurring in its natural habitat in the Atacama desert. It has been recently established that a low ascorbate oxidase activity may contribute to AsA accumulation in RNAi silenced oxidase lines of cherry tomato plants and that this transgenic material exhibits a higher salt-stress resistance [49]. It may therefore be interesting to quantify ascorbate oxidase activity in *S. chilense*. The other compound that participates in

the antioxidant metabolic cycle (glutathione-ascorbate) is GSH. In *S. chilense*, this antioxidant compound showed higher contents than in *S. lycopersicum* in absence of NaCl in the control solution (CK), but no differences were observed between these two species under stress conditions. The decrease in GSH contents could be due to an inhibition of the synthesis of this compound by a negative effect of the enzymatic activity of glutathione reductase (GR), an enzyme that is highly dependent on the availability of NAD(P)H and fulfills the function of maintaining the reduced pool of GSH in the cell [50]. Similar responses were reported by Zushi and Matsuzee [51] in different varieties of *S. lycopercicum*.

Proline (PROL) is also considered to act as an antioxidant contributing to ROS quenching beside enzymatic antioxidants such as SOD and APX. The lower proline concentration recorded in the wild species and the maintenance of SOD activity suggest that *S. chilense* has distinct and still unknown mechanisms to cope with salinity. At the enzymatic level, a decrease in APX despite the maintenance of SOD activities would lead to H_2O_2 accumulation in *S. lycopersicum*. Beside ethylene, H_2O_2 is an important compound involved in the fruit ripening process, initiating chlorophyll degradation by inhibiting the recovery of b-CAR that protect it [52]. In salt-treated plants of *S. lycopersicum*, however, we did not record any hastening of the maturation and ripening phases, and the H_2O_2 resulting from SOD activity and APX inhibition could thus be regarded as a symptom of toxicity. Murshed et al. [53] and Martínez et al. [31] reported an increase in APX enzyme activity under high salinity conditions in *S. lycopersicum* fruits and *S. chilense* leaves, respectively. As it is known, this increase is associated with a higher tolerance to oxidative stress caused by salinity. On the contrary, our results showed that the variety *S. lycopersicum* would be sensitive to salinity due to the reduction observed in its enzymatic activity. This same response was also reported by Martínez et al. [31] in leaves of the same variety. In relation to the enzymatic activity of SOD, Murshed et al. [53] did not observe effects of saline stress at fruit level in the tomato cultivar Micro-tom. On the other hand, Martínez et al. [31] observed that SOD in leaves (vegetative state) of *S. lycopersicum* and *S. chilense* did not show changes due to salinity effect at medium salinity levels (80 mM NaCl). However, a strong increase of SOD enzymatic activity in *S. chilense* was reported at significantly high salinity levels (160 mM NaCl). Compared to species of the same family (*Solanaceae*), Azuma et al. [54] reported reductions in SOD enzymatic activity in leaves and fruits of pepper plants under saline stress from 50 mM NaCl, with the highest reduction at fruit level. These phenomena would suggest the existence of a threshold from which the enzymatic activity associated with SOD in *S. chilense* would change the trend.

The maintenance of both APX and SOD activities in *S. chilense* confirms that the fruit oxidative status is more tightly regulated in the wild halophyte than in the cultivated glycophyte. This response suggests, once again, that this species is constitutively well-adapted to salinity.

From the perspective of human nutrition, salt effects on the fruit of *S. lycopersicum* should not necessarily be regarded as unfavorable: TSS is a good indicator of total soluble sugar, and an increase in TSS is itself an indicator of salt-induced improvement of fruit quality, as reported elsewhere [3,12,17,18,55]. Martínez et al. [12] observed that salt stress induced a decrease in the fruit osmotic potential of *S. lycopersicum* var. *cerasiforme*, which may explain why TTS were incremented in this species, indicating a possible solute accumulation for osmotic adjustment without driving cell expansion, as reported during fruit growth of unstressed plants [46]. It has been recently demonstrated that modification of the sugar profile in fruits of salt-treated tomatoes is directly related to protein N-glycosylation reprogramming and has a significant effect on fruit taste and firmness [56]. A higher-level of TA in the salt-resistant *S. chilense* could be regarded as a tolerance mechanism that may be related to stimulation of the Krebs cycle, allowing the production of additional energy required to cope with saline stress [47,57]. It is now well established that manipulation of central organic acids is a promising approach to improve tomato fruit yield. An increase in organic acids contributes to cell elongation during the elongation phase while accumulation of citric and malic acid plays a key role during ripening phase and may provide sugar through neoglucogenesis [47,53]. Increasing titratable acidity under salt-stress conditions is a major goal for tomato breeders, even if this parameter is strongly influenced by the type of culture system irrigated by saline water [53]. *Solanum chilense* may

thus constitute an interesting material providing key genes for improvement of this specific property in cultivated tomatoes.

5. Conclusions

The cultivated tomato species *S. lycopersicum* and its wild relative *S. chilense* display contrasting fruit behavior in response to salinity. Fruit growth was impaired in *S. lycopersicum*, but some qualitative traits were improved, such as TSS, TA and LYC content. Fruits of the cultivated species were affected in terms of antioxidative status, as indicated by a salt-induced decrease in FRAP index and APX activity. In contrast, fruit growth was not affected by salt-stress in *S. chilense*, and high antioxidant capacity was related to a constitutive higher concentration of ascorbate. Fruits of *S. chilense*, however, appeared unable to accumulate LYC and b-CAR. It is concluded that *S. lycopersicum* and *S. chilense* exhibit complementary behaviors for quantitative and qualitative fruit parameters in salt treatment and that interspecific crosses may be considered as a promising option for the improvement of salt resistance in tomatoes.

Conflicts of Interest: The authors declare no conflict of interest.

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