

Effect of titanium foliar applications on tomato fruits from plants grown under salt stress conditions

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Abstract

Salt stress affects plant metabolism, while beneficial elements such as titanium (Ti) may stimulate adaptive responses to mitigate salt stress. Here we evaluated the main effects of sodium chloride (NaCl 0, 50 and 100 mM) in the nutrient solution, and of titanium foliar spray (Ti 0.75 and 150 mg L⁻¹), as well as the interaction of these study factors, on tomato cv. 'Rio Supremo' performance in greenhouse. Plants were treated with NaCl during 80 d through automated drip irrigation; while eight Ti foliar sprayings were applied with a manual sprayer, at intervals of 10 d each. Yield and quality parameters of fruits were evaluated in the second cluster. NaCl reduced yield parameters, pH value and increased the titratable acidity (TA), electrical conductivity (EC), and total soluble solids (TSS), as well as the TSS/TA ration in the fruits. Ti did not affect yield parameters, though it reduced the pH and increased the TSS/TA ratio of fruits. NaCl and Ti have differential effects on fruit quality as separate factors, while the interaction of both factors revealed that Ti effects dependent on the presence of NaCl and its level in the nutrient solution. In conclusion, Ti did not mitigate the negative effects of saline stress on the evaluated yield parameters, but importantly, with moderate levels of NaCl in the nutrient solution (50 mM), Ti increased EC, TA and TSS of fruits.

Keywords: abiotic stress; beneficial elements; hormesis; salinity; Solanaceae; *Solanum lycopersicum*

Abbreviations: das - days after sowing; EC - electrical conductivity; TA - titratable acidity; TSS - total soluble solids

Introduction

Salinity is a critical problem worldwide, and its adverse effects in agriculture tend to increase, due to global climate change (Wichelns and Qadir, 2014). The total surface area of saline soils is estimated in 397 million hectares and that of sodic soils is approximately of 434 million hectares worldwide (FAO, 2016).

Tomato (*Solanum lycopersicum*) is cultivated practically in every country over the world. Given its high nutritional and nutraceutical properties, this vegetable has a great economic and dietary importance (Georgé *et al.*, 2011). In the food industry, titratable acidity, colour, electrical conductivity, firmness, pH, and total soluble solids are considered key indicators defining the quality of tomato fruits. Tomato fruit quality tends to improve in protected agricultural systems as compared to open-air systems, hence various production systems in greenhouses have been implemented, including hydroponics and fertigation, chemical and organic fertilization, and biostimulation by using beneficial elements (Casierra-Posada and Aguilar-Avenidaño, 2008; García-Sahagún *et al.*, 2009). Although beneficial elements are not essential for plants, they can stimulate positive responses in certain metabolic processes when applied at low dosages (Trejo-Téllez *et al.*, 2016). Furthermore, when plants are exposed to different environmental stresses, beneficial elements can induce tolerance, resistance, or defence responses that allow them to reach acclimatization and cope with stress factors (Gómez-Merino and Trejo-Téllez, 2018).

Titanium (Ti) is one of the elements categorized as beneficial elements (Gómez-Merino and Trejo-Téllez, 2018). This is a transition metal and represents the ninth most abundant element in the Earth's crust, making up 0.33% of its composition. Its use in agriculture began in the early 1930s. Since then, various studies have proven the beneficial effects of this element on metabolic, physiological, and morphological attributes in plants. For example, Ti increases the enzymatic activity of lipoxygenase and nitrate reductase in tomato seedlings as well as in other species (Sarmiento *et al.*, 1995; Hrubý *et al.*, 2002), and enhances the synthesis of ascorbic acid and capsanthene (Martínez *et al.*, 1993). Titanium increases the levels of malic acid and the chlorophyll concentration in chili pepper, and it induces the accumulation of photosynthetic pigments in strawberries, since it acts as a redox catalyser in the electron transport chain from the photosystem II to I (Carvajal *et al.*, 1994; Choi *et al.*, 2015). This element also favours the absorption of nutrients like N, K, Ca, and Mg, and especially Fe in pepper and tomato plants (Carvajal *et al.*, 1992; Hrubý *et al.*, 2002; Kleiber and Markiewicz, 2013). Likewise, Ti increases yield and quality in strawberry, as well as the growth of cotton plants stressed by drought (Choi *et al.*, 2015; Shallan *et al.*, 2016). Nevertheless, little is known about its effects on fruit quality in horticultural crops exposed to salt stress.

Exogenous application of some beneficial elements has been proved to ameliorate salt sensitivity, with a concomitant stimulation of plant growth, photosynthetic efficiency, and water use efficiency (Banerjee and Roychoudhury, 2018). Indeed, the application of Ti nanoparticles increased plant's tolerance to abiotic stress factors, including cold, drought and Cd toxicity (Lyu *et al.*, 2017). Importantly, beneficial elements may trigger hormesis, a biphasic dose response to an environmental agent characterized by a low dose stimulation or beneficial effect and a high dose inhibitory or toxic effect, representing an adaptive response of cells and organisms to a moderate stress (Mattson, 2007; Agathokleous and Calabrese, 2019; Agathokleous *et al.*, 2019).

Thus, we hypothesized that Ti can mitigate the negative effects caused by salt stress in tomato; and consequently, we expect positive effects of Ti reflected in improved yield attributes and fruit quality. So, the objective of this study was to evaluate the main effect of sodium chloride (applied at 0, 50, and 100 mM NaCl in the nutrient solution) and titanium (sprayed on the leaves in concentrations of 0, 75, and 150 mg L⁻¹), and their interactions, on yield and quality indicators of tomato fruits.

Materials and Methods

Experimental conditions

The experiment was carried out in an open hydroponic system (free-drain) in a tunnel-type greenhouse with zenith window, plastic cover, and anti-aphids mesh on the side walls. Irrigation was carried out using an automatized spaghetti drip system. Plastic tanks with a capacity of 200 L were installed in the greenhouse, each one provided with a ½ HP pump, a PVC branch with return to the tank regulated with a valve, and an outlet line with a ring filter. The system was connected to a 16 mm black tubing hydroponic irrigation flexible hose with three droppers per container, each dropper provided with a crosshead for four tubes and each tube with a stake.

During the experiment, the mean daytime and night-time temperatures were 31.7 and 15.1 °C respectively. The mean relative humidity was 30% in the day and 86.9% at night. The photoperiod duration was 11.3 h with a mean light intensity of 137 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Tomato cv. 'Río Supremo' seedlings were obtained from sowing seeds in 200 cavity plastic trays with peatmoss (particle size ≤ 6 mm; pH= 5.5 and electrical conductivity 0.6 dS m^{-1} determined in saturated paste extract) as substrate.

Treatments and experimental design

It was established a completely randomized experiment with a 3×3 -factorial arrangement. The first study factor was the sodium chloride (NaCl) concentration in the nutrient solution at three levels: 0, 50, and 100 mM, corresponding to electrical conductivities of 2.0, 7.0, and 12.0 dS m^{-1} (considering the composition of the nutrient solution). The experiment was set up and performed in hydroponics using the Steiner nutrient solution (Steiner, 1984). During the experimental phase, the irrigation volume per day was progressively increased from 500 to 800 mL according to crop requirements. The second study factor was foliar application of Ti at three levels (0, 75, and 150 mg L^{-1} from TiO_2). The combination of factors and levels resulted in nine treatments, and each treatment had nine replicates.

The experimental unit was a single plant, 61 days after sowing (das), planted in a black polyethylene bag, caliber 400 (30 x 30 x 30 cm). The substrate used was tezontle (a local volcanic gravel), with particle size between 5- and 6-mm; it is an inert substrate with pH 7.35, electrical conductivity of 0.15 dS m^{-1} and total pore space of 67.9% (Trejo-Téllez *et al.*, 2013); it does not contain organic matter and has a cation exchange capacity of 2.7 $\text{cmol}_c \text{kg}^{-1}$ (Cruz *et al.*, 2012).

The axillary suckers were removed to drive the plant to a single stem. The experiment was conducted up to the third cluster, so the evaluations were done in the second cluster.

Addition of NaCl to the nutrient solutions began 61 das, while the Ti foliar sprays began 62 das. All the foliar applications were done at 06:00 h with a manual sprayer, applying 50 mL of the solution per plant. In order to attain higher adherence of the solution to the leaves, an emulsifying agent containing approximately 40% of polyethylene glycol sorbitan monolaurate was added to the nutrient solution at a concentration of 0.5 g L^{-1} . During the experimental period (80 days), eight foliar applications of Ti were done at 10-day intervals each.

Evaluated variables

In order to evaluate the yield and quality variables, harvest began once the fruit reached the state of maturity known as "red" according to the UPOV (2018). The harvest was done manually, collecting, and analysing the fruits of the second cluster. The total number of fruits per cluster was counted. The fruits were subsequently weighed in an analytical scale to estimate the yield. Furthermore, the harvested fruits were measured for equatorial (mid-fruit) and polar (from the peduncle to the apex) diameters using a digital caliper.

To have higher representativity in the results of quality parameters, four composited samples (each one from three fruits of different plant) were analysed. The fruit juice was obtained with a juice extractor and determined for pH and electrical conductivity (EC) using a portable potentiometer. The juice was also

determined for titratable acidity (TA) following the methodology proposed by Boland (1990). Total soluble solids (TSS) were measured with a digital refractometer, placing a drop of juice in the reading cell of the device. The TSS and TA ratio was determined dividing the value of TSS by the value of TA.

Statistical analysis

Results are expressed as mean + SD of multiple measurements. Statistical analyses were carried out with the SAS statistical program (SAS, 2011). The significance of individual effects and interaction among different treatments for the parameters measured was assessed using an analysis of variance (ANOVA). The significance of difference between individual groups was analysed using the Tukey's test. For all analyses, the criterion of significance was set at $p \leq 0.05$.

Results

Sodium chloride (NaCl) as a single factor had a significant effect on yield parameters, while the main effect of Ti and the interaction NaCl \times Ti did not significantly affect the evaluated yield variables (Table 1).

Table 1. Significance of the study factors and their interactions on yield and chemical properties of fruit quality evaluated in the second cluster of tomato cv. 'Río Supremo'

Source of variation	Number of fruits		Fruit diameter		Yield
			Equatorial	Polar	
NaCl	<0.0001 *		<0.0001 *	<0.0001 *	<0.0001 *
Ti	0.4084 ns		0.1795 ns	0.7362 ns	0.5537 ns
NaCl \times Ti	0.9294 ns		0.3219 ns	0.3042 ns	0.6366 ns
CV	24.033		7.272	8.020	29.49
Source of variation	pH	Electrical conductivity (EC)	Total soluble solids (TSS)	Titratable acidity (TA)	TSS/TA ratio
NaCl	<0.0001 *	<0.0001 *	<0.0001 *	<0.0001 *	<0.0001 *
Ti	0.0002 *	0.2506 ns	0.0014 *	0.0353 *	0.0519 ns
NaCl \times Ti	<0.0001 *	<0.0001 *	<0.0001 *	<0.0001 *	<0.0001 *
CV	0.432	3.347	2.531	5.663	4.498

* = Significant ($p \leq 0.05$); ns = Not significant ($p > 0.05$)

Interestingly, the NaCl factor and its interaction with the Ti factor had positive effects on the evaluated quality variables. Moreover, the Ti factor also had a significant effect on most of the evaluated variables, except juice EC and TSS/TA ratio (Table 1).

Main effects of NaCl

In this study, the evaluated NaCl concentrations, i.e. 50 and 100 mM NaCl, resulted in EC means in the nutrient solution higher than 2.5 dS m^{-1} (i.e. 7.0 and 12.0 dS m^{-1}), which decreased tomato productivity by reducing the number of fruits per cluster and the diameter (equatorial and polar), and concomitantly lowered yield (Figure 1A-C). Specifically, 50 and 100 mM NaCl decreased the number of fruits in the second cluster by 34.4 and 57.0%, respectively, in comparison to the control (Figure 1A). Likewise, the addition of 50 and 100 mM NaCl to the nutrient solution decreased the equatorial diameter in 21.3 and 26.3%, respectively; while the polar diameter was reduced in 27.1 and 29.4%, respectively, in both cases as compared to the control (Figure 1B). Furthermore, the yields of the second cluster in plants treated with 50 and 100 mM NaCl were almost 69.0 and 84.8% lower than that of the control (Figure 1C).

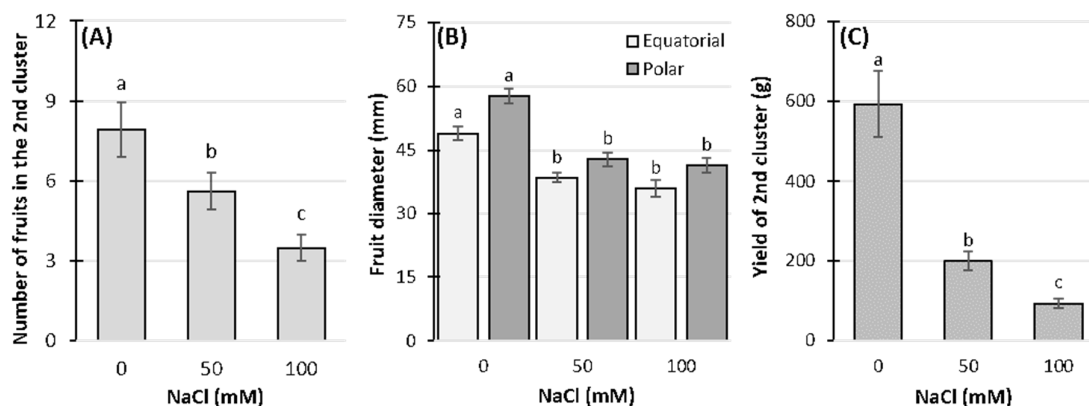


Figure 1. Effects of NaCl on yield parameters evaluated on the second cluster of tomato plants cv. 'Río Supremo'. (A) Number of fruits, (B) Equatorial and polar fruit diameters, (C) Yield of the second cluster. Means \pm SD with different letters in each figure are significantly different (Tukey, $p \leq 0.05$)

Sodium chloride significantly increased the acidity of the fruit juice and thus decreased the pH values of the juice by 2.9 and 3.4% when the plants were treated with 50 and 100 mM NaCl, respectively, in comparison to the control (Table 2).

Table 2. Main effect of the NaCl study factor on the chemical properties of fruit quality evaluated in the second cluster of tomato plants cv. 'Río Supremo'

NaCl (mM)	pH	EC (dS m^{-1})	TSS ($^{\circ}\text{Brix}$)	TA (% citric acid)	TSS/AT ratio
0	4.17 ± 0.015 a	5.12 ± 0.078 b	4.99 ± 0.069 c	0.44 ± 0.027 b	11.65 ± 0.420 b
50	4.05 ± 0.032 b	8.91 ± 0.593 a	9.17 ± 0.411 a	0.72 ± 0.045 a	13.05 ± 0.285 a
100	4.03 ± 0.018 b	8.80 ± 0.570 a	8.81 ± 0.482 b	0.73 ± 0.082 a	12.52 ± 0.695 a

Means \pm SD with different letters in each column are statistically different (Tukey, $p \leq 0.05$)

EC = electrical conductivity; TSS = total soluble solids; TA = titratable acidity

The addition of 50 and 100 mM NaCl in the nutrient solution also increased on average the EC of the juice by 72.9%, and $^{\circ}\text{Brix}$ by 80%, as compared to the control. So, 50 and 100 mM NaCl increased TA values in 63.6 and 65.9%, respectively, in comparison to the control. The ratio between TSS and TA was higher when the plants were treated with NaCl (Table 2).

Main effects of Ti

Titanium treatments did not significantly affect tomato yield parameters (Supplementary material 1).

Nevertheless, Ti significantly affected pH, TSS, and the ratio between TSS and TA. When applied in the range of 75 and 150 mg L^{-1} , Ti produced modest, but significant reductions in pH, between 0.7 and 0.5%, respectively, compared to the control. In the case of $^{\circ}\text{Brix}$, plants treated with 75 mg Ti L^{-1} decreased this parameter in 3.4% as compared to the control, while the application of 150 mg Ti L^{-1} did not affect this parameter regarding the control. Thus, the curve described in this variable as a function of the Ti dose applied displayed a "U" shape in a hormetic manner. Interestingly, Ti increased the TSS/AT ratio by 3.3 and 5.3% with doses 75 and 150 mg L^{-1} , respectively, as compared to the control (Table 3).

Table 3. Main effect of the Ti study factor on the chemical properties of fruit quality evaluated in the second cluster of tomato plants cv. 'Río Supremo'

Ti (mg L ⁻¹)	pH	EC (dS m ⁻¹)	TSS (°Brix)	TA (% citric acid)	TSS/AT ratio
0	4.10 ± 0.04 a	7.60 ± 1.10 a	7.73 ± 1.11 a	0.64 ± 0.12 a	12.06 ± 0.42 b
75	4.07 ± 0.03 b	7.71 ± 1.02 a	7.47 ± 0.99 b	0.64 ± 0.07 a	12.46 ± 0.29 ab
150	4.08 ± 0.04 b	7.53 ± 0.98 a	7.77 ± 1.05 a	0.61 ± 0.07 a	12.70 ± 0.70 a

Means ± SD with different letters in each column indicate statistical differences (Tukey, $p \leq 0.05$)

EC = electrical conductivity; TSS = total soluble solids; TA = titratable acidity

Effects of the interaction of the study factors (NaCl × Ti)

The evaluated yield parameters were not significantly influenced by the interaction NaCl × Ti (Supplementary material 2). When analysing the interactive effects of NaCl and Ti on the fruit quality indicators, Ti had differential effects on them, and they were dependent on the NaCl concentration in the nutrient solution (Figure 2).

In the absence of NaCl in the nutrient solution, foliar applications of 75 mg Ti L⁻¹ significantly decreased fruit pH, with respect to the control. This tendency is also observed when the plants were treated with 50 mM NaCl (Figure 2A).

Titanium had no significant effects on the EC value when plants were not treated with NaCl. Interestingly, with the low NaCl concentration evaluated (50 mM), the application of 75 and 150 mg Ti L⁻¹ significantly increased EC, with 30.3 and 32.2% increases respectively, compared against the treatment with the same level of NaCl and no Ti. Conversely, when the NaCl concentration was increased to 100 mM, Ti significantly decreased EC. In this last case, when applying 75 mg L⁻¹ Ti in plants exposed to 100 mM NaCl, the magnitude of the EC decrease was 17.4 and 25.2%, respectively, as compared to the application of 100 mM NaCl and no Ti (Figure 2B).

The effects of Ti on TA were inversely related to the effects observed in the pH value. In the absence of salinity, the application of 75 mg L⁻¹ Ti significantly increased TA of juice in comparison to the control. In the presence of high salinity (100 mM NaCl), the application of 75 and 150 mg Ti L⁻¹ decreased the value of titratable acidity in fruits by 32 and 34.7%, respectively, as compared to the application of 100 mM NaCl and no Ti (Figure 2C).

Total soluble solids (TSS) were directly related to those obtained in EC (Figure 2B), since the means increased significantly when applying Ti in the presence of 50 mM NaCl. Contrarily, in plants treated with 100 mM NaCl, Ti significantly decreased this variable. The lowest TSS means were observed in plants not exposed to NaCl, independently of the dose of Ti applied (Figure 2D).

The TSS/TA ratio increased significantly by spraying the leaves with Ti only in the presence of high salinity (100 mM NaCl), with increases of 25.9 and 25% with Ti sprayings of 75 and 150 mg L⁻¹, respectively, as compared to the control (without Ti) at the same level of salinity (Table 4).

Table 4. Effects of the interaction of the study factors (NaCl × Ti) on the TSS/TA ratio of the fruit juice evaluated in the second cluster of tomato plants cv. 'Río Supremo'

TSS/TA ratio			
NaCl (mM)	Ti (mg L ⁻¹)		
	0	75	150
0	12.1 ± 0.46 abcd	11.2 ± 0.15 cd	11.6 ± 0.54 bcd
50	13.4 ± 0.06 a	12.7 ± 0.38 abc	13.1 ± 0.24 ab
100	10.7 ± 0.10 d	13.5 ± 0.27 a	13.4 ± 0.19 a

TSS: Total soluble solids; TA: Titratable acidity. Means ± SD with different letters are significantly different (Tukey, $p \leq 0.05$)

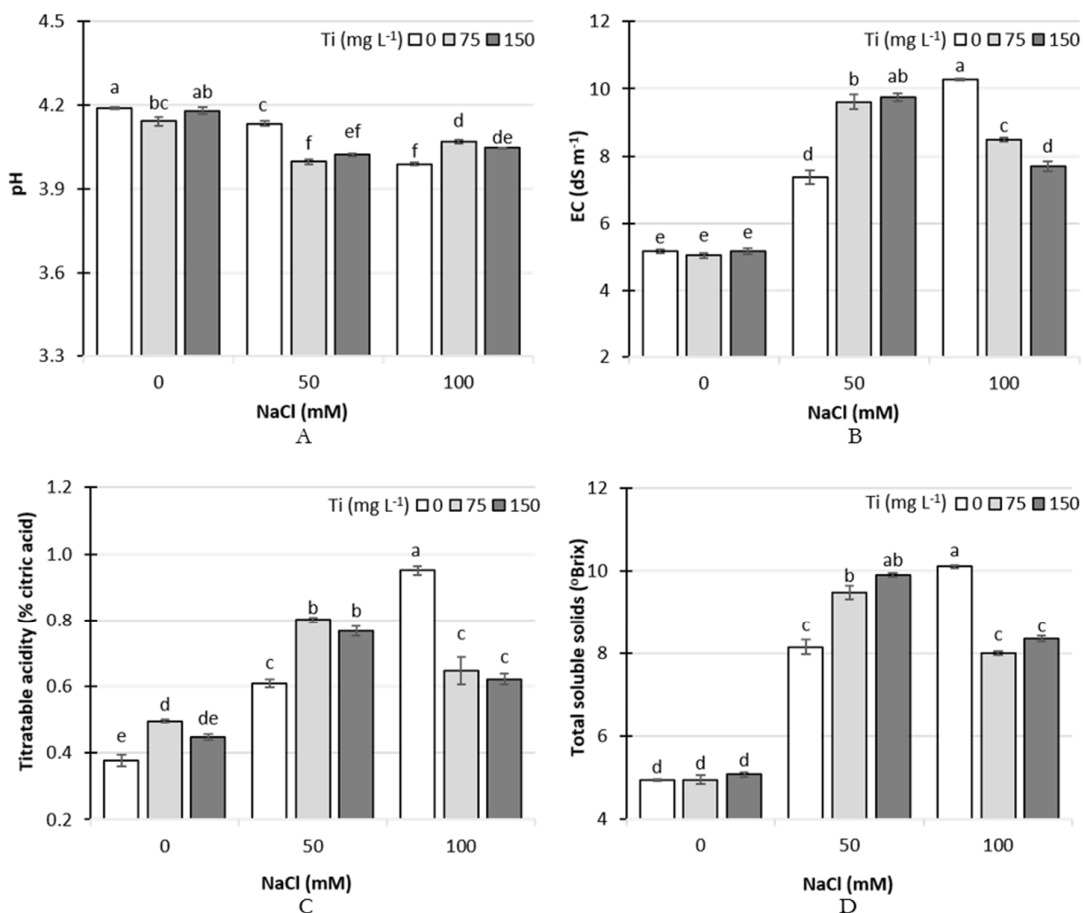


Figure 2. Effects of the interaction of the study factors (NaCl × Ti) on the chemical properties of fruit quality evaluated in the second cluster of tomato plants cv. 'Río Supremo'. (A) pH, (B) Electrical conductivity (EC), (C) Titratable acidity (TA), (D) Total soluble solids (TSS) Means ± SD with different letters in each subfigure are significantly different (Tukey, $p \leq 0.05$)

Discussion

The number of fruits (Figure 1A), fruit size (Figure 1B) and yield (Figure 1C) were reduced under our experimental conditions as a result of the NaCl levels tested. These decreases have been associated with reductions of the water potential in the root zone that causes water deficit, phytotoxicity of the Na⁺ and Cl⁻ ions, nutritional deficiencies due to depression in uptake and transport to the shoot and oxidative stress (Isayenkov and Maathuis, 2019).

Although salinity decreases yield in tomato, some fruit quality indicators are positively affected when increasing salt concentrations in the growth media (Zhang *et al.*, 2016), which was also observed in our study (Table 2). NaCl significantly increased the acidity of fruit juice (Table 2). In tomato, pH values of juice under 4.5 are desirable when the objective is industrialization, since this value may contribute to inhibit the proliferation of undesirable microorganisms in the final product (Giordano *et al.*, 2000). As the acidity of the fruits increased in the treatments with NaCl, so did the titratable acidity (TA) (Table 2). This means that the increase in TA caused by the addition of NaCl is negatively related with the pH value (Rezende-Fontes *et al.*, 2000).

Increases in °Brix observed in the NaCl treatments (Table 2), are caused by the osmotic effect generated by the low osmotic potential in the root area of the plants, which reduces water flow to the aerial parts of the plant including fruits (Wu and Kubota, 2008).

Under our experimental conditions, NaCl increased TSS/TA ratio (Table 2). This means that there was a higher proportion of TSS as the salinity increased, with respect to the TA. Moderate salinity fosters the enzymatic activity of sucrose invertase, which in turn produces higher accumulation of sugars. Also, the increase in TA in the fruits of plants treated with NaCl is because plants with high concentrations of total sugars, mainly reducing sugars, have a higher concentration of free organic acids and a lower concentration of hydrogen ions than do plants with low sugar levels (Getinet *et al.*, 2008).

Spraying the leaves with Ti decreased the pH of the fruit juice by 0.6%, and although the percentage difference was low, it was significant (Table 3). Low pH values in the fruits are positively related with a lower respiration rate thus maintaining better fruit quality for longer time (Tigist *et al.*, 2013). Consequently, low pH triggered by Ti may delay fruit senescence. Likewise, the polygalacturonase (PG) and expansins (EXP) enzymes that cooperatively dismantle the polysaccharide network of the cell walls of the fruits during ripening, allowing their softening (Jiang *et al.*, 2019), are pH- dependents. Indeed, the PG enzyme shows its maximum activity in the pH range of 4.4 to 4.8 (Verlent *et al.*, 2004); while EXP show their maximum activity at pH 4.0 (Wang *et al.*, 2008). Therefore, reductions in the pH of the fruit reduce the activity of both enzymes.

To further investigate the effect of Ti on fruit quality, it would be recommended to test its application to the roots in different systems, including, but not limiting to hydroponic nutrient solution, fertigation and drench, among others. Indeed, applying Ti to the roots is more effective in increasing the concentration of organic acids than applying it to the leaves (Simon *et al.*, 1991).

Importantly, TiO₂ has a photooxidative effect on ethylene in tomato during postharvest (Maneerat *et al.*, 2003; Maneerat and Hayata, 2006; Kaewklin *et al.*, 2018). Consequently, this process delays degradation of mature fruits and therefore prolongs shelf life. So far, there are no studies evaluating the effect of Ti on the synthesis of ethylene in tomato during different stages of fruit ripening.

Juice pH is closely related to the concentrations of some organic acids in the fruit (Bai *et al.*, 2015). Nonetheless, this response was not observed under our experimental conditions, since the main effect of Ti on TA was not significant (Table 3). Importantly, Ti may increase the synthesis of hydrocarboxylic acids (citric and malic) as well as ascorbic acid, which chelate Ti, representing a defence mechanism of the plant in response to exogenous Ti (Hrubý *et al.*, 2002). Therefore, in the presence of Ti, the plant would have a lower concentration of free organic acids. In tomato fruits, the main organic acids are citric, malic, and oxalic acids (Hernández-Suárez *et al.*, 2008). At harvest, the amount of organic acids depends on soluble solids and their rate of decomposition. Consequently, at harvest, the fruits have a higher concentration of acids, which decreases throughout maturation, since they are converted into sugars (Wongmetha *et al.*, 2015).

Titanium may also increase TSS due to the stimulation of the phosphofructokinase enzyme in tomato (Simon *et al.*, 1991). However, in strawberry, foliar applications of Ti had no effect on TSS nor on TA of the fruits but it did increase the concentrations of ascorbic acid anthocyanins (Skupień and Oszmiański, 2007). In apples, foliar Ti spraying (2.5 g ha⁻¹) had no effect on firmness, TSS, or TA (Wojcik and Klamkowski, 2005). In our study, TSS displayed a “U” shaped curve as a function of Ti concentration, since plants exposed to 75 mg Ti L⁻¹ significantly decreased in TSS, as compared to the control. Moreover, the TSS value observed with the high dose of Ti (100 mg Ti L⁻¹) was similar to that of the control (Table 3). The U-shaped curve usually refers to the nonlinear relationship between two variables, a dependent variable and an independent one (Lemmens, 2010). It is worthy to mention that two variables may have a linear correlation coefficient of zero, but in that case it does not imply that the variables are independent (Veličković, 2015). The occurrence of this U-shaped dose-response relationship is known as hormesis, a natural occurring phenomenon related to the concept of homeostasis. These concepts and their impacts are of paramount importance for biological adaptation to environmental stressors (Calabrese and Baldwin, 2001), as Ti could be at cell level.

Sugars and organic acids are the most common features associated with the taste of fruits, which are measured as TSS and TA, respectively. The ratio between these variables has an important impact on the acceptance of the fruits by the consumers (González-Agüero *et al.*, 2016). This ratio provides an indirect value of the fruit taste. In tomato, the optimum value of this ratio is 12.5, which is associated with good fruit taste (Beckles, 2012). In our study, the values closest to this ratio were found in the treatments with Ti. Moreover, there was a positive correspondence between the ratio of total soluble solids and titratable acidity and the concentration of Ti applied. This meant that such ratio increased by 3.3 and 5.3% with 75 and 150 mg Ti L⁻¹, respectively, compared to the control (Table 3).

The pH in fruit juice as a feature of tomato fruit quality ranges between 4.0 and 4.5 (Jones, 2007). Interestingly, values reported herein are within this interval. The lowest value (4.0) was registered in the treatment with 100 mM NaCl without foliar Ti application, which was statistically similar to that observed in fruits of plants treated with 50 mM NaCl and 75 mg L⁻¹ Ti. Instead, the highest value (4.2) was obtained in fruits of plants with no treatment of NaCl (Figure 2A).

Foliar application of 75 mg Ti L⁻¹ reduced pH of fruits in plants not exposed to salt stress. Similarly, both Ti dosis (75 and 150 mg L⁻¹), decreased fruit pH in plants treated with 50 mM NaCl (Figure 2A). These data demonstrate that without salt stress or under moderate salinity, Ti decreases fruit pH as the concentration of organic acids increases (Simon *et al.*, 1991). The effect of Ti on the decrease of pH in fruits is counteracted under high salinity conditions; thus, with the application of 100 mM NaCl, both Ti doses significantly increased the pH value of the fruits, with increases of 2.0 and 1.4% with 75 and 150 mg Ti L⁻¹, respectively, as compared to the treatment with 100 mM NaCl and no foliar application of Ti (Figure 2A).

Salinity increases EC and TSS values in tomato fruits (Rodríguez-Ortega *et al.*, 2019); however, in this study this increasing trend in EC and TSS by salinity was only observed in treatments without Ti; in other words, no effects of Ti on the EC and TSS of fruits of plants were observed without saline stress (Figures 2B and 2D). With moderate salinity (50 mM NaCl), EC and TSS of fruits were positively related to the concentration of Ti. On the contrary, with high salinity (100 mM NaCl), the Ti dose was inversely related to the EC value, while both Ti doses reduced TSS with high salinity (Figure 2B).

TA is a quality indicator that is dependent on the concentrations of organic acids in fruits; this variable plays a pivotal role in tomato maturation and taste (Huang *et al.*, 2015). In our study, in the same way as for EC and TSS, Ti effects on TA are dependent of salt stress concentration, since with 50 mM NaCl Ti increases TA; contrarily, with 100 mM NaCl, Ti reduces TA (Figure 2C). Consequently, the TSS/TA ratio increases significantly by spraying the leaves with Ti only in the presence of high salinity (100 mM NaCl; Table 4).

Although the responses produced by beneficial elements such as Ti are represented by biphasic models, at low-doses stimulation and high-doses of inhibitory effects (Gómez-Merino and Trejo-Téllez, 2018), these results show that the Ti effect depends too much on the intensity of salt stress. Hence, in addition to specific factors related to the species or variety, the intensity and duration of stress treatments can lead to different results (Colmenero-Flores and Rosales, 2013).

Conclusions

Salt stress induced by NaCl application in the nutrient solution negatively affected yield parameters in tomato, while titanium had no effects on these variables. In terms of fruit quality, NaCl supplied in the nutrient solution decreased the pH of the fruit and significantly increased titratable acidity (TA), electrical conductivity (EC), total soluble solids (TSS) and the TSS/TA ratio in tomato fruits. Foliar spraying of Ti on tomato plants significantly decreased the pH value of the fruit and increased the TSS/TA ratio.

The NaCl concentration in the nutrient solution influenced the effects of Ti. In the low NaCl dose evaluated (50 mM), Ti decreased fruit pH, while with 100 mM NaCl, Ti increased fruit pH. The same

tendency was observed regarding titratable acidity. Moreover, Ti increased the TSS/TA ratio in tomato, only under high salinity conditions (100 mM NaCl).

In conclusion, the foliar application of Ti to tomato plants did not mitigate the negative effects of the salt stress on yield parameters, though it did improve some fruit quality features. Hence, our study sets the tone for further investigation aimed at exploring new Ti sources, application timing and strategy, as well as concentrations in tomato and other vegetable crops grown under stress conditions. To this aim, more in-dept physiological, biochemical and molecular approaches have to be carried out in order to uncover the mechanisms triggered by Ti during the whole plant cycle, with special focus on fruit ripening. To date, the molecular mechanisms by which Ti regulates plant physiology and metabolism remain poorly characterized, especially under stress conditions. Hence, future perspectives and issues posed in here may contribute to a better biological and biochemical understanding of plant responses to beneficial elements such as Ti, and how these affect the traits of plant organs such as fruit; thereby, contributing to both molecular plant biology and industrial horticulture for better crop production.

Authors' Contributions

Conceptualization: LITT and FCGM; Formal analysis: VHCV and LITT; Funding acquisition: LITT, FCGM and GAG; Investigation: VHCV, JAHC and ACO; Methodology: LITT, FCGM and GAG; Supervision: LITT and GAG; Validation: LITT and JAHC; Writing - original draft: VHCV, LITT and FCGM; Writing - review and editing: LITT, FCGM and ACO. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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Supplementary Material

Supplementary material 1. Main effect of Ti on yield parameters evaluated in the 2nd cluster of tomato plants cv. 'Río Supremo'

Ti (mg L ⁻¹)	Number of fruits	Fruit diameter (mm)		Yield of 2nd cluster (g)
		Equatorial	Polar	
0	5.67 ± 1.30 a	39.91 ± 3.56 a	47.00 ± 4.86 a	306.30 ± 140.55 a
75	5.80 ± 1.18 a	41.80 ± 3.42 a	48.10 ± 4.37 a	301.16 ± 118.50 a
150	6.33 ± 1.20 a	41.60 ± 2.52 a	47.49 ± 3.85 a	331.86 ± 125.46 a

Means ± SD with the same letters in each column are not statistically different (Tukey, $p > 0.05$)

Supplementary material 2. Effect of the interaction of study factors (NaCl × Ti) on yield parameters evaluated in the second cluster of tomato plants cv. 'Río Supremo'

NaCl (mM)	Ti (mg L ⁻¹)	Number of fruits	Fruit diameter (mm)		Yield of 2nd cluster (g)
			Equatorial	Polar	
0	0	8.4 ± 0.97 a	48.9 ± 0.26 a	60.0 ± 1.11 a	670.7 ± 71.5 a
	75	8.2 ± 0.96 a	49.8 ± 1.53 a	58.4 ± 3.17 a	609.4 ± 46.8 a
	150	9.0 ± 0.61 a	47.5 ± 2.02 a	56.6 ± 1.95 a	646.4 ± 79.0 a
50	0	5.0 ± 1.00 a	37.3 ± 0.54 a	41.8 ± 0.45 a	160.6 ± 29.2 a
	75	5.6 ± 0.45 a	38.9 ± 1.46 a	42.6 ± 1.81 a	203.2 ± 17.4 a
	150	6.2 ± 0.55 a	39.5 ± 0.99 a	44.1 ± 2.29 a	234.2 ± 8.3 a
100	0	3.6 ± 0.45 a	33.6 ± 1.98 a	39.3 ± 1.42 a	87.6 ± 9.6 a
	75	3.6 ± 0.67 a	36.7 ± 2.37 a	33.3 ± 1.99 a	90.8 ± 12.4 a
	150	3.8 ± 0.42 a	37.8 ± 0.80 a	47.7 ± 1.70 a	114.9 ± 13.2 a

Means ± SD with the same letters in each column are not statistically different (Tukey, $p > 0.05$)