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EFFECTS OF COPPER NANOPARTICLES ON THE ORGANOLEPTIC, PHYSICOCHEMICAL AND NUTRACEUTICAL PROPERTIES OF GRAFTED TOMATO FRUIT

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Abstract

The changes in the metabolic and physiological processes that occur when a tomato plant's management, nutrition, and growth environment are modified, are reflected in modifications to the chemical and physical characteristics of its fruit. As such, the object of the present study was to evaluate the changes in the organoleptic properties and the physicochemical and nutraceutical quality of tomato fruit in response to the interaction between grafting and the application of copper nanoparticles (CuNPs). A total of six treatments with or without grafting and either a foliar application (50 g L^{-1}) or a substrate (5 mg/plant) of CuNPs were tested. The results show that grafting tomato plants in combination with substrate, application of CuNPs promotes an increase (21.3%) in fruit equatorial diameter, therefore the fruit of larger size and greater firmness (63.7%), and a lighter red color suggesting a decrease in the ripening index. The highest concentrations of nutraceutical compounds evaluated were recorded when the experimental factors were applied individually.

Key words: Graft, Antioxidant, nanofertilizer, *Solanum lycopersicum*.

Introduction

The use of alternative technologies and their integration into hoticultural cultivation systems, such as tomato, helps mitigate the problems that can cause not only reductions in production, but also deteriorations in the organoleptic or nutritional qualities of the fruits, for example, favorable results in crop production have been obtained with the use of grafting, an environmentally friendly technique (Geboloğlu *et al.*, 2013). The use of the appropriate rootstock can promote greater tolerance to root diseases, making, grafting a viable alternative to the use of pesticides for the control of soil pathogens (Ashok & Sanket, 2017). Additionally, grafting can promote greater nutrient absorption (Santa-Cruz *et al.*, 2002), thereby reducing the negative effects of an unbalanced nutrient supply, favoring photosynthetic activity, and activating the antioxidant response mechanism, which lead to improvements in plant growth, yields, and nutritional and organoleptic quality (Flores *et al*., 2010). There are even several reports about grafting that show better resistance to abiotic stress events, such as low temperatures, salinity and toxicity of heavy metals (Kyriacou *et al.*, 2017; Gaion *et al*., 2018, León-Calvario *et al.,* 2020).

Another technique that has recently taken off in agronomy is the use of nanotechnology in crop production systems, that is to say, the use of nano-scale mineral elements, which elicit different effects that their conventionally sized counterparts. Nanofertilizers have become more common every day since improving the efficiency of nutrient uptake leads to improvements in crop production and nutraceutical quality (Fraceto *et al*., 2016). Although the effects of nanoparticle (NP) application on different crops have been studied, the response varies according to the plant species, dose, type of application, and size and shape of the nanoparticles. Fu *et al*., (2014) mention that copper nanoparticles (CuNPs) promote antioxidant activity based on their concentration, which suggests that they could be used as biostimulants for the production of nutraceutical compounds. Other authors report that the application of CuNPs encapsulated in Chitosan (CS) hydrogel´s improves the growth and quality of tomatoes, increasing their lycopene content (Juaréz-Maldonado *et al*., 2016).

Although the use of these technologies separately has been associated with positive outcomes in some crops, there is not enough information on the effects elicited by the combination of the two. Only a single other study reported that the synergy between these two techniques improved the organoleptic quality of watermelon and stimulated the plant antioxidant defense mechanisms (González-Gómez *et al*., 2017). Operating under the hypothesis that the combined use of nano metals and grafting will have some effect on the quality of tomato fruit, the objective of the present study was to evaluate the changes in the organoleptic characteristics and the physicochemical and nutraceutical qualities of tomato fruit in response to both grafting and CuNP applications.

Methods and Materials

Location of the experiment: The study was carried out in diffuse plastic-covered greenhouses in the experimental agricultural fields belonging to the Antonio Narro Agrarian Autonomous University Department of Horticulture, located in Buenavista, Saltillo, Coahuila,

Mexico (25°22" N, 101°00" W) at 1742 m.a.s.l, during the 2019 spring-summer cycle. The nutraceutical characteristics of the fruits were analysed at the post-harvest laboratory of the Institute of Farming Science, Tulancingo, Hidalgo, Mexico.

Preparation of plant material and grafting: The tomato ball-type Piranha variety (Syngenta, Mexico) and Colosus RZ F1 (Rijk Zwaan, Netherlands) were grown from seeds, the latter being chosen as the rootstock. Both tomato varieties were sown in February 2019. The Piranha scions were sown first and 15 days later, the Colosus RZ F1 rootstocks were sown. Both were sown in 200 cell polystyrene trays filled with germination media (peat moss).

The cleft grafting method was used to join the scions and rootstocks. When the seedlings reached a stem diameter of 2 mm, the upper end of the rootstock seedling was removed, leaving only the cotyledons and a downward incision was made in between them. The upper ends of the scion seedlings were prepared by making a "V"-shaped incision of the same length as the rootstock incisions. The cut scion stems were inserted into the rootstock incision so that the two plants were in direct contact and a silicon clip was used to hold the two together while the graft took. After grafting, the seedlings were kept in a grafting chamber for six days in the dark, relative humidity of 80%, and a temperature of 25 °C. Afterwards, they were left in an acclimatization chamber for four days, under the same environmental conditions, but in the presence of light.

After grafting, the seedlings were transplanted to 10 kg polyethylene bags containing a 1:1 v/v mix of peat moss and pearlite, kept in a plastic tunnel greenhouse with an average daytime temperature of 27°C, average nighttime temperature of 20°C, and 60% relative humidity. The plants were grown at a density of three plants per square meter. The tomato plants were kept to a single, trained stem by pruning axillary shoots. Thinning fruit to four fruits per bunch was performed to regulate the load and the size of the tomato fruit.

A drip irrigation system was used to supply water to the crop. The system was run 11 times a day at 4 L h-1 and was adjusted according to the development of the plant using a Rain Bird irrigation controller. Steiner nutrient solution (Steiner, 1984) was used for crop fertilization. The concentration of nutrient solution was adjusted according to the stage of crop development: 25% during vegetative growth, 50% during flowering, 75% during fruit formation, and 100% during fruit maturation. Up to 5.8 L per plant were provided daily during times of peak demand. The pH of freshly prepared nutrient solution was adjusted to 6.8 using phosphoric acid.

Finally, at 90 days after transplanting, when the sixth bunch of fruit emerged, the apical bud was eliminated in order to prevent further growth and await the ripening of the tomato fruit.

Grafting and copper nanoparticle treatments: Six experimental treatment groups, with ten repetitions each, were employed: plants that were neither grafted nor treated with CuNPs (controls, T1), grafted plants untreated with CuNPs (T2), ungrafted plants with CuNPs applied to substrate (T3), grafted plants with CuNPs applied to substrate (T4), ungrafted plants with foliar application of CuNPs (T5), and grafted plants with foliar application of CuNPs (T6).

The application of NPC to substrate was performed when transplanting. After adding 2.5 L of growth substrate to a culture bag, 0.33 g of CuNP would be added to the substrate. This was repeated twice over until the complete dose of CuNPs was applied and the culture bags filled to their limit. The foliar application of CuNPs consisted of spraying an aqueous solution (50 mg L^{-1}) of CuNPs over the tomato plants in 0, 30, 60, and 90 days after transplant.

The copper nanoparticles used in this study were synthesized by the Center for Applied Chemistry Research, according to the methodology described in Ortega-Ortiz *et al.,* (2013). The NPs were 99.8% pure and possessed a spherical morphology with an average diameter of 25 nm.

Sample selection: Four fruit was selected among the ten plants in each treatment group. When these reached an average diameter of 4–5 mm, they were tagged and each week their equatorial diameters were measured with the help of an electronic Vernier caliper. Harvesting was done around 60 days after transplanting. Four completely red tomato fruit was randomly picked from the third bunches from each treatment group.

Physicochemical characteristic evaluation: The fresh weight of fruit (g) was recorded using a digital OHAUS scale (model YA501E). The longitudinal and equatorial diameters of all the selected fruit were measured with Vernier calipers. Fruit firmness (kg cm-2) was evaluated using a penetrometer (Fruit Pressure Tester FT 327).

A potentiometer (Hanna pHpe®5) was used to measure the pH. Total soluble solids (TSS) content was measured in °Brix using a digital refractometer (Hanna HI 96801) at 20°C. Titulable acidity (TA) was evaluated by titration with 0.1 N NaOH, using a phenolphthalein indicator solution (Anon., 2000), and expressed as a percentage of citric acid according to the AOAC method 942.15. The index of fruit ripeness was obtained from the ratio of TSS/TA.

Fruit color was measured with a HunterLab colorimeter (Minolta, CM508d, Minolta Camera Co. Ltd., Osaka, Japan). Values for luminosity (L*), green-red (a*), yellow-blue (b*) were obtained and used to calculate the chroma (C*) and color angle (°h).

Nutraceutical content evaluation: Samples of fruit pulp from the selected tomatoes from each treatment group were frozen in an ultrafreezer (Thermo Scientific 303 Ultrafreezer) at -82°C for 72 hrs. Subsequently, the frozen pulp samples were lyophilized (Labconco, FreeZone 6, Kansas City, MO, USA) over 72 hours at -84°C and 0.060 mbar. The samples were then ground in a knife mill (RTSCH GM 200, Germany) for 50 s in order to obtain a fine powder. A UV/Vis spectrophotometer (Varian CARY 100BIO, Turin, Italy) was used to determine the quantities of the various nutraceutical compounds.

Ascorbic acid content determination: The determination of ascorbic acid content was performed according to the method described by Dürüst *et al*., (1997) as modified by López-Palestina *et al*., (2018). The absorbance of the samples was measured with a UV/Vis spectrophotometer at 520 nm and the results were expressed as mg of ascorbic acid per g of fresh weight (mg AA g^{-1} FW).

Lycopene and carotene content determination: The concentration of lycopene and β-carotene was determined by spectrophotometry according to the method described by Goula & Adamopoulos (2005) with modifications. Fruit pulp samples (0.1 g) were mixed with 10 mL hexane and homogenized for 5 minutes using a vortex mixer. The mix was centrifuged at 10,000 xg for 10 min. (Thermo Scientific centrifuge, model ST 16R, Germany). Lycopene and β-carotene content were determined by measuring the supernatant absorbance at 503 nm and 478 nm, respectively.

Total antioxidant capacity determination: Two methods involving metastable radicals were used to determine the total antioxidant capacity (TAC). The first was the 2,2′-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) method described in Re *et al*., (1999), then measuring the absorbance at 734 nm.

The other method used was the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method developed by Brand *et al*., (1995). The absorbance was measured at 517 nm.

The results to ABTS and DPPH were expressed as µM Trolox equivalents per g fresh weight (µM TE g^{-1} FW).

Total phenol content determination: The method described by Singleton *et al.,* (1999) was used to determine the total phenol content. The mixtures were measured at a wavelength of 760 nm. The results were expressed in mg gallic acid equivalents per 100 g fresh weight (mg GAE 100 g^{-1} FW).

Flavonoid content determination: Flavonoid content was quantified according to the Dowd method, as adapted by Arvouet-Grand *et al*., (1994). The mixtures were measured at a wavelength of 415 nm. The flavonoid content was expressed as mg quercetin equivalents per 1 g fresh weight (mg QE g^{-1} FW).

Experimental design: The experimental variables chosen resulted in a 2x3 experimental factor matrix. The factors evaluated were the state of grafting (grafted or ungrafted) and the application of Cu-NPs (root, foliar, and no application), resulting in six experimental treatments with ten repetitions per treatment, set up in a randomized design. Each experimental unit was a single plant, from which four randomly chosen fruits were picked for quality analysis.

Statistical analysis: The collected data were subjected to analysis of variance (ANOVA) as well as least significant difference tests (LSD, $p \leq 0.05$) to compare the means of the evaluated factors.

Results

Fruit growth: The progression of fruit growth, as measured by equatorial diameter, week by week is shown in Fig. 1. From week 4 onwards, the effects of grafting on fruit size are evident, with a 15.7% increase in equatorial diameter. The positive effect is maintained throughout the following weeks. In week 7, the fruit from grafted plants with CuNPs applied to their substrate presented a 14.9% increase in diameter compared to fruit from ungrafted and untreated plants, and a 30.9% size increase compared to fruit from plants with only substrate application of CuNPs.

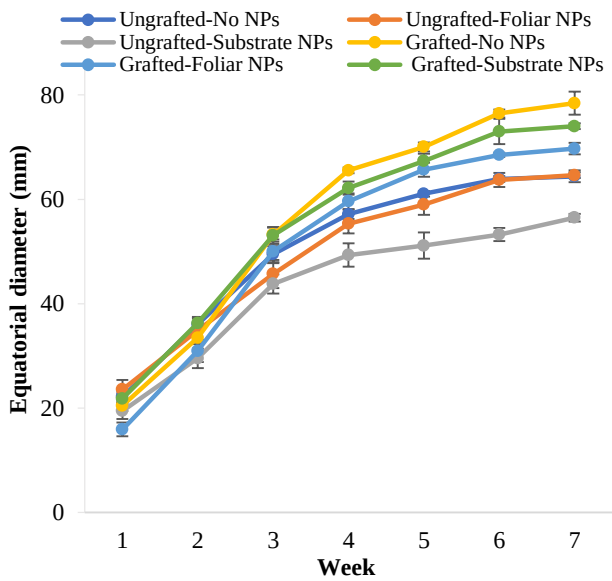

Fig. 1. Mean and standard error of equatorial diameter growth in tomato fruit from plants subjected to grafting and CuNP application.

Physicochemical characteristics: The use of grafting affected the weight and diameter of the tomato fruit, increasing the fresh weight by 62.6%, the longitudinal diameter by 18.65%, and the equatorial diameter by 23.73%. The results of the interactions between the experimental factors are shown in Table 1. It can be observed that the use of grafting with either form of CuNP application resulted in fruit weight and diameter increases.

The interplay between factors has also results in a positive effect on the luminosity (L) and hue angle (H°), components of the fruit color. Fruit from grafted plants with substrate CuNP application demonstrated an 18.4% improvement in these components compared to fruit from ungrafted plants with similar CuNP application. However, there is no appreciable difference in Chroma* between the different treatments.

Regarding fruit firmness, grafting either with or without substrate CuNP application resulted in positive increments in firmness (23.8% and 63.7%, respectively), compared to ungrafted plants with similar CuNP application.

The combination of grafting with nanoparticle application was not associated with an improvement in tomato fruit pH and TA, as seen in Table 2. Improvements in TSS concentrations and TSS/AT ratios were observed in fruit from ungrafted plants with foliar CuNP application (16.8% and 16.9%, respectively), compared to fruit from grafted plants with the same CuNP application.

Table 1. Physical characteristics of tomato fruit obtained from grafting and CuNP application.

Graft	CuNPs	FW (g)	LD (mm)	ED (mm)	L*	Color ° H	C*	Firmness (kg cm^{-2})
No	No	141.85ab	51.66b	63.34b	35.28ab	48.73a	27.99a	2.95b
	Foliar	56.4c	42.39b	47.30c	33.09abc	46.00a	27.61a	3.93ab
	Substrate	74.60c	44.73b	54.09c	30.31c	39.01b	28.68a	3.90ab
Yes	No	146.88ab	55.7a	67.74ab	32.20bc	42.21ab	28.17a	5.40a
	Foliar	148.93a	54.83a	71.66a	32.46bc	42.39ab	28.27a	4.13ab
	Substrate	129.18a	54.77a	64.46ab	35.89a	47.03a	27.38a	4.83a
ANOVA								
Graft		**	**	**	NS	NS	NS	0.019
CuNPs		**	NS	0.034	NS	NS	NS	NS
Graft+CuNPs		**	NS	**	**	0.013	NS	NS
CV (%)		19.93	8.39	8.10	6.38	10.49	5.95	27.2

LD (Longitudinal diameter), ED (Equatorial diameter), L (Luminosity (L*)), Chroma (C *), color hue (°h), ANOVA (analysis of variance), ** (Highly significant, p<0.01), CV (coefficient of variance). Means with the same letter do no present significant differences according to LSD (p≤0.05)

Table 2. Chemical characteristics of tomato fruit obtained from grafting and CuNP application.

Graft	CuNPs	pH	TSS	TA	TSS/TA
No	No	4.20a	4.53d	0.34a	13.64b
	Foliar	4.13ab	5.55a	0.34a	16.73a
	Substrate	4.21a	5.05bc	0.35a	14.69b
Yes	No	4.08ab	5.25ab	0.36a	14.70b
	Foliar	4.18b	4.75cd	0.34a	14.31b
	Substrate	4.03a	4.43d	0.34a	13.34b
ANOVA					
Graft		NS	0.039	NS	NS
CuNPs		NS	0.016	NS	NS
Graft+CuNPs		NS	**	NS	0.045
CV (%)		2.5	5.23	5.97	9.03

TSS (° Brix), TA (% citric acid), ratio TSS/TA (ripeness index), ANOVA (analysis of variance), ** (Highly significant, p<0.01), CV (coefficient of variance). Means with the same letter do no present significant differences according to LSD (p≤0.05)

Table 3. Nutraceutical compounds in tomato fruit obtained from grafting and CuNP application.

Graft	CuNPs	Vitamin C	CT	Lycopene	ABTS	DPPH	TF	Flavonoids
No	No	58.51c	8.44d	36.31e	2.40d	1.80d	21.17c	99.02c
	Foliar	66.49a	9.05c	42.50d	2.83a	2.14a	24.08a	105.83a
	Substrate	55.16d	11.17a	63.14a	1.75f	1.66e	17.91e	80.50e
Yes	No	61.08b	11.25a	59.53b	2.54c	1.89c	22.38b	102.37b
	Foliar	51.56e	10.83b	58.96c	2.73b	1.92b	20.58d	79.54e
	Substrate	42.41f	7.54e	36.23e	1.90e	1.66e	18.22e	93.28d
ANOVA								
Graft		**	**	**	**	**	**	**
CuNPs		**	**	**	**	**	**	**
Graft + CuNPs		**	**	**	**	**	**	**
CV (%)		1.03	1.41	0.33	1.10	0.93	1.05	0.87

FW (Fresh Weight), Vitamin C (mg Ascorbic Acid 100 g^{-1} FW), TC (Total Carotenoids, mg 100 g^{-1} FW), Lycopene (mg 100 g^{-1} FW), TAC by ABTS and DPPH (µM Trolox 1 g^{-1} PS), TF (Total Phenols, mg Gallic Acid 100 g^{-1} FW), Flavonoids (mg Quercetin 100 g^{-1} FW)), ANOVA (analysis of variance), ** (Highly significant, p<0.01), CV (coefficient of variance). Means with the same letter do no present significant differences according to LSD (p≤0.05)

Nutraceutical content: Regarding the vitamin C content, in the absence of CuNPs, the use of grafting alone increases the vitamin C content in tomato fruit by 4.3% compared to the ungrafted treatment. The results of the interactions between grafting and CuNP application are shown in Table 3. The fruit from ungrafted plants with foliar or substrate CuNP application presented the highest concentrations of vitamin C, with increases of 28.9% and 30.0%, respectively, compared to fruit from grafted plants with the same CuNP treatment.

As for lycopene and β-carotene content, grafting led to their increase (8.9% and 3.3%, respectively) compared to the ungrafted treatment. The data in Table 3 illustrates the interplay between grafting and CuNP application. In the absence of CuNPs, fruit from grafted plants have 63.9% and 33.29% greater lycopene and β-carotene content, respectively, than their ungrafted counterparts. Substrate application of CuNPs to ungrafted plants increases lycopene by 74.2% and β-carotene by 49.4%, compared to grafted plants with similar CuNP treatment.

The results from the TAC assays show that grafting alone has a positive effect on the total antioxidant capacity of tomato fruit. The ABTS assay for fruit from grafted plants demonstrated a 2.5% increase while the DPPH assay showed a 2.2% increase in TAC. Between the two different CuNP treatments, foliar application showed the more significant increases in TAC. In terms of the effects from combining grafting and CuNP application, fruit from ungrafted plants with foliar CuNP application presented with the highest TAC from among all the treatments. Compared to fruit from grafted plants with the same CuNP treatment, these had TAC increases of 3.6% and 11.4%, according to the respective ABTS and DPPH assays, while compared to fruit from ungrafted plants with CuNP application to substrate those increases were 61.7% and 28.9%, respectively.

Total phenol content increased by 5.3% and flavonoid content by 3.3% in fruit from grafted plants. Foliar application of CuNPs alone resulted in increases of 22.3% and 23.5%, respectively, for the same compounds. The data from the combination of the two factors shows that fruit from ungrafted plants treated with foliar CuNPs ended up with the highest concentrations of total phenols and flavonoids compared to all the other treatments. Those fruit had 17% and 33% more TF and flavonoid content, respectively, than fruit from grafted plants with the same CuNP treatment, and 34.4% more TF and 31.4% more flavonoid content than fruit from ungrafted plants with CuNPs applied to the substrate.

Discussion

Fruit growth: The patterns seen in fruit growth (Fig. 1), as measured by their diameter, correspond to the three phases of fruit development: cell division, cell expansion, and fruit ripening. Of those, the greatest growth was seen in the fruit from grafted plants with CuNPs in their substrate. The rootstock of grafted plants confers a more efficient radicular system with greater water and nutrient uptake capabilities (Pogonyi *et al*., 2005), which leads to the greater quantity of copper nanoparticles being absorbed through the roots. This is significant since nano scale copper is involved in the activation of plant growth hormone synthesis (Lira-Saldivar *et al*., 2016), which are directly implicated in cellular division and elongation, themselves processes that could lead to the increases in fruit diameter seen.

Physicochemical characteristics: The greatest increments in fruit fresh weight, longitudinal and equatorial diameters at harvest time were observed in tomato fruit from grafted plants and CuNP application in either form. This suggests that the increase in growth during the period of cellular expansion in fruit could be attributed to a combination of changes in biophysical processes such as water flow, turgor pressure, and osmotic tension, and agronomic factors: leaf area and canopy water demands and adjustments in root system hydraulic conductivity (Gambetta *et al*., 2012) stemming from the more efficient radicular system conferred by the rootstock and the introduction of copper nanoparticles, which promote the synthesis of auxins and cytokinins (Lira-Saldivar *et al*., 2016). Those compounds promote cellular division and elongation, which in turn are determining factors in the formation and development of fruit, resulting in fruit with greater weight and larger diameters.

Tomato fruit from grafted plants with copper nanoparticles applied to the substrate exhibited a light red color over more than 60% of their surface, in contrast to their counterparts from ungrafted plants with the same copper treatment and all the other treatments. This can be explained by the improved copper uptake of the rootstock radicular system. The higher concentration of copper causes changes in the expression of genes involved in the biosynthetic pathways responsible for the synthesis of the pigments that give tomatoes their red color (Chen, 2014). The intensity of the color was similar in all treatments, although this variable is not a reliable indicator of tomato fruit ripening, since it only indicates the purity or saturation of a single color. Even different colors can have the same chroma values (Barrett *et al*., 2010).

The increase in fruit firmness observed in this study suggests a variation in cell morphology and turgidity, combined with the ease of absorption of CuNPs due to the grafted rootstock, once absorbed, the nanoparticles cause the accumulation of some proteins that are responsible for fruit pericarp cell wall lignification, which are translocated to the fruit cell wall after CuNP absorption. This response is consistent with that reported by Juárez-Maldonado *et al*., (2016), who mention that the application of CuNPs in chitosan hydrogels improved the firmness of tomato fruit by 9%. Other studies have shown that application of CuNPs at 250 mg L^{-1} and 125 mg L^{-1} increase fruit firmness by 28.9% and 23.6%, respectively (López-Vargas *et al*., 2018).

On the other hand, pH and TA values are within the average range for tomato: 4.2–4.4 pH and 0.35–0.4% for TA (Bartell *et al.,* 2010). This suggests that either factor alone or in conjunction does not influence the acidity of tomato fruit. Increases in the TSS content of fruit are seen in plants treated with foliar CuNPs. In this case, the CuNPs enter the plant through the cuticle, epidermis, trichomes, and stomas reach the vascular system, where they generate changes in carbohydrate metabolism,

leading to greater concentrations of organic acids and greater capacity to accumulate sucrose during fruit ripening, at the expense of fructose and glucose (Magwaza & Opara, 2015). The observed response coincides with Juárez-Maldonado *et al*., (2016), whom report a delay in ripening and improved shelf life of tomato fruit following CuNP or CuNP with chitosan application. Consequently, the changes seen in the ratio of fruit TSS/TA after foliar application of CuNPs to ungrafted plants suggests that the nano scale copper leads to a reduction in acidity and influences the fruit respiration process, resulting in a delay in ripening.

Nutraceutical content: When it comes to the nutraceutical content of the tomato fruit, the use of grafting increased the vitamin C content, in part because the precursor to ascorbate is glucose, which in this case is derived from a high production of photoassimilates, obtained from an improved photosynthesis thanks to the abundant foliar area of grafted plants (Flores *et al.,* 2010). However, when it comes to the interaction between the two factors, ungrafted plants treated with foliar CuNPs also have elevated vitamin C levels. This is due to the copper's ability to complex with the ascorbate, which apart from being the most abundant antioxidant compound in the plant also has great reductive potential (Reátegui *et al.,* 2008). Thus, the copper precipitates a rapid regeneration of ascorbate and a redistribution of the vitamin C content to other plant organs, such as the fruit. This result is in line with those reported by López-Vargas *et al*., (2018), who observed increased vitamin C concentration in tomato fruit following foliar CuNP treatment. These results suggest that the synthesis of vitamin C in plants may be induced by the presence of copper.

In this study, grafting was also seen to improve the carotenoid and lycopene content of tomato fruit, given that lycopene is a precursor to other carotenoids. The use of grafting and the vigor it confers leads to an increase in chlorophyll, whose decomposition leads to the synthesis of other plant pigments as it is the biomolecule primarily responsible for tissue coloration changes (López-Camelo & Gómez, 2004). However, after assessing the interaction between the two experimental factors, the highest concentrations of lycopene and carotenoids were found in fruit from ungrafted plants with CuNPs in their substrate. This behavior suggests that in the presence of copper, genes related to the synthesis of lycopene and carotenoids are active, leading to differential biochemical profiles in tomato fruit (Chen, 2014). Other reports corroborate this result, with a 12% lycopene increase in tomato fruit seen after application of CuNPs with chitosan (Juárez-Maldonado *et al.,* 2016) and a similar increase in watermelon from grafted plants with 0.4 mg of CuNPs in their substrates (González-Gómez *et al*., 2017).

Greater antioxidant capacity was observed after foliar application of CuNPs in ungrafted plants. This could be caused by the presence of CuNPs, which stress that plant and lead to the generation of reactive oxygen species that in turn activate the plant's defense mechanisms and result in the production of antioxidant compounds to stabilize the oxidative agents (González-Gómez *et al*., 2017). Similar increases in the total antioxidant capacity of tomato fruit following foliar application of CuNPs have been previously reported (López-Vargas *et al.,* 2018). Likewise, a similar response is observed in the concentrations of phenolic compounds and flavonoids of fruit from plants treated with foliar CuNPs. These compounds are some of the most important antioxidants involved in regulating the production of oxidative species and protecting the cellular membranes from oxidative damage, which can lead to a delay in fruit senescence (Khatun *et al*., 2008). As such, the combination of grafting and foliar application of CuNPs not only improves antioxidant activity, but also leads to a delay in fruit ripening.

Conclusions

Grafting in conjunction with substrate CuNPs promotes fruit diameter growth, starting at the fourth week and reaching a greater increase in size by the seventh week. Fruit firmness is also improved and a dominant light red color suggests delay in ripening, which favor a longer fruit shelf life. Heavier and larger (longer, wider) tomato fruit was obtained from grafted plants with CuNPs applied to their foliage.

Grafting alone, without CuNP application, improves carotenoid content. This includes provitamin A and lycopene, which led to fruit with a more intense red color and higher concentrations of those antioxidant compounds. Adding CuNPs to the substrate of ungrafted plants only improves carotenoid content and lycopene.

Vitamin C content, the primary source of antioxidants in fruit, is improved by the use of grafting in the absence of CuNPs. Foliar application of CuNPs of ungrafted plants results in fruit with greater TSS and lower acidity, as well as improved vitamin C content, total antioxidant capacity, and phenolic compound concentrations (total phenols, flavonoids). This improved nutraceutical compound profile favors cellular membrane protection against oxidation, which manifests as a delay in fruit ripening.

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