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Article

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Abstract: Malnutrition problems are related to diets that lack one or more essential elements, which it is extremely important to food enrichment to improve the content of compounds effective for health. The objective of this study is to evaluate the effect of the addition of Ca²⁺, K⁺ and Mg²⁺ on the induction of nutraceutical quality in grafted tomatoes grown in the NFT (Nutrient Film Technique) system. In the experiment, two factors were evaluated: plants with and without grafting and the foliar application of 10 ml L⁻¹ Ca²⁺, 4 ml L⁻¹ K⁺ and 10 ml L⁻¹ Mg²⁺ cations separately for each case, in addition to the control. The treatments were evaluated using a completely randomized design, with 5 repetitions per treatment. The interactive effects between grafting and foliar application of Ca²⁺ induced a greater content lycopene, β-carotene and total flavonoids in fruits of tomato. The interactive effects between the graft and the Mg²⁺ foliar applications obtained the best results in the phenol content. As regards, capacity DPPH and ABTS, the graft decreased concentration, increasing the content of these through foliar applications of calcium. Regarding the enzymatic variables, graft factor increased activity catalase, glutathione peroxidase and phenylalanine ammonium lyase, obtained the best results graft interaction and foliar application of calcium and magnesium. The results obtained demonstrate the influence of the use of grafts and foliar applications of calcium and magnesium as tools that could improve the nutraceutical quality content of tomato crop.

Keywords: enzymatic antioxidants; non-enzymatic antioxidants; graft; foliar

1. Introduction

Several studies have demonstrated the effects of the minerals Ca²⁺, K⁺ and Mg²⁺ on the detoxification of reactive oxygen species (ROS) such as superoxide radicals (O²⁻), hydrogen peroxide (H₂O₂) and hydrogen peroxide (H²O₂). The excessiveness of ROS causes oxidative damage to the deoxyribonucleic acid (DNA), proteins, and lipids [2–4]. Exogenous applications of mineral elements such as Ca²⁺, K⁺ and Mg²⁺ benefit the growth, crop yield and the quality of agricultural products, since each mineral element plays a key role in the physiological processes of the plant [1]. The Ca²⁺ is the main component of cell walls and plays an important role in membrane permeability; it also improves the growth and development of plant germination and pollen. It also increases the enzymatic speed, accelerating mitosis, increasing cell size, and producing good-quality fruit [5]. Also, acts as an important second messenger in plant cells in response to various endogenous or environmental cues [6].

For its part, Mg^{2+} plays a central role in chlorophyll biosynthesis and CO_2 fixation, activating the enzymes involved in photosynthesis and ATP biosynthesis, favoring the biosynthesis of nucleic acids and proteins. In addition, Magnesium is also involved in the transport of metabolic substances [7].

Similarly, K^+ plays an important role in the quality of the fruit by being involved in metabolic processes. Such as enzyme activation, protein synthesis, and membrane transport processes, since K^+ has a strong mobility in plants, carrying out an important role in the regulation of cellular osmotic pressure and the balance of cations and anions in the cytoplasm; Through these processes, K^+ participates in the regulation of stomatal opening and closing, cell elongation, and other important physiological processes [8].

The amount and type of nutrients supplied to the tomato can influence not only its yield but also its nutrient content, flavor and postharvest storage quality, however, to maintain high production levels and high quality, it is necessary to use a high amount of fertilizers and large volumes of water, which increase production costs and negatively affect the environment and human health [9]. Therefore, the use of tools such as recirculating nutrient solution systems seems to be the most rational solution for this type of problem. Sambo *et al.*, [10] established a nutrient solution that covers all the nutritional requirements of plants; it maximizes both the yield and the quality of agricultural products. In this sense, hydroponic cultivation systems also allow a better reproducibility in plant growth and yield as well as in the quality of the agricultural products in terms of nutraceuticals content [11]. Grafting provides higher yield, quality and tolerance to avoid or reduce yield loss caused by biotic and abiotic stress [12].

2. Materials and Methods

2.1 Experimental site and design

The work was carried out in a greenhouse conditions in the Department of Horticulture at Universidad Autónoma Agraria Antonio Narro, in Saltillo, Coahuila, México (latitude $25^{\circ}21'23.4''$, longitude $101^{\circ}02'10.6''$ and 1760 m above sea level. The conditions inside the greenhouse were 4.5 W/m^2 of solar radiation, a day maximum temperature of $37^{\circ}C$ and a minimum of $21^{\circ}C$, and relative humidity of 40%. The experiment started on February 16, 2021 and ended on August 3 of the same year. In the experiment, two factors were evaluated: plants with and without grafting and the foliar treatments whit recommended doses of $10\text{ ml L}^{-1}\text{ Ca}^{2+}$, $4\text{ ml L}^{-1}\text{ K}^{+}$ and $10\text{ ml L}^{-1}\text{ Mg}^{2+}$ separately for each case, in addition to the controls (grafted and non-grafted plants without foliar applications). The experimental design used for this experiment was completely randomized with 5 repetitions per treatment one plant per repetition. An analysis of variance and a comparison of means test were performed according to Fisher's LSD test ($p \leq 0.05$) with the statistical program InfoStat (InfoStatversion 1.0).

The pH adjustment was 6.8 and the electrical conductivity (EC) was 1.8 dS m^{-1} . Hoagland's solution was used whose sources of mineral elements with a concentration of 100% were: 500 mg L^{-1} of $Ca(NO_3)_2$, 0.083 mL L^{-1} of H_3PO_4 (53%), 0.311 mL L^{-1} of HNO_3 (55 %), 355 mg L^{-1} of KNO_3 , 302 mg L^{-1} of K_2SO_4 , and 197 mg L^{-1} of $MgSO_4$. It needs to be mentioned that 130 liters of water were used for a NFT (Nutrient Film Technique) station, whose concentration of the nutrient solution varied according to the phenological phase of the crop (vegetative development, flowering and fruit filling) with 50, 70 and 100% concentration.

The density of 9 plants per m^2 was established. In respect of treatments, the product AMIFOL K has been used as a source of potassium, with a composition of 31.0% w/w potassium and 5.12% free amino acids (w/w), the recommended dosage of 4 ml L^{-1} with an application every 15 days. The product HUMISOIL CA-16 has been used as a source of calcium, whose composition is based on 16% (w/w) calcium, 8% (w/w) nitrogen, 1.30% (p/p) phytohormones, 2% free amino acids (w/w), humic and fulvic acids 12% (w/w) and extenders and conditioners 60% (w/w), the recommended dosage of 10 ml L^{-1} with one application of each 15 days. Finally, as a source of Magnesium, the product HUMISOIL Mg-14 has been used, whose composition is based on magnesium at 14% (p/p), nitrogen 8% (w/w), phytohormones 1.30% (w/w), free amino acids 2% (w/w), humic and fulvic acids 2% (w/w)

and extenders and conditioners 51.70% (p/p), in the same way, the recommended dosage of 10 ml L⁻¹ with an application every 15 days. It was a total of 8 applications of the treatments per crop cycle.

2.2. Plant Material

As plant material, ball-type tomato seeds of the "LEZAFORTA" variety were used, a plant easily adaptable to temperate climate conditions, with excellent foliar coverage, continuous ties and short internodes, "FORTAMINO" tomato was used as a rootstock, a plant with an extra-vegetative habit, which has excellent foliar coverage for conditions of high luminosity and high temperatures. It allows lengthening the productive cycles and provides more growth to the tomato bunches. Both materials belonging to the commercial house Enza Zaden.

2.3. Graft

The LEAFORTA variety was sown in February 2021 in a 200-cavity polystyrene tray with peat moss as substrate and 10 days later, the FORTAMINO rootstock was sown in a 200-cavity tray, using peat moss as substrate. The reason for sowing the rootstock 10 days later was due to its characteristic greater vigor and vegetative growth, which allowed a better adaptation of the shoot size and stem width. This action made it possible to equalize the diameters of the stems, benefiting the union (grafting) of both plant structures. The grafting process was carried out when the plants reached a stem diameter of 3mm, which was obtained 32 days after planting the variety. For this, the splicing technique was used Lee *et al.* [13] which consists of a cut at an angle of 45° to the two plants to form the union of the stems, for the fastening of the union point of the graft. They used special 2-mm-wide silicone tweezers. Subsequently, the grafted plants were kept for 15 days in an acclimatization chamber, where they were kept in conditions of darkness and relative humidity of 95% for a period of 6 days, five with 50% shade and the rest of the days without shade, and a temperature ranging between 25 and 35 °C (Figure 1a). For the recovery of the plants, the application of the Phos Green Campbell product was carried out with a composition of N 12.790%, P₂O₅ 61.60%, Ca 01.05%, Mg 01.02%, Fe 0.190%, Zn 0.130%, Mn 0.11%, Cu 0.09%, B 0.11%, Mo 0.01%, S 1.480% and Co 0.01% to achieve timely nutrition at critical moments of plant development, at a dose of 1 g L⁻¹, were applied daily to the foliage with a manual sprinkler.

2.4. Transplant to NFT System

The transplant to the NFT (Nutrient Film Technique) system was carried out 15 days after grafting in the month of March 2021. The seedlings were removed from the substrate and their roots were rinsed with water. Subsequently, a fungicide was applied to the base of the seedlings as a preventive treatment against disease-causing microorganisms. Subsequently, the plants were transferred to plastic baskets for hydroponics of 3 inches in diameter. For the adjustment of the plant inside the basket, a polyurethane sponge was raised, leaving the root free so that it had contact with the nutrient solution (Figure 1b).



(a)



(b)

Figure 1. Treatments application: (a) management of scions and rootstocks; (b) transplant to NFT system.

2.5. Sample process for biochemical analysis

From each plant, five representative tomatoes with commercial maturity were harvested by treatments, making a total of 40 samples. Each sample consisted of 50 g of tomato stored in plastic jars and kept in an ultra-freezer at $-80\text{ }^{\circ}\text{C}$ for 48 h. Subsequently, they were lyophilized at $-80\text{ }^{\circ}\text{C}$ with a vacuum pressure of 0.020 mbar for 72 h. The samples were extracted and macerated with a porcelain mortar and stored in plastic jars. The extraction was performed with 100 mg of lyophilized and macerated tissue that was placed in 2 mL microtubes, then 2.0 mL of the 1:1 solution. A mixture of water:acetone was added and stirred for 30 seconds. Subsequently, the sample was sonicated for 5 min and centrifuged at 12,000 rpm for 10 min at $4\text{ }^{\circ}\text{C}$. The supernatant was removed with plastic syringes, filtered through 0.45 micron pore filters and stored in microtubes.

2.5.1. Antioxidant Capacity

Antioxidant capacity was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid). For this, the methodology proposed by Sykłowska-Baranek *et al* [14]. The absorbances were obtained in the Microplate Reader (Biotek, Model ELx808™) at 540 nm. The determination of antioxidants by ABTS was carried out by the spectrophotometric method [15]; absorbance was quantified in a UV-VIS spectrophotometer (UNIQUE, model 2150, Dayton, USA) at 754 nm. The results were expressed in mmol equiv TROLOX/mg sample.

2.5.2. Lycopene and β -carotene

Lycopene and β -carotene concentrations were quantified using the equations proposed by Nagata and Yamashita [15] for antioxidants in tomato fruits.

$$\text{Lycopene (mg/100 gFW)} = -0.0458 A_{663} + 0.372 A_{505} - 0.0806 A_{453}$$

$$\beta\text{-carotene (mg/100 gFW)} = 0.216 A_{663} - 0.304 A_{505} + 0.452 A_{453},$$

The results were expressed in milligrams per 100 grams of dry weight (mg g^{-1})

2.5.3. Total Phenols

It was carried out according to the methodology proposed by Yu & Dahlgren [16]. The quantification was carried out according to Sultana & Anwar [17], and NsorAtindana *et al.* [19]. The absorbance will be prolonged in a UVVIS spectrophotometer at 750 nm. The absorbances were interpolated in the equation obtained from calibration curve with gallic acid (1-12.5 ppm), the results were grains in milligrams Gallic Acid Equivalents per 100 grams of weight dry ($\text{mg GAE } 100\text{ g}^{-1}\text{ PS}$)

2.5.4. Total flavonoids

It was carried out by the Dowd method, adapted by Arvouet-Grand *et al.* [20]. The absorbance was read at a wavelength of 415 nm in a UV-VIS spectrophotometer. The total flavonoid content was determined using a calibration curve with quercetin (0 to 50 ppm) in methanol, the results were expressed in equivalent milligrams of Quercetin per 100 grams of dry weight ($\text{mg EQ } 100\text{ g}^{-1}\text{ PS}$).

2.5.5. Enzymatic antioxidants

Catalase (CAT): its activity was quantified by measuring two reaction times, time 0 (T0) and time 1 (T1), by the spectrophotometric method Cansev *et al.* [21]. The reaction was carried out at $20\text{ }^{\circ}\text{C}$ under constant stirring, the consumption of H_2O_2 was read at 270 nm in a UV-VIS spectrophotometer (UNIQUE, model 2150, Dayton, USA). The difference of the absorbances was interpolated in the equation of the calibration curve made with H_2O_2 (20 to 200 mM). The results were reported as specific activity ($\text{U g}^{-1}\text{ protein}$).

Glutathione peroxidase (GPX): it was determined with the methodology Flohé & Günzler [22] with H₂O₂ as substrate. The absorbances were determined in a UV-VIS spectrophotometer (UNICO, model 2150, Dayton, USA) at 412 nm and these were interpolated in the equation of the calibration curve made with GSH (0.02 to 1 mM). It was reported as specific activity (U g⁻¹ proteins).

Ascorbate peroxidase (APX): it was measured at two times T₀ (initial time) and T₁ (one minute reaction time) according to Nakano & Asada [23]. It was reported as specific activity (U g⁻¹ proteins).

Phenylalanine ammonium lyase (PAL): determined according to Sykłowska-Baranek *et al.* [14]. Absorbance at 290 nm was determined in a UV-VIS spectrophotometer (UNIQUE, model 2150, Dayton, USA). The absorbances were interpolated in the equation obtained from the calibration curve with transcinnamic acid (0.01-0.8 mg ml⁻¹). The results were reported as specific activity (U g⁻¹ proteins).

3. Results

3.1. Non-enzymatic variables

3.1.1. Antioxidant Capacity DPPH and ABTS

The results show that the plants with the grafting factor presented a lower DPPH anti-oxidant capacity, decreasing more than double in most treatments ($p < 0.05$), between grafted and ungrafted plants. Presenting a higher antioxidant capacity ($p < 0.05$) the plants treated with 10 ml L⁻¹ Ca²⁺ without the graft factor, increasing by 23% compared to control 2, no significant difference was observed between the treatments. Regarding the ABTS antioxidant capacity, the same trend was obtained, since the plants with the graft factor decreased antioxidant content, obtaining the best results again for the plants treated with 10 ml L⁻¹ Ca²⁺ without the graft factor, increasing by 8% compared to control 2. Once again, no significant difference was observed between the application of calcium and magnesium treatments (Table 1).

3.1.2. Lycopene and β -carotene content

The interactive effects between the graft and the Ca²⁺ concentrations obtained significant differences ($p < 0.05$) in the amount of lycopene, where the grafted tomato and the application of 10 ml L⁻¹ Ca²⁺ presented an increase of 52% and 67% in lycopene content compared to the control treatments 1 and 2 respectively. Similarly, the graft factor with the application of magnesium induced a higher lycopene content compared to control treatments 1 and 2, increasing 13% and 24% respectively. According to the interaction of the graft and the applications of potassium in the lycopene content, only a slight increase of this antioxidant can be observed compared to the controls. In general terms, a greater increase in this antioxidant is observed in grafted plants (Table 1).

Regarding the content of β -carotene content, the same trend can be observed, since the grafting factor with foliar applications of calcium and magnesium obtained the best results. Also observe an increase of this antioxidant in the grafted plants.

3.1.3. Phenol content

The interactive effects between the graft and the 10 ml L⁻¹ Mg²⁺ foliar applications obtained the best results in the phenol content ($p < 0.05$), increasing by 27.43% compared to its control without grafting (Table 1). Although magnesium increases the content of this antioxidant in ungrafted plants, no significant differences were obtained between treatments.

3.1.4. Flavonoid content

The interactive effects between grafting and Ca²⁺ foliar applications obtained the best results in flavonoid content ($p < 0.05$), increasing by 30% and 34% compared to the control treatments 1 and 2 respectively. As shown in the Table 1, the graft did not have a very significant effect on the content of this antioxidant, observing a greater effect with the foliar application of calcium.

Table 1. Comparison of means of graft, doses of different treatments on non-enzymatic variables.

Factor	Treatment	Total Flavonoids mg/g ⁻¹	Total Phenols mg/g ⁻¹	Lycopene mg/100g ⁻¹	β -caroteno mg/100g ₁	DPPH (mM- ET/mg ⁻¹ sample)	ABTS (mM-ET/mg ⁻¹ sample)
Grafted	Control 1	33.43bc	3.89a	26.18c	4.41b	6.61e	12.51b
Grafted	10 ml L ⁻¹ Ca ²⁺	51.07a	3.13bc	39.94a	5.64a	16.19c	17.41ab
Grafted	4 ml L ⁻¹ K ⁺	27.61c	3.28bc	26.66bc	3.66c	7.80d	9.86c
Grafted	10 ml L ⁻¹ Mg ²⁺	37.69b	4.01a	29.61b	4.77ab	7.35de	8.32c
Ungrafted	Control 2	35.54b	2.91c	23.79cd	3.48cd	23.40b	20.28a
Ungrafted	10 ml L ⁻¹ Ca ²⁺	38.69b	3.02bc	19.47de	3.06d	30.13a	22.14a
Ungrafted	4 ml L ⁻¹ K ⁺	30.12c	3.09bc	18.36e	2.71d	16.82c	17.59ab
Ungrafted	10 ml L ⁻¹ Mg ²⁺	32.08c	3.43b	20.53d	2.72d	7.67de	8.32c

Means with different letters are significantly different, LSD Fisher mean comparison test = (p > 0.05).

3.2. Enzymatic variables

3.2.1. Catalase

The plants grafted with the foliar application of magnesium presented the highest activity, increasing by 41 and 45% compared to controls 1 and 2 respectively, while the plants ungrafted presented the least activity, no significant differences were observed between the application of the treatments (Table 2).

3.2.2. Glutathione peroxidase

The plants grafted with the foliar application of calcium presented the highest activity, increasing by 35 and 42% compared to controls 1 and 2 respectively, similarly the most of the ungrafted plants presented the lowest activity of this enzyme, no significant differences were observed between the application of the treatments (Table 2).

3.2.3. Ascorbate peroxidase

Regarding the activity of this enzyme, no significant differences were observed, behaving similarly to the different treatments.

3.2.4. Phenylalanine ammonium lyase

The grafting factor caused the highest PAL activity, increasing by 8% between grafted and ungrafted plants, observing that the plants treated with calcium obtained the greatest increase. Regarding the treatments, no significant differences were observed (Table 2).

Table 2. Comparison of means of graft, doses on enzymatic variables.

Factor	Treatment	CAT Ug ⁻¹ PT	GPX Ug ⁻¹ PT	APX Ug ⁻¹ PT	PAL Ug ⁻¹ PT
Grafted	Control 1	12.12b	9.34bc	8.50a	20.28a
Grafted	10 ml L ⁻¹ Ca ²⁺	9.65bc	14.48a	3.45ab	22.14a
Grafted	4 ml L ⁻¹ K ⁺	15.30b	11.99ab	6.79ab	17.59ab
Grafted	10 ml L ⁻¹ Mg ²⁺	20.84a	12.13ab	3.23ab	13.24bc
Ungrafted	Control 2	11.34b	8.33c	3.45ab	12.51bc

Ungrafted 10 ml L ⁻¹ Ca ²⁺	7.88bc	7.47d	2.05b	9.86c
Ungrafted 4 ml L ⁻¹ K ⁺	5.21c	6.89d	3.31ab	17.41ab
Ungrafted 10 ml L ⁻¹ Mg ²⁺	4.57c	11.26abc	3.23b	8.32c

CAT: catalase, GPX: Glutathione peroxidase, APX: Ascorbate peroxidase and PAL: Phenylalanine ammonium lyase. Means with different letters are significantly different, LSD Fisher mean comparison test = ($p > 0.05$).

4. Discussion

4.1. Non-enzymatic variables

4.1.1. Antioxidant Capacity DPPH and ABTS

In general, they are based either on the ability to eliminate the radicals (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), ABTS and 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl, DPPH [24]. Comparing our results from the ABTS and DPPH (techniques quantifies the hydrophilic compounds) assays, a general decrease in antioxidant capacity in grafted plants is observed. Results of the combination of the LEZAFORTA variety and the FORTAMINO rootstock proved do not have an effect on the increase in antioxidant capacity. The results showed that the plants treated with foliar applications of calcium obtain the best effects. These results agree with previous works. Vinkovic *et al.* [25] when determining the antioxidant activity determined by the DPPH method (2,2-diphenyl-1-picrylhydrazyl) the variety was significantly higher in antioxidant activity compared to rootstocks and grafted plants. Greathouse *et al.* [26] obtained as results the ABTS assay did not show a correlation between the antioxidant capacities of any of the combinations of tomato varieties and rootstocks. Yeo *et al.* [27] point out that the antioxidant properties of tomatoes depend largely on the lycopene content, since it is the main water-soluble antioxidant of tomato and contribute to the antioxidant activity of the water-soluble fraction. These results indicate that certain combinations of tomato varieties and productive rootstocks may influence the antioxidant capacity. Regarding the effect of Calcium, Thwin *et al.* [28,29], obtained similar results, when treating cherry tomato and persimmon fruits with CaCl₂; they observed increases in antioxidant activities ABTS and DPPH for all treatments.

4.1.2. Lycopene and β -carotene content

The different nutritional components of tomato fruits, the antioxidant group composed of carotenoids as lycopene and β -carotene are crucial for human health as they stimulate the immune response, reduce adverse effects of stress. Additionally, the effect has been documented beneficial of carotenoids against some degenerative diseases. [30].

The results obtained show an increase in the content of lycopene and β -carotene in the interactive effects between the graft and the foliar application of 10 ml L⁻¹ Ca²⁺, obtained a maximum content of 39.94 mg/100g⁻¹, of lycopene at the level of tomato paste product with a content that ranges from 5.40–150mg/100⁻¹ g [31]. Similarly, to the β -carotene content previous sources mention a content of 0.23–4.06 mg 100 g⁻¹. Orchard [32], obtained as result a maximum content of 5.64mg/100g⁻¹. Moreno *et al.* [30] obtained similar results, showing that grafting increased the content of carotenoids (lycopene, β -carotene and total carotene) in the fruits, mentioning that the changes observed in the content of lycopene is the result of the differential regulation of genes responsible for the biosynthesis of secondary metabolites. This behavior is regulated according to the rootstock/variety combination [33].

As regards the effect of calcium on the increase of lycopene Abdelhameed and Abdelhady [34] obtained similar results, where the lycopene content increased with foliar application treatments with calcium. Mazumder *et al.* [35] significant differences were observed in the lycopene content with the highest concentrations of CaCl₂. Verma *et al.* [36] indicate that this element is crucial for plant growth, having a direct role in physiological and biochemical systems. Also, calcium has a favorable effect on human health, has been associated not only with the prevention of hypertensive disorders and blood pressure reduction but also with cholesterol levels and prevention of osteoporosis [37].

According to our results, the application of potassium did not have a significant effect on the content of carotenoids, a possible answer is based on the results obtained by Taber *et al.* [38] since by inducing a higher fertilization with K⁺ in various tomato cultivars they were able to verify that the degree of response of the content of certain carotenoids such as lycopene and β -carotene depended on the genotype

4.1.3. Phenol content

This group of phytochemical compounds is of great nutritional interest due to its contribution to the maintenance of human health, mainly associated with antioxidant activity and antinutritive properties [39]. Kolečka *et al.* [40] obtained similar results, the phenol content, in tomato fruits were significantly affected by grafting, but the alteration of these parameters was dependent on genotype. Other studies also show [25] since when using different rootstocks, total phenols in tomato fruits did not show a significant effect. Maršič *et al.* [41] mention that the graft can influence the phenolic concentrations of the fruits, however, these the authors did not provide concise information on these mechanisms, obtaining as results a wide range of phenols in the fruits of 3 commercial varieties of aubergine and a native variety grafted on tomato. Other studies have found that biofortification with magnesium through the edaphic [42–44] induce an increase significant in the content of phenols in crops such as potatoes, green beans and tomato. Several authors mention that magnesium is a crucial component of many metabolic and signaling pathways [45], the effects of Mg imbalances in metabolic processes seem to occur rapidly, Alsharafa [46] showed that hydrogen peroxide was significantly increased after 72h in deficient nutrient solution of Mg²⁺.

4.1.4. Flavonoid content

Flavonoids are a class of phenolic compounds whose health properties derive from their antioxidant characteristics as free radical scavengers; the structural and electrochemical properties of flavonoids suppress lipid peroxidation and protect the membrane structure by reducing lipid oxidation [47]. Some more specific functions have been reported in the health human, effects on cancer prevention, anti-inflammatory and antiviral activities, and its positive effect on vascular protection [48]. Ahmad *et al.* [49] by applying calcium exogenously, they induced gene expression for polyphenol biosynthesis, increasing the flavonoid content to what corresponds to a greater ROS removal capacity; Aghdam *et al.* [50] mention that when applying calcium in cherry tomato fruits stimulate the accumulation of flavonoids by activating their biosynthetic pathways carried out via the shikimate-phenylpropanoid pathways.

Regarding the effect of grafting on the increase of these secondary metabolites, various studies reported variations in the content of flavonoids [51,52]. Discrepancies between these studies and our findings may be due to genetic factors, culture system, and/or environmental differences.

4.1.5. Effect of potassium on antioxidant Non-enzymatic

Based on previous studies, an increasing level of K fertilization may influence a lower concentration of antioxidants such as lycopene, β -carotene, and total phenols [53]. Ehret *et al.* [54] mentions factors, such as the environment, temperature or light intensity, can affect or reverse the effects of K fertilization in tomatoes. Based on these results from previous work, we justify the low effect that this element had on the non-enzymatic antioxidant action.

4.2. Enzymatic variables

4.2.1. Catalase

Silva *et al.* [55] mentioned that catalase is considered an enzyme involved in the cell defense process against high H₂O₂ production that takes place after grafting process, generated during lignification, in tomato plants. The, grafting method used in this study induced stress to affect the activity of the enzyme catalase. Previous studies mention, that grafted tomato plants showed a

significant increase in H₂O₂ on day 8, and catalase activity increased in parallel [56]. Therefore, it is considered that catalase could be mainly involved in cell defense against the high level of H₂ O₂ production observed at this stage.

Similarly, these results are consistent with previous research, Sakhonwasee and Phingkasan, [57] along with tending to stimulate development, Mg²⁺ has many functions in plant life that are valuable for the biochemical characteristics of plants. Tang *et al.* [58] report lower CAT activity in leaves deficient in Mg²⁺. The authors hypothesized that their findings may reflect a lower rate of photorespiration in Mg deficient leaves, since CAT is mainly located in the peroxisome, where it is involved in removing most of the H₂O₂ generated by photorespiration.

4.2.2. Glutathione peroxidase

Glutathione peroxidase (GPx) it's a potent antioxidant as it decompose H₂O₂ to H₂O protecting the biological molecules damage, inactivation, cross-linking and fragmentation, and peroxidation [59]. In this study, ungrafted plants presented the lowest activity of this enzyme, where the plants grafted with the foliar application of calcium presented the highest activity, similar results were reported Xu *et al.* [60]. These authors reported a lower activity of antioxidant enzymes in ungrafted plants than grafted plants. Pugalendhi [61], considering grafting increases the level of Reactive Oxygen Species (ROS) that stimulate defense antioxidant enzymes viz., catalase and peroxidase. Similar to activity of catalase plants foliarly treated with calcium and magnesium obtained higher results. These results for antioxidant activities were similar to previous investigations for determine the efficiency of use Ca²⁺ and Mg²⁺ roles to the biochemical characteristics [62,63], where, the fertilization with Ca²⁺ and Mg²⁺ had a positive effect on antioxidant production in tomato and cassava.

4.2.3. Ascorbate peroxidase

Ascorbate peroxidase is also an H₂O₂ scavenging enzyme and is essential for the protection of chloroplasts and other cellular constituents from damage caused by H₂O₂ and hydroxyl radicals (OH) [64]. Several studies have confirmed the increase in the activity of antioxidant enzymes is greater in grafted plants [65]. However, in this study we were unable to verify these results as grafting did not have an effect on the enzyme ascorbate peroxidase. Gálvez *et al.* [66] these authors reported the activity of the enzyme ascorbate peroxidase decreased significantly on plants grafted. According to Shehata *et al.* [67] mentions, the antioxidant enzymes as ascorbate peroxidase were significantly affected by the use of genotypes when grafting.

4.2.4. Phenylalanine ammonium lyase

Phenylalanine ammonium lyase (PAL) is a key enzyme in the phenylpropanoid pathway responsible for the biosynthesis of many secondary metabolites, such as anthocyanins, flavanols, and lignins [68]. According to our results, the induction of PAL can be related to scion-rootstock compatibility. Pereira *et al* [69] evaluated the effects of different rootstock-scion interactions in PAL activity in different grafted Prunus species, showing significant differences related to the activity of PAL between different combinations scion and rootstock. In another study, Prabpree *et al.* [70] evaluated the expression of PAL and the phenolic content in grafts on different rubber rootstocks, indicating that the expression of PAL differs between the combinations of grafts investigated at the transcriptional. Previous research suggested [71] exogenous calcium concentration enhances pathogen resistance in tomato seedlings by activation of PAL gene expression.

4.2.5. Effect of potassium on antioxidant enzymes

In research works, the increase in the activity of these enzymes was verified with the application of potassium. Liang *et al.* [72], when applying K, improved the catalase activities (CAT) in ginger (*Zingiber officinale*) in the same way, Zheng *et al.* [73] mention that the application of an adequate amount of KNO₃ detoxified ROS by increasing the activities of SOD, CAT and POD enzymes in *Triticum aestivum* under saline stress. However, multiple sources mention [74,75], when inducing a

higher potassium content, a reduction in the activities of antioxidant enzymes was, these authors mention that potassium could decrease the activity of antioxidant enzymes, perhaps by eliminating free radicals, therefore, the decrease of free radicals result in decreased activity of the antioxidant systems Waraich *et al.* [76] suggests that the use of exogenous K could decrease ROS formation by maintaining plant photosynthetic electron transport and decreasing the action of NADPH oxidase. Based on these results from previous work, we justify the low effect that this element had on enzymatic activity.

5. Conclusions

This study aimed to evaluate the effects of the addition of Ca²⁺, K⁺ and Mg²⁺ on the induction of nutraceutical quality in grafted tomato and grown in the NFT. The results showed the combination of the LEZAFORTA variety and the FORTAMINO rootstock induced a slight increase in variables such as lycopene, β-caroteno and the activity of antioxidant enzymes (CAT, GPX, PAL), obtain the best results the graft interaction and the foliar application the of 10 ml L⁻¹ Ca²⁺ and 10 ml Mg²⁺ L⁻¹. In the content of phenols and flavonoids, only the interaction of the graft factor and the foliar application of calcium and magnesium had an impact significant. Therefore, the combination of the use of grafts and foliar application of Ca²⁺ and Mg²⁺ increases the levels of nutraceutical components in tomato fruits.

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