horticultura brasileira

Research

RODRÍGUEZ-MORAN, H; DELGADO-MARTÍNEZ, R; CABRERA-DE LA FUENTE, M; BENAVIDES-MENDOZA, A; ROBLEDO, V; SANDOVAL-RANGEL, A; GONZALES-MORALES, S. Nutraceutical Quality of Grafted Tomato and Cultivated in the NFT System. *Horticultura Brasileira* v.43, 2025. Elocation e288224. DOI: http://dx.doi.org/10.1590/s0102-0536-2025- e288224

# Nutraceutical quality of grafted tomato and cultivated in the NFT system

**Héctor Rodríguez-Moran[1,2]; Rafael Delgado-Martínez[2]; Marcelino Cabrera-de la Fuente[3*]; Adalberto Benavides-Mendoza[3]; Valentín Robledo[3]; Alberto Sandoval-Rangel[3]; Susana Gonzales-Morales[4]**

[1]Universidad Autónoma Agraria Antonio Narro, Saltillo 25315, México; hecrodriguez@docentes.uat.edu.mx.. [2]Facultad de Ingeniería y Ciencias, Universidad Autónoma de Tamaulipas, Centro Universitario Adolfo López Mateos, Victoria, Tamaulipas 87120, México; rdelgado@docentes.uat.edu.mx. [3]Departamento de Horticultura, Universidad Autónoma Agraria Antonio Narro, Saltillo 25315 México; cafum7@yahoo.com; abenmen@gmail.com; robledo3031@gmail.com; asandovalr16@gmail.com. [4]Secretaría de Ciencia, Humanidades, Tecnología e Innovación - Universidad Autónoma Agraria Antonio Narro, Calzada Antonio Narro 1923, CP. 25315. Saltillo, Coahuila, México; qfb_sgm@hotmail.com (*author for correspondence)

## ABSTRACT

Malnutrition problems are related with diets lacking one or more essential elements, which are extremely important for 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 $Ca^{2+}$, $K^+$ and $Mg^{2+}$ 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 $Ca^{2+}$, 4 mL/L $K^+$ and 10 mL/L $Mg^{2+}$ cations separately for each case, in addition to the control (grafted and non-grafted plants without foliar applications). The treatments were evaluated using a completely randomized design, with 5 repetitions per treatment. The interactive effects between grafting and foliar application of $Ca^{2+}$ induced a greater content of lycopene, β-carotene and total flavonoids in tomato fruits. The interactive effects between the graft and the $Mg^{2+}$ foliar applications obtained the best results in the phenol content. About the antioxidant capacity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′ -Azino-bis-[3-ethylbenzothiazoline-6-sulfonic acid (ABTS)], the graft decreased the concentration, increasing the content of these through foliar applications of calcium. Regarding the enzymatic variables, graft factor increased the activity of catalase, glutathione peroxidase and phenylalanine ammonium lyase and obtained the best results of graft interaction and foliar application of calcium and magnesium. The foliar application of K obtained a low response on antioxidant defense systems. The obtained results demonstrate the influence of grafts and foliar applications of calcium and magnesium as tools that could improve the nutraceutical quality of tomato.

**Keywords:** *Lycopersicon esculentum*, enzymatic antioxidants, non-enzymatic antioxidants.

## RESUMO

**Qualidade nutracêutica de tomate enxertado e cultivado no sistema NFT**

Problemas de desnutrição estão relacionados a dietas carentes de um ou mais elementos essenciais, sendo de extrema importância o enriquecimento alimentar para melhorar o teor de compostos efetivos para a saúde. O objetivo deste estudo é avaliar o efeito da adição de $Ca^{2+}$, $K^+$ e $Mg^{2+}$ na indução da qualidade nutracêutica em tomates enxertados, cultivados no sistema NFT (Nutrient Film Technique). No experimento, foram avaliados dois fatores: plantas com e sem enxertia e a aplicação foliar de 10 mL/L de cátions $Ca^{2+}$, 4 mL/L $K^+$ e 10 mL/L $Mg^{2+}$ separadamente para cada caso, além da testemunha (plantas enxertadas e não enxertadas sem aplicação foliar). Os tratamentos foram avaliados utilizando delineamento inteiramente casualizado, com 5 repetições por tratamento. Os efeitos interativos entre a enxertia e a aplicação foliar de $Ca^{2+}$ induziram maior teor de licopeno, β-caroteno e flavonoides totais nos frutos de tomateiro. Os efeitos interativos entre a enxertia e as aplicações foliares de $Mg^{2+}$ obtiveram os melhores resultados no teor de fenóis. Quanto à capacidade antioxidante do ácido 2,2-difenil-1-picrilhidrazil (DPPH) e do ácido 2,2′-azino-bis-[3-etilbenzotiazolina-6-sulfônico (ABTS)], o enxerto diminuiu a concentração, aumentando o teor destes por meio de aplicações foliares de cálcio. Em relação às variáveis enzimáticas, o fator enxerto aumentou a atividade da catalase, glutationa peroxidase e fenilalanina amônio liase, e obteve os melhores resultados da interação enxerto e aplicação foliar de cálcio e magnésio. A aplicação foliar de K obteve baixa resposta nos sistemas de defesa antioxidante. Os resultados obtidos demonstram a influência do uso de enxertos e aplicações foliares de cálcio e magnésio como ferramentas que podem melhorar a qualidade nutracêutica do tomateiro.

**Palavras-chave:** *Lycopersicon esculentum*, antioxidantes enzimáticos, antioxidantes não enzimáticos.

**Received on October 2, 2024; accepted on March 5, 2025**

Several studies have demonstrated the effects of the minerals $Ca^{2+}$ $K^+$ and $Mg^{2+}$ on the detoxification of reactive oxygen species (ROS) such as superoxide radicals ($O^{2-}$), and hydrogen peroxide ($H_2O_2$). The excessiveness of ROS causes oxidative damage to the deoxyribonucleic acid (DNA), proteins, and lipids (Ravi *et al*., 2023; Kobayashi *et al*., 2018). Exogenous applications of mineral elements such as $Ca^{2+}$ $K^+$ and $Mg^{2+}$ 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 (Andersson *et al*., 2017). $Ca^{2+}$ 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 (Riveras *et al*., 2015). Also, acts as an important second messenger in plant cells in response to various endogenous or environmental cues (Zhang *et al*., 2014).

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 (Wang & Xing, 2017).

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, $K^+$ 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 (Daoud *et al*., 2020).

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 (Hoogesteger & Wester, 2017). 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*. (2019) 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 (Skrypnik *et al*., 2019). Grafting provides higher yield, quality and tolerance to avoid or reduce yield loss caused by biotic and abiotic stress (Huang *et al*., 2015).

## MATERIAL AND METHODS

### Experimental site and design

The work was carried out in a greenhouse in the Department of Horticulture at Universidad Autónoma Agraria Antonio Narro, in Saltillo, Coahuila, México (25°21´23´´; 101°02´11´´; 1760 m altitude). The conditions inside the greenhouse were 4.5 W/m$^2$ of solar radiation, a day maximum temperature of 37°C and a minimum of 21°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 with recommended doses of 10 mL/L $Ca^{2+}$, 4 mL/L $K^+$ and 10 mL/L $Mg^{2+}$ separately for each case, in addition to the controls (control 1= grafted and control 2= 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. Hoagland's solution was used whose sources of mineral elements with a concentration of 100% were: 500 mg/L of $Ca(NO_3)$, 0.083 mL/L of $H_3PO_4$ (53%), 0.311 mL/L of $HNO_3$ (55%), 355 mg/L of $KNO_3$, 302 mg/L of $K_2SO_4$, and 197 mg/L of $MgSO_4$. It needs to be mentioned that 130 liters of water were used for the 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/m$^2$ was established. In respect of treatments, the product AMIFOL K was used as potassium source, composited of 31.0% w/w potassium and 5.12% free amino acids (w/w), at the recommended dosage of 4 mL/L, applied every 15 days. The product HUMISOIL CA-16 was used as calcium source, composed of 16% (w/w) calcium, 8% (w/w) nitrogen, 1.30% (p/p) phytohormones, 2% (w/w) free amino acids, 12% (w/w) humic and fulvic acids and 60% (w/w) extenders and conditioners, at the recommended dosage of 10 ml/L, one application each 15 days. Finally, the product HUMISOIL Mg-14 was used as magnesium source, whose composition is based of 14% (p/p) magnesium, 8% (w/w) nitrogen, 1.30% (w/w) phytohormones, 2% (w/w) free amino acids, 2% (w/w) humic and fulvic acids and extenders and 51.70% (p/p) of conditioners, in the same way, at the recommended dosage of 10 mL/L, applied every 15 days. A total of 8 applications of the treatments were made per crop cycle.

**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 belong to the commercial house Enza Zaden.

**Graft**

The LEZAFORTA variety was sown on 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 plants were grafted after reaching 3 mm stem diameter, which was obtained 32 days after planting the variety. For this, the splicing technique was used according to Lee *et al*. (1994), 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. We 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. For the recovery of the plants, we applied the Phos Green Campbell product, composed of N 12.790%, $P_2O_5$ 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 the dose of 1 g/L. The product was daily applied to the foliage with a manual sprinkler.

**Transplant to NFT system**

The transplant to the NFT (Nutrient Film Technique) system was done 15 days after grafting in 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 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, allowing contact with the nutrient solution.

**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°C for 48 h. Subsequently, they were lyophilized at -80°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 water: acetone solution 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°C. The supernatant was removed with plastic syringes, filtered through 0.45-micron pore filters and stored in microtubes.

**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.* (2012) was used. 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 (Miller *et al*., 1993); 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.

**Lycopene and β-carotene**

Lycopene and β-carotene concentrations were quantified using the equations proposed by Nagata & Yamashita (1992) for antioxidants in tomato fruits.

$$\text{Lycopene (mg/100 g FW)} = -0.0458\ A663 + 0.372\ A505 - 0.0806\ A453$$

$$\beta\text{-carotene (mg/100 g FW)} = 0.216\ A663 - 0.304\ A505 + 0.452\ A453,$$

The results were expressed in milligrams per 100 grams of dry weight (mg/100 g).

**Total phenols**

The total phenols were evaluated according to the methodology proposed by Yu & Dahlgren (2000). The quantification was carried out according to Sultana & Anwar (2009), and Nsor-Atindana *et al*. (2012). The absorbance 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 dry weight (mg GAE/100 g PS).

**Total flavonoids**

The total flavonoids were evaluated by the Dowd method, adapted by Arvouet-Grand *et al*. (1994). The absorbance was read at 415 nm wavelength 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 (mg EQ/100 g PS).

**ENZYMATIC ANTIOXIDANTS**

**Catalase (CAT)**

Its activity was quantified by measuring two reaction times, time 0 (T0) and time 1 (T1), by the spectrophotometric method of Cansev *et al*. (2011). The reaction was carried out at 20°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 (U/g protein).

**Glutathione peroxidase (GPX)**

Was determined through the methodology of Flohé & Günzler (1984) with $H_2O_2$ 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). The results were reported as specific activity (U/g proteins).

**Ascorbate peroxidase (APX)**

Was measured at two times T0 (initial time) and T1 (one-minute reaction time) according to Nakano & Asada (1987). The results were reported as specific activity (U/g proteins).

**Phenylalanine ammonium lyase (PAL)**

Was determined according to Sykłowska-Baranek *et al*. (2012). 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). The results were reported as specificc activity (U/g proteins).

## RESULTS AND DISCUSSION

**NON-ENZYMATIC VARIABLES**

**Antioxidant capacity DPPH and ABTS**

In general, they are based 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 (Mareček *et al*., 2017). The results show that the plants with the grafting factor presented a lower DPPH antioxidant 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 $Ca^{2+}$ without the graft factor, increased by 23% compared to control 2 (non-grafted plants without foliar applications). 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 $Ca^{2+}$ 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). The results from the ABTS and DPPH (techniques quantifies the hydrophilic compounds) assays, presented a general decrease in antioxidant capacity in grafted plants. Results of the combination of the LEZAFORTA variety and the FORTAMINO rootstock do not influence the increase in antioxidant capacity. The results showed that the plants treated with foliar applications of calcium obtained the best effects. These results agree with previous works. Vinkovic *et al.* (2011) determined the antioxidant activity by the DPPH method (2,2-diphenyl-1-picrylhydrazyl) and concluded that the variety was significantly higher in antioxidant activity compared to rootstocks and grafted plants. Greathouse *et al.* (2021) obtained as results that the ABTS assay did not show a correlation between the antioxidant capacities of any of the combinations of tomato varieties and rootstocks. Yeo & Shahidi (2019) 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.* (2020) and Bagheri *et al.* (2015) obtained similar results, when treating cherry tomato and persimmon fruits with $CaCl_2$; they observed increases in antioxidant activities of ABTS and DPPH for all treatments.

**Lycopene and β-carotene content**

The interactive effects between the graft and the $Ca^{2+}$ concentrations obtained significant differences ($p<0.05$) in the amount of lycopene, where the grafted tomato and the application of 10 mL/L $Ca^2$ presented an increase of 52% and 67% in lycopene content compared to the control treatments (control 1= grafted and control 2= non-grafted plants without foliar applications) respectively. Similarly, the graft factor with the application of magnesium induced a higher lycopene content compared to control 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, the same trend can be observed, since the grafting factor with foliar applications of calcium and magnesium obtained the best results. Also, an increase of this antioxidant in the grafted plants was observed.

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 (Moreno *et al.*, 2019).

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 $L^{-1}$ $Ca^{2+}$. A maximum content of 39.94 mg/100$g^{-1}$ of lycopene was obtained at the level of tomato paste product with a content that ranges from 5.40–150mg/$100^{-1}$ g (Imran *et al.*, 2020). Similarly, to the β-carotene content, previous sources mention a content of 0.23-4.06 mg/100 g. Orchard *et al*. (2021) obtained as result a maximum content of 5.64 mg/100 g. Moreno *et al*. (2019) 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 are 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 (Lang & Nair, 2019).

As regards the effect of calcium on the increase of lycopene, Abdelhameed & Abdelhady (2018) obtained similar results, where the lycopene content increased with foliar application treatments with calcium. Mazumder *et al*. (2021) observed significant differences in the lycopene content with the highest concentrations of $CaCl_2$. Verma *et al*. (2019) 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, being associated not only with the prevention of hypertensive disorders and blood pressure reduction but also with cholesterol levels and prevention of osteoporosis (Onakpoya *et al*., 2011).

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.* (2008) 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.

**Phenol content**

The interactive effects between the graft and the 10 mL/L $Mg^{2+}$ 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.

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 (Rehman-Shah *et al*., 2021). Koleška *et al*. (2018) obtained similar results: the phenol content in tomato fruits was significantly affected by grafting, but the alteration of these parameters was dependent on genotype. Other studies also show (Vinkovic *et al.*, 2011) that, when using different rootstocks, total phenols in tomato fruits did not show a significant effect. Maršič *et al.* (2014) mention that the graft can influence the phenolic concentrations of the fruits, however, these 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 applied via edaphic route (Lopez, 2019: Ciscomani *et al.*, 2021; Fiorentini *et al.*, 2021) induce a significant increase 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 (Fiorentini *et al*., 2021). The effects of Mg imbalances in metabolic processes seem to occur rapidly. Alsharafa (2017) showed that hydrogen peroxide was significantly increased after 72 h in deficient nutrient solution of $Mg^{2+}$.

**Flavonoid content**

The interactive effects between grafting and $Ca^{2+}$ foliar applications obtained the best results in flavonoid content ($p<0.05$), increasing by 30% and 34% compared to control 1 and 2 respectively. As shown in 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.

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 (Eren *et al.*, 2018). Some more specific functions have been reported in human health, effects on cancer prevention, anti-inflammatory and antiviral activities, and its positive effect on vascular protection (Kaleem & Ahmad, 2018). Ahmad *et al.* (2018), by applying calcium exogenously, induced gene expression for polyphenol biosynthesis, increasing the flavonoid content to what corresponds to a greater ROS removal capacity; Aghdam *et al.* (2013), 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 (Milenkovic *et al.*, 2018; Marsic *et al.*, 2018). Discrepancies between these studies and our findings may be due to genetic factors, culture system, and/or environmental differences.

**Effect of potassium on non-enzymatic antioxidants**

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 (Sonntag *et al.*, 2020). Ehret *et al.* (2013) mention that 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.

**Table 1.** Comparison of results obtained on grafted and ungrafted plants and doses of different minerals applied foliar, on non-enzymatic variables. Saltillo, Coahuila. Universidad Autónoma Agraria Antonio Narro. 2021.

| Factor | Treatment | Total flavonoids (mg/g) | Total phenols (mg/g) | Lycopene (mg/100 g) | β-carotene (mg/100 g) | DPPH (mM-ET/mg sample) | ABTS (mM-ET/mg sample) |
|---|---|---|---|---|---|---|---|
| Grafted | Control 1 | 33.43bc | 3.89a | 26.18c | 4.41b | 6.61e | 12.51b |
| Grafted | 10 mL/L $Ca^{2+}$ | 51.07a | 3.13bc | 39.94a | 5.64a | 16.19c | 17.41ab |
| Grafted | 4 mL/L $K^{+}$ | 27.61c | 3.28bc | 26.66bc | 3.66c | 7.80d | 9.86c |
| Grafted | 10 mL/L $Mg^{2+}$ | 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 $Ca^{2+}$ | 38.69b | 3.02bc | 19.47de | 3.06d | 30.13a | 22.14a |
| Ungrafted | 4 mL/L $K^{+}$ | 30.12c | 3.09bc | 18.36e | 2.71d | 16.82c | 17.59ab |
| Ungrafted | 10 mL/L $Mg^{2+}$ | 32.08c | 3.43b | 20.53d | 2.72d | 7.67de | 8.32c |

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

## ENZYMATIC VARIABLES

**Catalase**

Plants grafted with foliar applications of magnesium presented the highest activity, increasing by 41 and 45% compared to controls 1 and 2 respectively, while the ungrafted plants presented the least activity, no significant differences were observed between the application of the treatments (Table 2). Silva *et al*. (2016) mentioned that catalase is considered an enzyme involved in the cell defense process against high $H_2O_2$ 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 $H_2O_2$ on day 8, and catalase activity increased in parallel (Fernández-Garcia *et al*., 2004). Therefore, it is considered that catalase could be mainly involved in cell defense against the high level of $H_2O_2$ production observed at this stage. Similarly, these results are consistent with previous research of Sakhonwasee & Phingkasan (2017), who mention that, along with tending to stimulate development, $Mg^{2+}$ has many functions in plant life that are valuable for the biochemical characteristics of plants. Tang *et al*. (2012) report lower CAT activity in leaves deficient in $Mg^{2+}$. 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 $H_2O_2$ generated by photorespiration.

**Glutathione peroxidase**

Plants grafted with foliar application of calcium presented the highest activity, increasing by 35 and 42% compared to controls 1 and 2 respectively; similarly, 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). Glutathione peroxidase (GPx) is a potent antioxidant as it decomposes $H_2O_2$ to $H_2O$ protecting the biological molecule's damage, inactivation, cross-linking and fragmentation, and peroxidation (Al-Madboly *et al.*, 2020). In this study, ungrafted plants presented the lowest activity of this enzyme, where the plants grafted with foliar application of calcium presented the highest activity; similar results were reported by Xu *et al.* (2005). These authors reported a lower activity of antioxidant enzymes in ungrafted plants than in grafted plants. Pugalendhi *et al.* (2021) considered that grafting increases the level of Reactive Oxygen Species (ROS) that stimulates defense antioxidant enzymes. Similarly, activity of catalase plants foliar treated with calcium and magnesium obtained higher results. These results for antioxidant activities were like the previous investigations to determine the efficiency of the use of $Ca^{2+}$ and $Mg^{2+}$ on the biochemical characteristics (Alrashidi *et al.*, 2022; Djabou *et al.*, 2018), where the fertilization with $Ca^{2+}$ and $Mg^{2+}$ had a positive effect on antioxidant production in tomato and cassava.

**Ascorbate peroxidase**

Regarding the activity of this enzyme, no significant differences were observed, behaving similarly to the different treatments. Ascorbate peroxidase is also an $H_2O_2$ scavenging enzyme and is essential for the protection of chloroplasts and other cellular constituents from damage caused by $H_2O_2$ and hydroxyl radicals (OH) (Mehla *et al.*, 2017). Several studies have confirmed that the increase in the activity of antioxidant enzymes is greater in grafted plants (Liu *et al.*, 2014). However, in this study we were unable to verify these results, as grafting did not influence the enzyme ascorbate peroxidase. Gálvez *et al*. (2021) reported that the activity of the enzyme ascorbate peroxidase decreased significantly on grafted plants. According to Shehata *et al*. (2020), the antioxidant enzymes as ascorbate peroxidase were significantly affected when using grafted genotypes.

**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).

Phenylalanine ammonium lyase (PAL) is a key enzyme in the phenylpropanoid pathway responsible for the biosynthesis of many secondary metabolites, such as anthocyanins, flavonols, and lignins (Feduraev *et al*., 2020). According to our results, the induction of PAL can be related to scion-rootstock compatibility. Pereira *et al.* (2014) 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 of scion and rootstock. In another study, Prabpree *et al.* (2018) 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 that (Huiyun & Xiangge, 2018) exogenous calcium concentration enhances pathogen resistance in tomato seedlings by activation of PAL gene expression.

**Effect of potassium on antioxidant enzymes**

Research studies have confirmed an increase in the activity of these enzymes following the application of potassium. Liang *et al*. (2007) observed that potassium application enhanced catalase (CAT) activity in ginger (*Zingiber officinale*). Similarly, Zheng *et al*. (2018) reported that the application of an adequate amount of $KNO_3$ detoxified reactive oxygen species (ROS) by increasing the activities of SOD, CAT, and POD enzymes in *Triticum aestivum* under saline stress. However, several studies (Soleimanzadeh *et al*., 2010; Amjad *et al*., 2016) have reported that an increase in potassium content induces a reduction in antioxidant enzyme activity. 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.* (2012) suggest 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.

**Table 2.** Comparison of means obtained on grafted and ungrafted tomato plants, on doses of enzymatic variables. Saltillo, Coahuila. Universidad Autónoma Agraria Antonio Narro. 2021.

| Factor | Treatment | CAT (U/g PT) | GPX (U/g PT) | APX (U/g PT) | PAL (U/g PT) |
|---|---|---|---|---|---|
| Grafted | Control 1 | 12.12b | 9.34bc | 8.50a | 20.28a |
| Grafted | 10 mL/L $Ca^{2+}$ | 9.65bc | 14.48a | 3.45ab | 22.14a |
| Grafted | 4 mL/L $K^{+}$ | 15.30b | 11.99ab | 6.79ab | 17.59ab |
| Grafted | 10 mL/L $Mg^{2+}$ | 20.84a | 12.13ab | 3.23ab | 13.24bc |
| Ungrafted | Control 2 | 11.34b | 8.33c | 3.45ab | 12.51bc |
| Ungrafted | 10 mL/L $Ca^{2+}$ | 7.88bc | 7.47d | 2.05b | 9.86c |
| Ungrafted | 4 mL/L $K^{+}$ | 5.21c | 6.89d | 3.31ab | 17.41ab |
| Ungrafted | 10 mL/L $Mg^{2+}$ | 4.57c | 11.26abc | 3.23b | 8.32c |

CAT= catalase; GPX= glutathione peroxidase; APX= ascorbate peroxidase; PAL= phenylalanine ammonium lyase. Means with different letters are significantly different, LSD Fisher mean comparison test ($p>0.05$).

This study aimed to evaluate the effects of the foliar application of $Ca^{2+}$, $K^{+}$ and $Mg^{2}$ on the induction of nutraceutical quality on grafted and ungrafted tomato plants, grown in the NFT system. The results showed that the combination of the LEZAFORTA variety and the FORTAMINO rootstock induced a slight increase in variables such as lycopene, β-carotene and the activity of antioxidant enzymes (CAT, GPX, PAL). Also, we obtained the best results through the graft interaction and the foliar application of 10 mL/L $Ca^{2+}$ and 10 mL/L $Mg^{2+}$. About the content of phenols and flavonoids, only the interaction of the graft factor and the foliar application of calcium and magnesium had a significant impact. Therefore, the combination of the use of grafts and foliar application of $Ca^{2+}$ and $Mg^{2}$ increases the levels of nutraceutical components in tomato fruits.

## AUTHORS' CONTRIBUTIONS

Conceptualization, H.M.R.M and M.C.D.L.F.; methodology, R.D.M. and A.S.R.; software, S.G.M.; validation, H.M.R.M and M.C.D.L.F.; formal analysis H.M.R.M; investigation, H.M.R.M and A.B.M; resources, R.D.M.; data curation, H.M.R.M; writing-original draft preparation, H.M.R.M; writing-review and editing, H.M.R.M and M.C.D.L.F.; visualization, V.R.T. and A.S.R; supervision, M.C.D.L.F. and V.R.T.; project administration, R.D.M.; funding acquisition, M.C.D.L.F. All authors have read and agreed to the published version of the manuscript.

## STATEMENTS AND DECLARATIONS

**Conflicts of interest:** The authors declare that there is no conflict of interest.

**Consent for publication:** All authors give their consent for the publication of the manuscript to Horticultura Brasileira.

## DATA AVALAIBILITY

Data will be made available upon request to the corresponding author.

## REFERENCES

ABDELHAMEED, A; ABDELHADY, M. 2018. Response of tomato plant to foliar application of calcium and potassium nitrate integrated with different phosphorus rates under sandy soil conditions. *Egyptian Journal of Soil Science*.

AGHDAM, MS; DOKHANIEH, AY; HASSANPOUR, H; FARD, JR. 2013 - Enhancement of antioxidant capacity of cornelian cherry (*Cornus mas*) fruit by postharvest calcium treatment. *Scientia Horticulturae* 161: 160-164.

AHMAD, PABD: ALLAH, EF; ALYEMENI, MN; WIJAYA, L; ALAM, P; BHARDWAJ, R; SIDDIQUE, KHM. 2018. Exogenous application of calcium to 24-epibrassinosteroid pre-treated tomato seedlings mitigates NaCl toxicity by modifying ascorbate-glutathione cycle and secondary metabolites. *Scientific Reports* 10: 8.

AL-MADBOLY, LA; ALI, SM; FAKHARANY, EME; RAGAB, AE; KHEDR, EG: ELOKELY, KM. 2020. Stress-based production, and characterization of glutathione peroxidase and glutathione S-transferase enzymes from *Lactobacillus plantarum*. *Frontiers in Bioengineering and Biotechnology* 8: 78.

ALRASHIDI, AA; ALHAITHLOUL, HA; SOLIMAN, MH; ATTIA, MS; ELSAYED, SM; ALI, MM; SADEK, AM; FAKHR, M. 2022. Role of calcium and magnesium on dramatic physiological and anatomical responses in tomato plants. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*.

ALSHARAFA, KY. 2017. Mineral deficiencies influence on tomato leaves: Pigments, hydrogen peroxide and total phenolic compounds contents. *Plant Omics* 10: 78-87.

AMJAD, M; AKHTAR, J; MURTAZA, B. 2016. Differential accumulation of potassium results in varied salt-tolerance response in tomato (*Solanum lycopersicum* L.) cultivars. *Horticulture, Environment and Biotechnology* 57: 248-258.

ANDERSSON, MSA; SALTZMAN, PS; VIRK; PFEIFFER, WH. 2017. Progress update: crop development of biofortified staple food crops under HarvestPlus. *African Journal of Food, Agriculture, Nutrition and Development* 17: 11905-11935.

ARVOUET-GRAND, A; VENNAT, B; POURRAT, A; LERGRET, P. 1994. Standardisation dun extrait de propolis et identification des principaux constituants. *Journal de Pharmacie de Belgique* 49: 462-468.

BAGHERI, M; ESNA-ASHARI, M; ERSHADI, A. 2015. Effect of postharvest calcium chloride treatment on the storage life and quality of persimmon fruits (*Diospyros kaki* Thunb.) cv. 'Karaj'. *International Journal of Horticultural Science and Technology* 2: 15-26.

CANSEV, A; GULEN, H; ERIS, A. 2011. The activities of catalase and ascorbate peroxidase in olive (*Olea europaea* L. Cv. Gemlik) under low temperature stress. *Horticulture, Environment and Biotechnology* 52 (2).

CISCOMANI-LARIOS, JP; SÁNCHEZ-CHÁVEZ, E; JACOBO-CUELLAR, JL; SÁENZ-HIDALGO, HK; ORDUÑO-CRUZ, N; CRUZ-ALVAREZ, O; ÁVILA-QUEZADA, GD. 2021. Biofortification efficiency with magnesium salts on the increase of bioactive compounds and antioxidant capacity in snap beans. *Ciência Rural* 51(6).

DAOUD, B; PAWELZIK, E; NAUMANN, M. 2020. Different potassium fertilization levels influence water-use efficiency, yield, and fruit quality attributes of cocktail tomato - A comparative study of deficient-to-excessive supply. *Scientia Horticulturae* 272: 109562.

DJABOU, AS; QIN, Y; THADDÉE, B; FIGUEIREDO, PG; FEIFEI, A; CARVALHO, LC; OMOKOLO, DN; LI, K; NIEMENAK, N; CHEN, S. 2018. Effects of calcium and magnesium fertilization on antioxidant activities during cassava postharvest physiological deterioration. *Crop Science* 58: 1385-1392.

EHRET, DL; USHER, K; HELMER, T; BLOCK, G; STEINKE, D; FREY, B; KUANG, T; DIARRA, M. 2013. Tomato fruit antioxidants in relation to salinity and greenhouse climate. *Journal of Agricultural and Food Chemistry* 61: 1138-1145.

EREN, GB; INCE, E; GURER, OH. 2018. *In vitro* antioxidant/prooxidant effects of combined use of flavonoids. *Natural Poduct Research* 32: 1446-1450.

FEDURAEV, P; SKRYPNIK, L; RIABOVA, A; PUNGIN, A; TOKUPOVA, E; MASLENNIKOV, P; CHUPAKHINA, G. 2020. Phenylalanine and tyrosine as exogenous precursors of wheat (*Triticum aestivum* L.) secondary metabolism through PAL-associated pathways. *Plants* 9: 476.

FERNÁNDEZ-GARCIA, N; CARVAJAL, M; OLMOS, E; GRAFT. 2004. Unión formation in tomato plants: peroxidase and catalase involvement. *Annals of Botany* 93: 53-60.

FIORENTINI, D; CAPPADONE, C; FARRUGGIA, G; PRATA, C. 2021. Magnesium: biochemistry, nutrition, detection, and social impact of diseases linked to its deficiency. *Nutrients* 13: 1136.

FLOHÉ, L; GÜNZLER, WA. 1984. Assays of glutathione peroxidase. *Methods in Enzymology* 105: 114–120.

GÁLVEZ, A; ALBACETE, A; MARTÍNEZ-ANDÚJAR, C; AMOR, FM: LÓPEZ-MARÍN, J. 2021. "Contrasting rootstock-mediated growth and yield responses in salinized pepper plants (*Capsicum annuum* L.) are associated with changes in the hormonal balance. *International Journal of Molecular Sciences* 22: 3297.

GREATHOUSE, J; HENNING, S; SOENDERGAARD, M. 2021. Effect of grafting rootstock on the antioxidant capacity and content of Heirloom tomatoes (*Solanum lycopersicum* L.) in hydroponic culture. *Plants* 10: 965.

HOOGESTEGER, J; WESTER, P. 2017. Regulating groundwater use: The challenges of policy implementation in Guanajuato, central Mexico. *Enviromental Science & Policy* 77: 107-113.

HUANG, W; LIAO, S; LV, H; KHALDUN, ABM; WANG, Y. 2015. Characterization of the growth and fruit quality of tomato grafted on a woody medicinal plant, *Lycium chinense. Scientia Horticulturae* 197: 447-453.

HUIYUN, YU; XIANGGE, DU. 2018. Differential regulation of calmodulin, phenylalanine ammonia-lyase, and salicylic acid in response to *Botrytis cinerea* infection in tomato with different Ca2+ concentrations. *Journal of Plant Nutrition* 41: 1104-1118.

IMRAN, M; GHORAT, F; UL-HAQ, I; UR-REHMAN, H; ASLAM, F; HEYDARI, M; SHARIATI, MA; OKUSKHANOVA, E; YESSIMBEKOV, Z; THIRUVENGADAM, M; HASHEMPUR, MH; REBEZOV, M. 2020. Lycopene as a natural antioxidant used to prevent human health disorders. *Antioxidants* 9:706.

KALEEM, M; AHMAD, A. 2018. Flavonoids as nutraceuticals. In: GRUMEZESCU, MA; HOLBAN, AM (eds). *Therapeutic, probiotic, and unconventional foods*. Academic Press; Cambridge, MA, USA: pp.137-155.

KOBAYASHI, NI; OGURA, T; TAKAGI, K; SUGITA, R; SUZUKI, H; IWATA, R. 2018. Magnesium deficiency damages the youngest mature leaf in rice through tissue-specific iron toxicity. *Plant and Soil* 428: 137-152.

KOLEŠKA, I; HASANAGIĆ, D; TODOROVIĆ, V; MURTIĆ, S; MAKSIMOVIĆ, I. 2018. Grafting influence on the weight and quality of tomato fruit under salt stress. *Annals of Applied Biology* 172: 187-196.

LANG, KM; NAIR, A. 2019. Effect of tomato rootstock on hybrid and Heirloom tomato performance in a midwest high tunnel production system. *Hortscience* 54: 840-845.

LEE, JM. 1994. Cultivation of grafted vegetables I. 1994. *Current Status, grafting methods, and benefits*. *HortScience* 29: 235-239.

LIANG, TB; WANG, ZL; WANG, RJ; LIU, LL; SHI, CY. 2007 Effects of potassium humate on ginger root growth and its active oxygen metabolism. *Ying Yong Sheng Tai Xue Bao* 18: 813-817.

LIU, J, LI, J; SU, X; XIA, Z. 2014. Grafting improves drought tolerance by regulating antioxidant enzyme activities and stress-responsive gene expression in tobacco. *Environmental and Experimental Botany* 107: 173-179.

LOPEZ, M .2019. Phenolic compounds, carotenoids and antioxidant activity in diploid potato with foliar fertilization of magnesium and manganese. National University of Colombia Bogotá Campus Faculty of Agronomy Department of Agronomy National University of Colombia Bogotá Campus Faculty of Sciences Faculty of Agronomy Department of Agronomy.

MAREČEK, VÍT; MIKYŠKA, A; HAMPEL, D; ČEJKA, P; NEUWIRTHOVÁ, J; MALACHOVÁ, A; CERKAL, R. 2017. ABTS and DPPH methods as a tool for studying antioxidant capacity of spring barley and malt. *Journal of Cereal Science*. 73: 40-45.

MARŠIČ, NK; MIKULIČ-PETKOVŠEK, M; ŠTAMPAR, F. 2014. Grafting influences phenolic profile and carpometric traits of fruits of greenhouse-grown eggplant (*Solanum melongena* L.). *Journal of Agricultural and Food Chemistry* 62: 10504-10514.

MARSIC, N; VODNIK, D; MIKULIC-PETKOVSEK, M; VEBERIC, R; SIRCELJ, H. 2018. Photosynthetic traits of plants and the biochemical profile of tomato fruits are influenced by grafting, salinity stress, and growing season *Journal of Agricultural and Food Chemistry* 66: 5439-5450.

MAZUMDER, MNN; MISRAN, A; DING, P; WAHAB, PEM; MOHAMAD, A. 2021. Preharvest foliar spray of calcium chloride on growth, yield, quality, and shelf-life extension of different lowland tomato varieties in Malaysia. *Horticulturae* 7: 466.

MEHLA, N; SINDHI, V; JOSULA, D; BISHT, P; WANI, SH. 2017. An introduction to antioxidants and their roles in plant stress tolerance. In: *Reactive oxygen species and antioxidant systems in plants: role and regulation under abiotic stress*; KHAN, MIR; KHAN, NA (eds). Springer: Singapore; pp.1-23.

MILENKOVIC, L; MASTILOVIC, J; KEVREŠAN, Z; JAKŠIC, A; GLEDIC, A; ŠUNIC, L; STANOJEVIC, L. 2018. Tomato fruit yield and quality as affected by grafting and shading. *Journal of Food Science and Nutrition* 4: 42.

MILLER, NJ; RICE-EVANS, C; DAVIES, MJ; GOPINATHAN, V; MILNER, A. 1993. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clinical Science* 84: 407-412.

MORENO, MM; VILLENA, J; GONZÁLEZ-MORA, S; MORENO, C. 2019. Response of healthy local tomato (*Solanum lycopersicum* L.) populations to grafting in organic farming. *Scientific Reports* 9: 4592.

NAGATA, M; YAMASHITA, I. 1992. Simple method for simultaneous determinations of chlorophyll and carotenoids in tomato fruit. *Nippon Shokuhin Kogyo Gakkaish* 39: 925-928.

NAKANO, Y; ASADA, K. 1987. Purification of ascorbate peroxidase in spinach chloroplasts; its inactivation in ascorbate-depleted medium and reactivation by monodehydroascorbate radical. *Plant and Cell Physiology* 28: 131-140.

NSOR-ATINDANA, J; ZHONG, F; MOTHIBE, KJ; BANGOURA, ML; LAGNIKA, C. 2012. Quantification of total polyphenolic content and antimicrobial activity of cocoa (*Theobroma cacao* L.) *Beans Shells Pakistan Journal of Nutrition* 11: 574-579.

ONAKPOYA, IJ; PERRY, R; ZHANG, J; ERNST, E. 2011. Efficacy of calcium supplementation for management of overweight and obesity: Systematic review of randomized clinical trials. Nutrition Reviews 69: 335-343.

ORCHARD, CJ; COOPERSTONE, JL; GAS-PASCUAL, E; ANDRADE, MC; ABUD, G; SCHWARTZ, SJ; FRANCIS, DM. 2021. Identification and assessment of alleles in the promoter of the Cyc-B gene that modulate levels of β-carotene in ripe tomato fruit. *Plant Genome* 14: e20085.

PEREIRA, IS; MESSIAS, RS; CAMPOS, ÂD; ERREA, P; ANTUNES, LEC; FACHINELLO, JC. 2014. Growth characteristics and phenylalanine ammonia-lyase activity in peach grafted on different *Prunus* spp. *Biologia Plantarum* 58: 114-120.

PRABPREE, A; SANGSIL, P; NUALSRI, C; NAKKANONG, K. 2018. Expression profile of phenylalanine ammonia-lyase (PAL) and phenolic content during early stages of graft development in bud grafted *Hevea brasiliensis*. Biocatalysis and Agricultural Biotechnology 14: 88-95.

PUGALENDHI, L; S BHARATHI, R; PRIYA, MS; VELMURUGAN. 2021. Biochemical and quality attributes of grafted tomato (*Solanum lycopesicum* L.). *Pharma Innovation* 10: 333-338.

RAVI, B; FOYER, CH; PANDEY, GK. 2023. The integration of reactive oxygen species (ROS) and calcium signaling in abiotic stress responses. *Plant and Cell Environment*

SHAH, R; ABDUR, M; RAHMAT, K; ALI, AHMED, M. 2021. Phytochemical assessment, total phenolic content, cytotoxic, antioxidant and antidiabetic activities of *Hyoscyamus insanus*. *Journal of the Chilean Chemical Society* 66: 5285-5290.

RIVERAS, E; ALVAREZ, JM; VIDAL, EA; OSES, C; VEGA, A; GUTIÉRREZ, RA. 2015. The calcium ion is a second messenger in the nitrate signaling pathway of *Arabidopsis*. *Plant Physiology* 169: 1397-1404.

SAKHONWASEE, S; PHINGKASAN, W 2017. Effects of the foliar application of calcium on photosynthesis, reactive oxygen species production, and changes in water relations in tomato seedlings under heat stress. *Horticulture, Environment and Biotechnology* 58: 119-126.

SAMBO, P; NICOLETTO, C; GIRO, A; PII, Y; VALENTINUZZI, F; MIMMO, T; LUGLI, P; ORZES, G; MAZZETTO, F; ASTOLFI, S; TERZANO, R; CESCO, S. 2019. Hydroponic solutions for soilless production systems: issues and opportunities in a smart agriculture perspective. *Frontiers in Plant Science* 10: 923.

SHEHATA, SA; OMAR, HS; ELFAIDY, AGS; EL-SAYED, SSF; ABUARAB, ME; FEDURAEV, P; SKRYPNIK, L; RIABOVA, A; PUNGIN, A; TOKUPOVA, E; MASLENNIKOV, P; CHUPAKHINA, G. 2020. Phenylalanine and tyrosine as exogenous precursors of wheat (*Triticum aestivum* L.) Secondary metabolism through PAL-associated pathways. *Plants* 9: 476.

SILVA, ES; MENEZES, D; SILVA, E; GOTO, R; LIMA, G. 2016. Different methods of grafting and activity of antioxidant enzymes in tomato. *Revista Brasileira de Ciências Agrárias* 11: 267-271. 10.5039/agraria.v11i4a5392.

SKRYPNIK, L; NOVIKOVA, A; TOKUPOVA, E. 2019. Improvement of phenolic compounds, essential oil content and antioxidant properties of sweet basil (*Ocimum basilicum* L.) depending on type and concentration of selenium application.

SOLEIMANZADEH, H; HABIBI, D; ARDAKANI, MR; PAKNEJAD, F; REJALI, F. 2010. Effect of potassium levels on antioxidant enzymes and malondialdehyde content under drought stress in sunflower (*Helianthus annuus* L.). *American Journal of Agricultural and Biological Sciences* 5: 56-61.

SONNTAG, F; BUNZEL, D; KULLING, SE; PORATH, I; PACH, F; PAWELZIK, E; SMIT, I; NAUMANN, M. 2020. Effect of potassium fertilization on the concentration of antioxidants in two cocktail tomato cultivars. *Journal of Applied Botany and Food Quality* 93: 34-43.

SULTANA, FB; ANWAR, MA. 2009. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules* 14: 2167-2180.

SYKŁOWSKA-BARANEK, K; PIETROSIUK, A; NALIWAJSKI, MR. 2012. Effect of l-phenylalanine on PAL activity and production of naphthoquinone pigments in suspension cultures of *Arnebia euchroma* (Royle) Johnst. *In Vitro Cellular & Developmental Biology - Plant* 48: 555-564.

TABER, H; VEAZIE, PP; LI, S; WHITE, W; RODERMEL, S; XU, Y. 2008. Enhancement of tomato fruit lycopene by potassium is cultivar dependent, *HortScience* 43: 159-165.

TANG, N; LI, Y; CHEN, LS. 2012. Magnesium deficiency-induced impairment of photosynthesis in leaves of fruiting *Citrus reticulata* trees accompanied by up-regulation of antioxidant metabolism to avoid photo-oxidative damage. *Journal of Plant Nutrition and Soil Science* 175: 784-793.

THWIN, YW; CHANG, MS; HONG, Y; LIM, S. 2020. Effects of 1-MCP and calcium chloride treatments on quality maintenance of full-ripe cherry tomatoes. *Korean Journal of Food Preservation* 27: 433-445.

VERMA, G; SRIVASTAVA, D; TIWARI, P; CHAKRABARTY, D. 2019. ROS modulation in crop plants under drought stress. Reactive oxygen, nitrogen and sulfur species in plants: Production, metabolism, signaling and defense mechanisms.

VINKOVIC, VRCEKI; SAMOBORV, BOJICM; SARICM, M; VUKOBRATOVICM, ERHATICR; HORVATD; MATOTANZ. 2011. The effect of grafting on the antioxidant properties of tomato (*Solanum lycopersicum* L.). *Spanish Journal of Agricultural Research* 9: 844-851.

WANG, X., XING, Y. 2017. Evaluation of the effects of irrigation and fertilization on tomato fruit yield and quality: a principal component analysis. *Scientific Reports* 7: 1-13.

WARAICH, EA; AHMAD, R; HALIM, A; AZIZ, T. 2012. Alleviation of temperature stress by nutrient management in crop plants: A review. *Journal of Soil Science and Plant Nutrition* 12: 221-244.

XU, SL; CHEN, QY; CHEN, XQ; GAO, JS; LI, SH. 2005 Effect of grafting on 'Giashi' muskmelon yield, and quality of grafted muskmelon. *Fujian Journal of Agricultural Sciences* 21: 354-359.

YEO, J; SHAHIDI, F. 2019. Critical re-evaluation of DPPH assay: presence of pigments affects the results. *Journal of Agricultural and Food Chemistry* 67: 7526-7529.

YU, Z; DAHLGREN, RA. 2000: Evaluation of methods for measuring polyphenols in conifer foliage. *Journal of Chemical Ecology* 26: 2119-2140.

ZHANG, L; DU, L; POOVAIAH, BW. 2014. Calcium signaling and biotic defense responses in plants. *Plant Signaling & Behavior* 11: e973818.

ZHENG, Y; AIJUN, J; TANGYUAN, N; JIALIN, X; ZENGJIA, L; GAOMING, J. 2018. Potassium nitrate application alleviates sodium chloride stress in winter wheat cultivars differing in salt tolerance. *Journal of Plant Physiology* 165: 1455-1465.

**Author's ORCID:**

**Héctor Rodríguez Moran** - https://orcid.org/0009-0009-2852-7967
**Rafael Delgado Martínez** - https://orcid.org/0000-0001-9945-5985
**Marcelino Cabrera De la Fuente** - https://orcid.org/0000-0002-3292-2793
**Adalberto Benavides Mendoza** - https://orcid.org/0000-0002-2729-4315
**Valentín Robledo Torres** - https://orcid.org/0000-0001-5235-6584
**Alberto Sandoval Rangel** - https://orcid.org/0000-0003-4651-7980
**Susana González Morales** - https://orcid.org/0000-0001-5734-6086