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TOMATO GRAFTED AND CULTIVATED IN SALINE MEDIUM AND ITS RELATION ON NUTRACEUTICAL COMPOUNDS OF THE FRUITS

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Abstract

The fruit of tomato is one of the highly consumed vegetables in the world, because of its increasing nutraceutical quality and positive effects on the health of the consumer. The objective of this research was to increase the content of antioxidants in tomato fruits, without significantly affecting yields, inducing abiotic stress in grafted and ungrafted tomato plants, subjecting to four different concentrations of salt (NaCl). The use of grafts increased the lycopene content and the activity of the enzyme ascorbate peroxidase (APx). The concentrations of 50, 75 and 100 mM de NaCl increased the content of vitamina C and the activity of superoxide dismutase (SOD) and APx. However, they reduced the lycopene content and the yields of the culture. The fruits obtained of the grafted plants subjected to 100 mM NaCl showed the highest amount of total phenols. The saline-free treatments had higher lycopene content, while the activity of SOD was increased with the graft and 100 mM NaCl. The maximum yields were obtained in grafted plants without salt stress.

Key words: Grafting, Antioxidant enzymes, *Solanum lycopersicum*.

Introduction

Tomato is the most fruit consumed vegetables around the world (Bashir *et al.*, 2017), whose source of antioxidants is mainly lycopene, in addition to ascorbic acid and phenolic compounds, which have positive effects on human health (Giovanelli & Paradise, 2002), because they are associated with the reduction of the incidence of cardiovascular diseases (Riboli & Norat, 2003).

The antioxidants play an important role in the sequestration of oxidizing agents (Mittler *et al*., 2004), produced in plants constantly in response to various metabolic pathways that are carried out in different cellular organelles mainly in chloroplasts, mitochondria, and peroxisomes (Navrot *et al.*, 2007) that are controlled by various antioxidant mechanisms to mitigate oxidative damage to lipids, DNA and DNA proteins (Chu *et al.*, 2016). Some of the first defensive responses against free radicals are enzymatic, such as the activation of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), and non-enzymatic antioxidants, which are phenolic compounds, which intervene in the speed to neutralize molecules of free radicals or molecules that induce the production of other free radicals (Ighodaro & Akinloye, 2017).

The production and increase of reactive oxygen species (ROS) are triggered by different conditions that generate biotic or abiotic stress (Foyer *et al.*, 2017). To protect themselves from oxidative damage, plants must ensure that their antioxidant systems are highly efficient at producing both non-enzymatic and enzymatic antioxidants balanced their activity (Khan & Panda, 2008). High salinity significantly restricts the harvests of crops (Queiros *et al*., 2011) by inducing osmotic stress, nutritional inequity, and ion toxicity (Cambrollé *et al*., 2011). Under these conditions at the cellular level ROS are continuously generated as a result of metabolism of the plants (Tester & Davenport, 2003). Plants are obligated to adapt to these suboptimal environmental conditions by making changes to their physiological, molecular, and metabolic functions to activate their antioxidant systems and protect themselves from harm (Apel & Hir, 2004).

Grafting is a technique that has been used as an alternative production system for crops under different stress conditions. Initially, the use of the graft mitigates the damage of soil pathogens (Ke & Saltveit, 1988), but recently it is used to improve the absorption of nutrients (Ahmedi *et al*., 2007) and the tolerance to cold and warm temperatures (Zhou *et al.*, 2007).

It has also been suggested that grafting helps in improving salt tolerance by reducing ionic stress and increasing water absorption (Flores *et al*., 2010). The objective of the study was to induce modifications in the yield and antioxidant systems of tomato fruits obtained by grafting and grown in high salt conditions.

Methods and Materials

Location of the experiment: The research was conducted in a curved roof greenhouse (40-70% relative humidity) of the Antonio Narro Autonomous Agrarian University, Department of Horticulture, in Saltillo, Coahuila, Mexico (latitude 25° 21´ 12.8´´ N, longitude 101° 01´ 51.9´´ W), in a warm steppe climate (semi-arid) (Köppen, 1936). The maximum temperature was 37°C and the minimum was 3°C during the cultivation cycle.

Plant material: For the scion, the Lezaforta tomato was chosen. It is a beef indeterminate variety, characterized by round, globular fruits weighing between 250-300 g. The highly vigorous Fortamino tomato was used as the rootstock. This variety is resistant to *Fusarium oxysporum f. lycopersici*, races 0, 1, and 2, and is recommended for saline soils. Both varieties were sourced from the plant breeding company, Enza Zaden.

Seedling production: The scion was sown in May 2017, and 6 days later, the rootstock was sown. Grafting was performed when the plants had a stem of approximately 3.0 mm diameter, after 25 days scion sowing.

Scions were splice grafted to their rootstocks (Lee *et al.,* 2010) by making 45° cuts to both plants and joining them. Afterwards, the grafted plants were held in an acclimatization chamber for 15 days, with constant relative humidity of 95% and temperature between 25-35°C, to promote scarring of the graft.

Plant cultivation: The transplants were performed 22 days after grafting to a NTF hydroponic system, consisting of 6-inch PVC piping with a 10° decline, through which the nutrient solution was constantly re-circulated. Re-circulation was achieved with a submergible pump (BIOPRO model H-450) placed in the nutrient solution collection tank.

Nutritional regime: Steiner nutrient solution was supplied to plants at different concentrations, according to their phenologic stage: 25%, at the beginning of vegetative growth; 50%, during vegetative growth; 75%, during blooming and fruit setting; and 100%, during fruit filling and harvest (Steiner, 1961). Each concentration of NaCl (0, 50, 75 and 100 mM) was added, with respective electrical conductivities (EC) of 2.5, 8.4, 10.5 and 15.3 mS / cm ± 0.2 as a saline stress inducer.

Response variables

Number of fruits and yields: The plants' productive cycle was allowed to progress until the sixth flower cluster emerged. At that stage, the plant tip was cut to prevent further growth. The fruits were harvested 77 days after transplanting, from September 6, 2017 until December 8, 2017. Fruits were picked conformable to the USDA guidelines (Anon., 1975) indicatinge that the fruits would be with a uniform red color over 90% of their surface. Afterwards, fruits were counted and weighed on a portable scale (OHAUS model YA501E) in order to estimate the yield based on production volume.

Total soluble solid (TSS) content: Total soluble solid content was measured immediately upon harvesting in tomato fruits having uniform red color. A drop of internal pulp was collected, deposited in a digital refractometer (HI model 96811), and the TSS concentration recorded in percent of soluble solids.

Vitamin-C content: Vitamin C content was determined volumetrically (Padayatty *et al.,* 2001). 20 g of fresh tomato fruit were weighed out and placed in a mortar. The sample was completely ground adding 10 ml HCl (2%). Afterwards, 100 ml of distilled water were added, the mixture was homogenized, and then filtered. The exact filtrate volume was deposited in a 250 ml Erlenmeyer flask, and a 10 ml aliquot of filtrate was taken. The aliquot was titrated with 10^{-3} N 2,6-dichlorophenolindophenol (DCPIP), dispensed from a 10 ml volumetric pipette, until the solution sustained a rose color for 30 seconds. The content was calculated in mg of ascorbic acid / 100 g of fresh weight based on the amount of DCPIP consumed.

Biomolecule extraction: Freshly harvested tomato fruits were frozen at -4°C immediately after picking. Samples were disposed by placing 20 g of fruit in plastic cups with perforated lids and lyophilized in a FreeZone 2.5 Liter Benchtop Freeze Dry System (LABCONCO) for 48 hours. The lyophilized tissue was manually macerated in a porcelain mortar. Afterwards, 200 mg of macerated tissue was mingled with 20 mg of polyvinylpyrrolidone (Sigma®) and 1.5 ml of 0.1 M phosphate buffer (pH 7-7.2) before sonicating (Ultrasonic Cleaner Branson 1510) for 5 minutes. Subsequently were centrifuged at 12,500 rpm for 10 min at 4°C (Labnet Prism Refrigerated Microcentrifuge), the supernatant decanted, and filtered through a nylon membrane (PVDF 0.45 µm). Finally, the filtrate was diluted 1:15 with phosphate buffer (Ramos *et al*., 2010).

Phenolic compounds: Folin-Ciocalteau reagent was used to quantify total phenol content (Rivero *et al*., 2001). 1 ml of 1:1 water-acetone was added to 200 mg of lyophilized and macerated tomato fruit and then centrifuged at 10,000 rpm at 4°C for 10 min, and the supernatants were decanted. Reactions were prepared by adding 50 µl of extract, 200 µl of 1M Folin-Ciocalteau reagent, 500 µl of 20% (w/v) Na_2CO_3, and 5 ml of distilled water to a test tube. Afterwards, the reactions were incubated at 45°C for 30 min. Total phenol content was determined by measuring the reactions in a G10S UV-Vis spectrophotometer (Thermo Fisher Scientific) with a wavelength of 750 mm and concentrations were extrapolated from a anteriorly prepared calibration curve of gallic acid units (mg l^{-1}).

Lycopene: A previously reported technique for lycopene quantification was used (Bunghez *et al.,* 2011). Lycopene was extracted from tomato fruit by adding 1.5 ml hexane to 1 g of dried tissue in a 2 ml tube and vortexing for 30 seconds. Afterwards, the samples were sonicated for 5 min, centrifuged at 10,000 rpm for 10 min at 4°C, and finally, the supernatants were decanted and filtered through a nylon membrane (PVDF 0.45 µm). Lycopene content was measured spectrophotometrically with a wavelength of 472 nm. Concentrations were determined from an calibration curve made with lycopene standard (Sigma-Aldrich).

Protein concentration determination: Protein concentrations were determined by Coomassie Brilliant Blue assay. 1 ml of tomato fruit extract was mingled with 1 ml of Coomassie Brilliant Blue dye in a test tube. The samples were incerted in plastic cells and measured in a spectrophotometer with an absorbance wavelength of 450 nm. The recorded absorbances were used to determine protein concentrations by comparing them against a previously prepared bovine serum albumin (mg kg^{-1}) calibration curve (Bradford, 1976).

Superoxide dismutase (SOD): The determination of SOD activity was performed with an SOD Assay Kit-WST (Sigma-Aldrich), which is based on a water-soluble tetrazolium salts (WST-1) colorimetric assay (SIGMA-ALDRICH, 2014). WST-1 when reduced with a superoxide anion produced by the reaction of xanthine and xanthine oxidase (XO), produced a water-soluble formazan dye. The inhibition of WST-1 oxidation is due to the neutralization of superoxide radicals by SOD. The results of the assay were expressed as percentages of inhibition.

Ascorbate peroxidase (APx): The technique for quantification of APx activity was previously described (Nakano & Asada, 1981). Essentially, 0.1 mL of biomolecule extract, 0.5 ml of ascorbate (10 mg l^{-1}), and 1 mL of H_2O_2 (100 mM) were added to a centrifuge tube, at 22°C, for 1 minute. The reaction is stopped with 0.4 mL H_2SO_4 (5% v/v). The rate of ascorbate consumption was quantified spectrophotometrically at 266 nm, and the activity units expressed in mM ascorbate min^{-1}/ total proteins.

Experimental design: The experimental design took into consideration two factors: grafting and salt concentrations.

Plants were either grafted or non-grafted, and four different concentrations of NaCl (0, 50, 75, and 100 mM) were used. The treatments were evaluated according to a 2x4 factorial array and set up in a completely randomized block design with 16 experimental units per treatment, for a total of 128 plants. The non-grafted-0-mM was used as the complete control and the grafted-0-mM treatments was used as the grafted control.

Statistical analysis: Statistical analyses of the data were performed with the Infostat 2017 software. Analysis of variance and a comparison of means according to least significant difference (LSD; $p \leq 0.05$) were performed.

Results

Fruit quantity, yield, and TSS

Graft: The grafting on tomato plants did not induce significant difference in fruit quantity, yields, or total soluble solids (TSS) (Fig. 1).

NaCl concentration: The addition of NaCl in the nutrient solution induced significant differences in the weight of the tomatoes, tomato yields, and TSS. The number of fruits per plant was negatively affected by NaCl concentration in a proportional manner. The plants not subjected to salt stress produced the highest amount of fruits per plant. Plants in the 75 and 100 mM treatment groups demonstrated a 41.9% and 52% reduction in fruits per plant, respectively (Table 1). Fruit yields behaved similarly, as increasing concentrations of NaCl significantly reduced fruit yield. Again, salt-free treatment plants have the highest yields, while those from the 75 and 100 mM treatments had their yields reduced by 92.12% and 91.72%, respectively, as related to the control (Table 1). Conversely, the TSS content increased with salt stress. The greatest value of TSS content was seen in the 100 mM treatment group, with a 194.5% increase related to the control (Fig. 2).

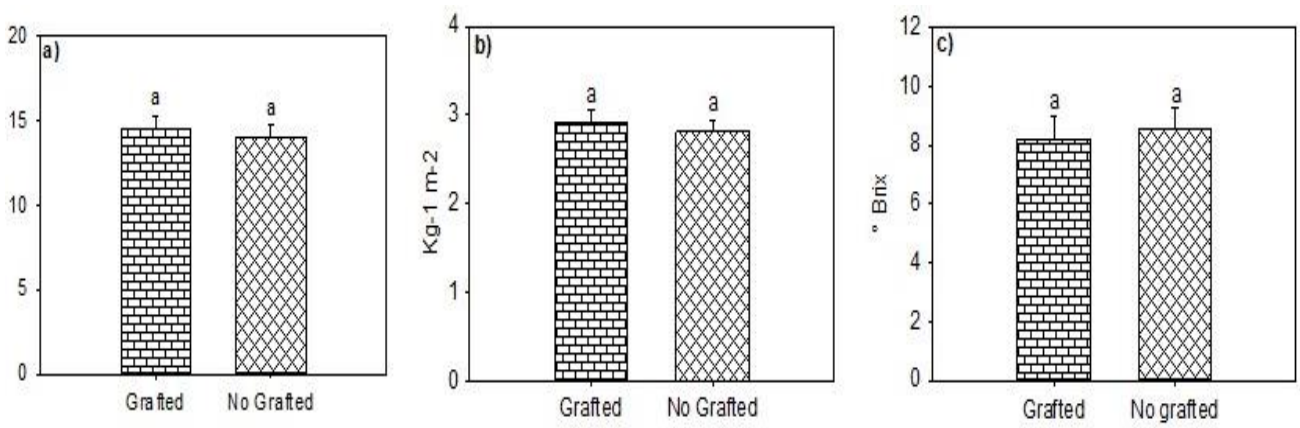

Fig. 1. Effect of the graft on: a) Number of fruits per plant, b) Yield and c) Total soluble solids. The line above the bar represents the standard error.

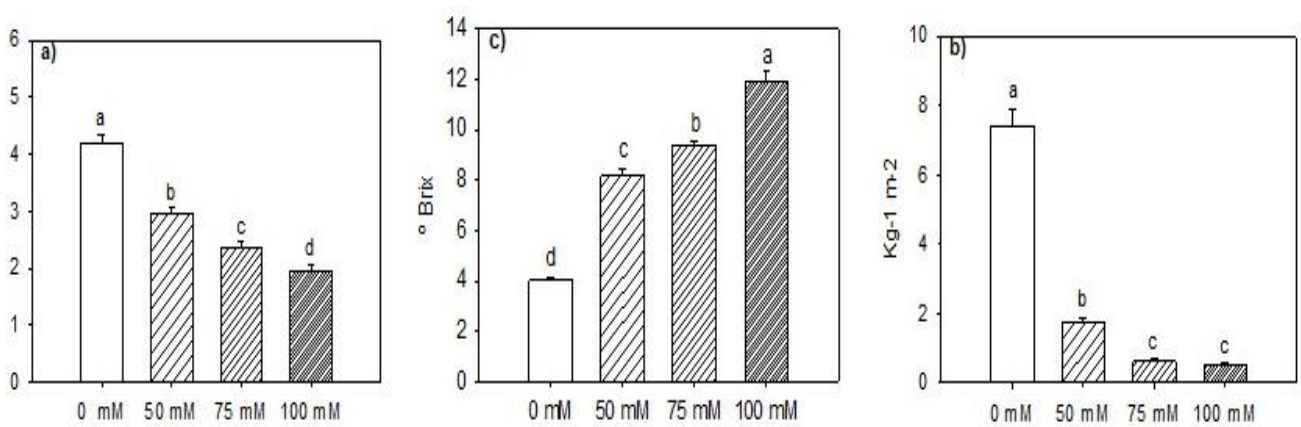

Fig. 2. Effect of NaCl on production tomato: a) Number of fruits per plant, b) Yield and c) Total soluble solids. The line above the bar represents the standard error.

Table 1. Effect of grafting and NaCl concentration on fruit weight, yields, and total soluble solids.

Factor	Treatment	#FP	Yield	TSS
Graft and NaCl	Grafted Control	21.69a	8.19a	3.99d
	Yes-50	14.88b	1.73c	8.84b
	Yes-75	11.63c	0.65d	9.53b
	Yes-100	10.00c	0.60d	11.75a
	Total Control	20.25a	6.66b	4.08d
	No-50	14.69b	1.79c	7.53c
	No-75	11.88c	0.59d	9.24b
	No-100	9.44c	0.47d	12a
Significance		0.80 ns	0.12 ns	0.17 ns
VC		24.71	34.59	14.71

#FP= Number of fruits per plant; TSS=total soluble solids (Brix); Yield (Kg^{-1} m^{-2})). VC= variation coefficient. Different letters in the columns indicate significant differences, according to the LSD test ($p<0.05$). The values represent the means of the treatments. The levels of significance represent the values of P: $p>0.05$ = ns, not significant; $p<0.05$ = *, significant; and $p<0.01$ = **, highly significant

Graft-NaCl concentration interaction: The interactions between grafting and the different NaCl concentrations (0, 50, 75 and 100 mM) were assessed. The grafted control had the maximum number of fruits, with an increase of 7.1%, while the plants from the non-grafted, 100 mM treatment had the maximum decrease in fruit number (53.3%) when related to the controls (Table 1).

The grafted control also had a 22.97% increase in yield, related to the absolute control (non-grafted, 0 mM). The non-grafted, 100 mM treatment group had the lowest yields, with a 92.94% decrease related to the non-grafted, 0 mM control (Table 1).

TSS content exhibited an inverse trend. Those treatments with greater fruit numbers and yields presented with lower TSS content, while those with the least fruit numbers and yields had the greatest TSS content. The non-grafted, 100 mM treatment resulted in a 194% increase in TSS content, as related to the non-grafted, 0 mM control. The grafted control group had the lowest TSS content, with a 2.2% decrease related to the other controls (Table 1).

Vitamin-C, Total phenols, Lycopene, SOD, and APx

Graft: Grafting only modified lycopene content and APx activity. No significant differences were seen in the other quantified antioxidants (Fig. 3). Grafting increase lycopene content with a 9.1% increase, as related to non-grafted plants, but resulted in a 16.72% decrease in APx activity.

NaCl concentration: The salt concentration in the nutrient solution significantly affected vitamin C content, lycopene, APx, and SOD activity, but not total phenol content (Fig. 4). Increases in salt concentration resulted in corresponding increases in fruit vitamin C content. Fruit from the 100 mM treatment exhibited an 82% augmentation in vitamin C content, related to the controls (Fig. 4).

Lycopene content did not benefit from increased salt concentrations. Plants that were not subjected to salt stress had greater quantity of this antioxidant. The 100 mM treatment group exhibited reductions in lycopene content of up to 36%. Conversely, higher salt concentrations increased SOD activity. The same 100 mM treatment induced in a 20% increase in SOD activity, compared to controls (Fig. 4). The activity of APx also increased whith salt stress. The addition of 50 mM NaCl increased APx activity the most (24.7%), compared to the controls.

Graft-NaCl concentration interaction: The non-grafted, 100 mM treatment augmented vitamin C content by 103%, compare to the complete control (non-grafted, 0 mM), which had the least amount of vitamin C (Table 2). Phenolic compounds were increased in the grafted, 100 mM treated plants by 19.7%, as related to the complete control, while the lowest values were observed in the grafted controls, which exhibited a 20% reduction in phenolic compounds. The greatest increase in lycopene was seen in the control groups. The grafted control had a 0.36% increase in lycopene, related to the complete control, while both grafted and non-grafted plants when treated with 100 mM NaCl had lycopene reductions of 27.49% and 46.15%, respectively.

The enzyme activity of SOD was increased with salt stress, as evidenced by the 18.28% rise in the non-grafted, 100 mM treatment group and the 10.71% decrease in the grafted control group. APx activity also augmented under high salinity conditions. The non-grafted, 100 mM treatment generated a 39.7% APx activity increase (Table 2).

Discussion

Number of fruits, yields, and total soluble solids content: Soil salinity leads to ionic stress (primarily due to Na^+, Cl^-, and SO_4), osmotic stress, and secondary strains, such as nutritional imbalances, since high concentrations of Na^+ disrupt the osmotic equilibrium, leading to a water deficit and preventing water absorption by the plant (Türkan & Demiral, 2009).

While tomato is a moderately salt tolerant plant, it is not exempted from the negative effects of high soil salinity. This is one of the principal environmental factors that limits global crop productivity (Arzani, 2008). The grafting of crops onto rootstocks with high-yield genotypes is one environmentally amiable technique used to avoid or reduce production losses caused by high salinity (Yetisir & Uygur, 2010).

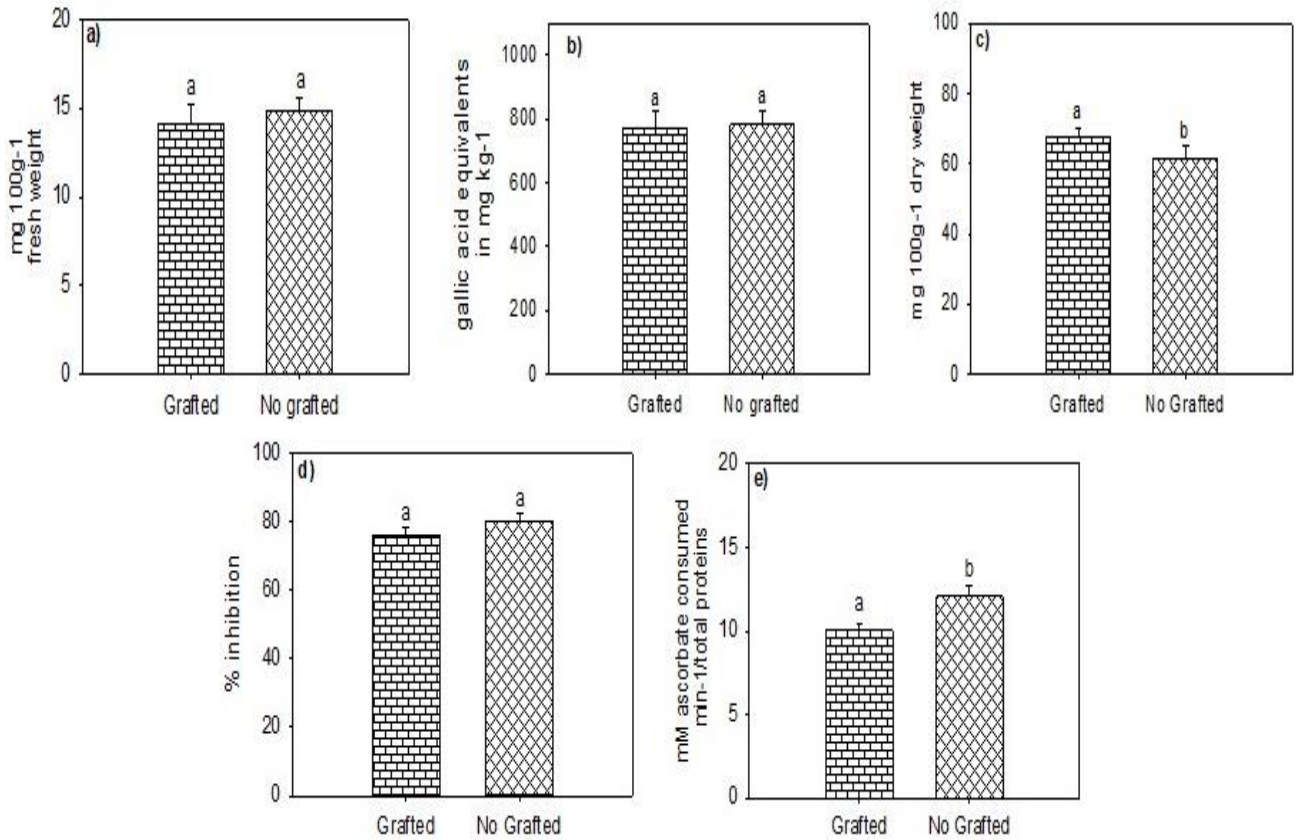

Fig. 3. Effect of the graft on nutraceutic compounds: a) Vitamin C, b) Total phenols, c) Lycopene, d) Superoxide dismutase and e) Ascorbate peroxidase. The line above the bar represents the standard error.

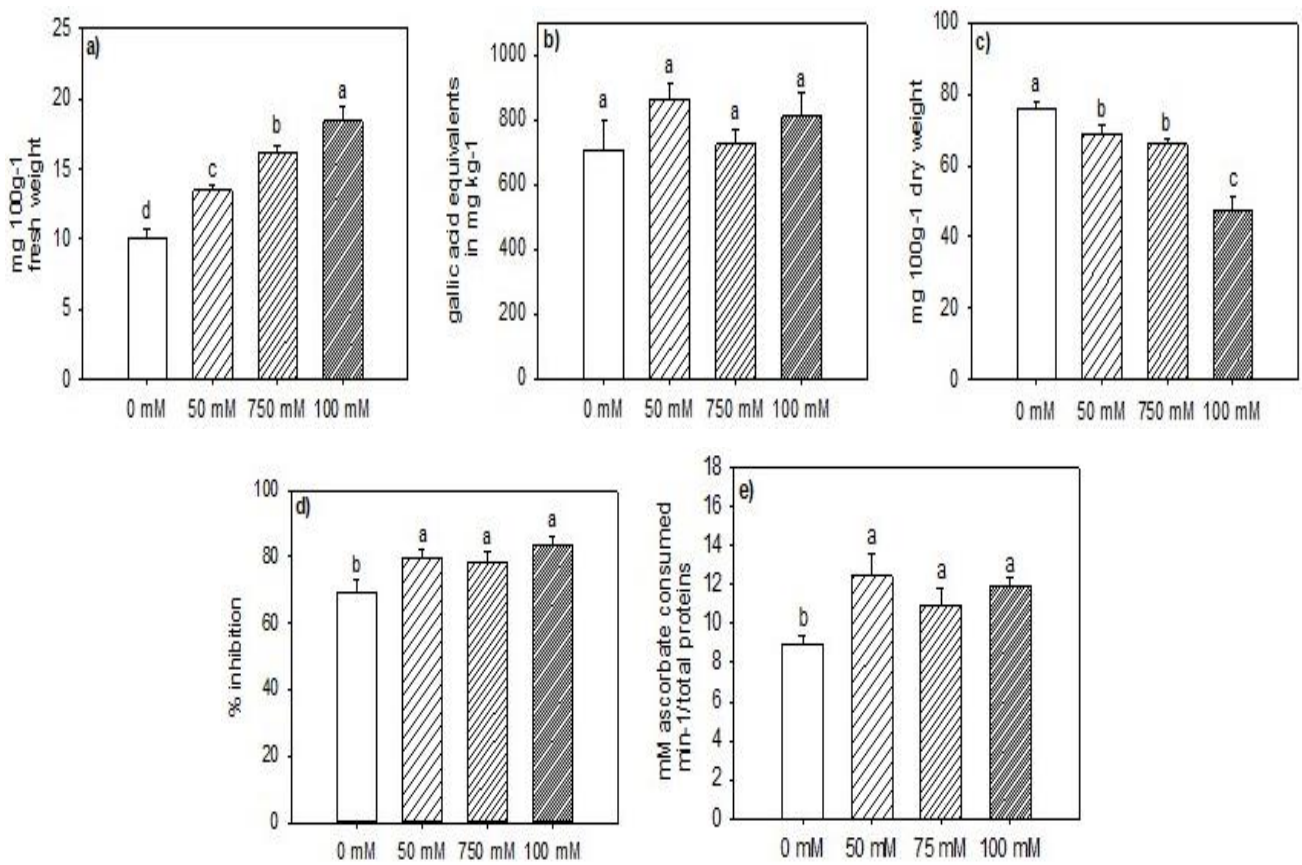

Fig. 4. Effect of NaCl: a) Vitamin C, b) Total phenols, c) Lycopene, d) Superoxide dismutase and e) Ascorbate peroxidase. The line above the bar represents the standard error.

Table 2. Effect of grafting and NaCl concentration on tomato fruit antioxidant content and enzymatic activity.

Factor	Treatment	Vit. C	TP	Lycopene	SOD	APx
	Graft Control	10.91de	628.47c	75.89a	65.57c	9.01e
	Yes-50	14.53bc	826.09abc	72.36ab	80.39ab	10.05cde
	Yes-75	15.93ab	736.92abc	67.80ab	77.27abc	9.44de
Graft-NaCl	Yes-100	18.05a	941.76a	54.82c	79.99ab	11.74bcd
	Complete Control	9.21e	786.32abc	75.61a	73.44bc	8.92e
	No-50	12.38cd	905.62ab	65.49d	79.34ab	14.80a
	No-75	16.36ab	712.82abc	64.37d	79.88ab	12.50ab
	No-100	18.72a	679.08bc	40.71c	86.87a	12.08bc
Significance		0.37 ns	0.0031**	0.11 ns	0.68 ns	0.0214*
VC		13.56	16.48	8.83	10.47	14.89

Vit. C= (mg ascorbic acid / 100g^{-1} fresh weight); TP= Total Phenols (gallic acid equivalents in mg kg^{-1}); Lycopene= (mg 100g^{-1} dry weight); SOD=Superoxide dismutase (% inhibition); APx= acorbate peroxidase (mM ascorbate consumed min^{-1}/total proteins). VC= variation coefficient. The values represent the means for each treatment. Different letters in columns indicated significant differences according to the LSD of means test ($p<0.05$). The levels of significance represent the values of P: $p>0.05$ = ns, not significant; $p<0.05$ = *, significant; and $p<0.01$ = **, highly significant

Grafting provides an alternative way to improve salt tolerance-as evaluated by harvested fruit yield-in a commercial tomato hybrid (Martinez-Rodriguez *et al*., 2008), but the reported results indicated that grafting did not induce significant differences when related to their non-grafted counterparts. Grafted and non-grafted cantaloupe also showed similar sensitivity to salt stress (Edelstein *et al*., 2005). The greatest tolerance to saline conditions is frequently related with the root system. The radicular system is the critical part of the plant, as it must cope with soil stresses, such as salinity (Fullana-Pericàs *et al.,* 2018). As such, the rootstock's qualities are primarily responsible for mitigating the detrimental effects of salt stress on shoot growth (Kyriacou & Rouphael, 2018).

Saline stress had a negative result on the tomato yield, similar reductions in the yields of sand-grown, grafted cucumber (Colla *et al*., 2012) and hydroponically grown zucchini (Rouphael *et al.,* 2006) are also reported when subjected to high salt conditions. This is because of the different restrictions caused by salinity, resulting in a biphasic growth response (Schwarz *et al*., 2010). The first phase arise rapidly, caused by the osmotic stress induced by the salt outside the plants. The second phase takes longer time to develop and results from salt toxicity within the plants as cells exceed their ability to sequester the salts in the vacuole (Munns, 2005).

Concerning fruit quantity, the quantity of fruits per plants is not affected by moderate salinity, as the decrease in yields are a result of smaller fruit sizes (Li *et al.,* 2001). This was contrary to our observed results, wherein both the fruit quantity and size were greatly affected by salt concentration, with aggregate salt concentration leading to greater reduction in quantity. A previous study (Van Ieperen, 1996) also observed a decrease in harvested fruit quantity, leading to a drop in fruit yield, potentially due to a decline in the number of flowers per plant at increased salt concentrations (Magán *et al.,* 2005).

The TSS content is very important for processing quality tomato, conferring a greater market value. Moderate salt concentrations (up to 86 mM NaCl) can significantly increase TSS content, thereby compensating for reduced yields with greater market value (Rouphael *et al.,* 2018). In the this study, TSS content benefitted from saline conditions, with increases in salinity leading to concomitant TSS increases, although at the cost of reduced yields. A simultaneous gain in both fruit yields and TSS content in tomato cultivars has proved difficult to achieve (Bai & Lindhout, 2007). This is due to the inverse relationship between fruit yield and TSS content-the latter is favored in saline conditions (Martinez-Rodriguez *et al.,* 2008). It is suggested that due to the reason non-grafted plants exhibit the highest levels of TSS is because they suffer the most from the impediment in the water uptake, as evidenced by reduced water content in their fruits (Flores *et al*., 2010).

Antioxidant content and activity: Free radical production occurs naturally due to the metabolic processes taking place in the plant chloroplasts, mitochondria, and peroxisomes are important sources of intracellular ROS (Navrot *et al.,* 2007). Saline conditions can promote excessive ROS production (Asada, 2006). The antioxidant compounds and enzymes present in tomato fruit have been shown to provide some degree of protection against naturally occurring oxidative stress (Torres *et al*., 2006). The plants have developed many biochemical and molecular mechanisms in order to combat the detrimental effects of salt stress (Parida & Das, 2005). Although, the results of high salt concentrations on phytochemical content can be indirect (Dorais *et al.,* 2008). Great levels of salt restrict plant growth, leading to reductions in foliar area and rate of photosynthesis, and an increased exposure of fruit to excessive sunlight (Kyriacou & Rouphael, 2018).

Vitamin C content is influenced by many factors, including environmental conditions during crop growth, post-harvest storage, physiological diseases, and mechanical damage (Cortés *et al*., 2008). A rise in the electrical conductivity of nutrient solution (up to 30 mM) containing NaCl and a low watering frequency in bell pepper plants resulted in augmented vitamin C content of red peppers, while green peppers did not exhibit significant changes in their vitamin C content (Marin *et al*., 2009). In that case, the abiotic salt stress appeared to

favor vitamin C content, as increased salt concentrations led to augmented vitamin C content in the fruit. A study involving mandarins reported greater vitamin C content in mandarins subjected to low temperatures (Qiu & Wang, 2015), possibly because vitamin C is the most important water-soluble antioxidant. It accounts for more than 65% of the antioxidant and anti-radical activity in many fruits (Klimczak *et al.,* 2007).

Phenolic compounds provide defense against oxidative and reductive reactions, are capable of chelating metal ions, and function as hydrogen donors (Diaz *et al*., 2012). Induction of total phenol production and increased antioxidant activity caused by salt stress has been reported in strawberry (Cardeñosa *et al*., 2015). Three tomato cultivars showed a 62% increase in phenolic compounds caused by high exposure to UV radiation (Toor *et al.,* 2006). In this research, phenolic compound content did not benefit from grafting nor salt stress, but rather from the combination of both. The greatest quantities of phenolic compounds were register for those plants that were both grafted and subjected to the highest salt concentration. Some author suggested that these increases in phenolic compounds could be adjudicated to the greater light intensity the fruits are exposed to when the foliar area is decreased under salt stress. Increased sunlight intensity and duration of exposure may provoke photo-oxidative stress, which induces biosynthesis of protective biomolecules, such as phenols, to mitigate the produced ROS (Rouphael *et al.,* 2018).

The grafted tomato plants showed elevated levels of lycopene. A similar finding was reported for grafted watermelon, particularly when pumpkin and wild watermelon were used as rootstock (Kong *et al.,* 2017). Grafting is known to exert diverse effects on the amassing of lycopene in plants, although the precise mechanisms are still unknown (Lv *et al*., 2015). It is supposed that the rootstock may affect lycopene metabolism at the transcriptional level (Guo *et al*., 2015). Salt stress did not confer any beneficial effect upon lycopene concentration, as unstressed plants ended up with greater concentrations of lycopene. Another study in which tomato plants were grown in salt stress reported that lycopene begins to increase rapidly under conditions of high electrical conductivity (EC). They attributed this to a premature ripening and rise of lycopene immediately prior to harvest caused by osmotic stress (Wu & Kubota, 2008). Conversely, a study utilizing various concentrations of silver nitrate ($AgNO_3$) found greater lycopene concentrations in the plants exposed to the lowest $AgNO_3$ concentration (30 mg l^{-1}) and reported decreasing lycopene concentrations as abiotic stress increased (Cabrera-De La Fuente *et al*., 2014). Temperatures above 30°C have also been noted to affect lycopene synthesis negatively (Lurie *et al.,* 1996). The temperature may have affected lycopene synthesis since the daily average temperature during the day was greater than 30°C. It was worth noting that the NaCl-stressed plants had greatly reduced foliar areas, allowing sunlight to strike the tomato fruits directly, while the fruit of unstressed plants stayed in the shade provided by the foliage.

An increase in antioxidant enzyme activity while under salt stresses could be demonstrative of greater ROS production, as accumulation of these species activates the protective mechanisms used to mitigate oxidative damage resulting from abiotic stress (Meloni *et al*., 2003).

SOD is a metalloprotein with an essential role in the restrain of ROS levels (Santos *et al.,* 2018). SOD is first in detoxification and the principal powerful cellular antioxidant thanks to its speed for controlling O_2^{-*} (Willems *et al*., 2016). It is an endogenous antioxidant enzyme that serves as the first line of defense against ROS by catalyzing the dismutation of the superoxide radical (O_2^{-*}) into molecular oxygen (O_2) and hydrogen peroxide (H2O2) (Ighodaro & Akinloye, 2017). According to our observations, the incidence of salt appears to promote SOD activity, as greater enzyme activity is seen in saline conditions. Another study reported increased SOD activity in different triticale genotypes at different stages of growth (Rasouli & Kiani-Pouya, 2015). Increased SOD activity in salt stressed tomato plants-especially in non-grafted or auto-grafted plants-had also been previously studied (He *et al*., 2009). Similar results have also been observed in grafted plants when subjected to heat stress, leading to the suggestion that ROS production under stress conditions is lower in grafted plants than non-grafted or auto-grafted plants (Rivero *et al.,* 2003). Work carried out with two bean genotypes under saline conditions of 150mM obtained similar results on SOD activity showing an increase in the activity of this enzyme in the two genotypes (Alzahrani *et al.,* 2019), the activity of SOD in bean was higher than that shown in plants grafted and ungrafted tomato.

The enzymes APx, dehydroascorbate reductase (DHAR), and glutathione reductase (GR) are important antioxidant enzymes involved in the ascorbate-glutathione cycle. During this cycle, APx reduces H_2O_2 to H_2O and O_2 using ascorbate as a reducer (Noctor & Foyer, 1998). A prior experiment involving grafted and non-grafted tomato plants exposed to different NaCl concentrations (0, 50, 100, and 150 mM) were found to increase APx activity at the higher concentrations (100 and 150 mM), while grafting did not exert significant changes to APx activity (He *et al.,* 2009). In this research, we report greater APx activity in the 50 mM treatment group, with significant differences in non-grafted plants when related to their grafted counterparts.

Modifications in the responses of antioxidant enzymes to saline stress have been noted. The activity of CAT and DHAR was reduced in salt stressed cucumber, while SOD, APx, GPOD, and GR showed greater activity (Zhu *et al*., 2004). Increased SOD and APx activities with salt stress were similarly reported in this study. Catalase and APx, among other antioxidant enzymes, neutralize the H_2O_2 produced by the activity of SOD (Balfagón *et al.,* 2018). Our results also indicate that APx activity increased along with the increase in SOD activity.

Conclusions

The grafted plants improve the yields and the content of lycopene when they are not in saline conditions. Salinity diminishes yields in grafted and ungrafted plants but in general increases the content of some antioxidants. The interplay between grafting and salinity in some cases may favor the contention of phenolic compounds.

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