

Agronomic biofortification with selenium improves the yield and nutraceutical quality in tomato under soilless conditions

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Abstract

Selenium (Se) is an essential element for humans. Its consumption comes from food of animal or vegetal origin; whose content varies widely depending on its availability in soil or anthropogenic sources. Biofortification improves food nutritional quality, and its consumption has a positive influence in human health. Thus, the objective of this research was to assess agricultural biofortification with Se in tomato fruit and its effects on yield, nutraceutical quality, and antioxidant capacity. Five Se doses (0, 2, 4, 6, and 8 mg L⁻¹) in the form of sodium selenite (Na₂SeO₃) were added in a nutritional solution in a hydroponic system. The results obtained indicated that agricultural biofortification with Se applied in the nutritional solution improved yield, nutraceutical quality, and Se concentration in tomato fruit. The optimum Se dose that maximized yield and nutraceutical quality, as well as the recommended consumption concentration in tomato fruit in this study was 2 mg L⁻¹ (Na₂SeO₄) because higher doses decreased yield and bioactive compound biosynthesis. Incorporating Se in the nutritional solution is an alternative to increase phytochemical compound biosynthesis in tomato fruit and yield with the possibility of improving public health with its consumption.

Keywords: biofortification; nutraceutical quality; *Solanum lycopersicum* L.

Introduction

Selenium (Se) is an essential micronutrient for humans (Hu *et al.*, 2019), its consumption comes from food of animal and plant origin, which show variations in their Se content due to the availability of this microelement in soil or the synthetic sources applied anthropogenically (Utoiu *et al.*, 2017). According to the World Health Organization (WHO), Se consumption in human diet should be from 55-200 µg/day per adult

(Stefani *et al.*, 2020). Se prevents cell damage, thyroid alterations, mental confusion, depression, mutations, cancer, among others (Vinceti *et al.*, 2018; Kavcic *et al.*, 2020; Motesharezadeh *et al.*, 2020). Nevertheless, 15% of the population has been estimated to show deficiencies of this micronutrient (Nedelkov *et al.*, 2020), which represents a health problem at world level (Zou *et al.*, 2019).

One of the strategies to decrease Se deficiency is its incorporation in food through agronomic biofortification, which consists in increasing Se content in edible parts of plants through synthetic fertilization with sodium selenite (Na_2SeO_4) (Moretti *et al.*, 2013; Das *et al.*, 2019). Biofortification with Na_2SeO_4 has been performed successfully in different cultivations, affecting harvest quality and yield (Motesharezadeh *et al.*, 2020; Rady *et al.*, 2020; Zieba *et al.*, 2020). On the other hand, tomato (*Solanum lycopersicum* L.) is one of the most consumed horticultural species at world level whether fresh or as processed product (Andrejiová *et al.*, 2019; Martínez-Damián *et al.*, 2019). Tomato is considered a functional food rich in fiber, containing a wide variety of bioactive compounds that are beneficial for human health (Wakchaure *et al.*, 2020). These bioactive compounds may be increased by adding Na_2SeO_4 in the nutritional solution. However, the application dose of this microelement should be determined to increase tomato nutritional and nutraceutical properties. Therefore, the objective of this research was to determine the effect of Se biofortification in yield, nutraceutical quality, and antioxidant capacity in tomato fruit.

Materials and Methods

Plant material and growing conditions

This study was performed in a greenhouse located in the Instituto Tecnológico de Torreón, México at 24°30' and 27° N latitude, 102°00' and 104°40' W longitude at 1120 m.a.s.l. Tomato cv. Aquila seeds were used, germinated, and transplanted to pots containing a substrate based on river sand and perlite (80:20 vol/vol). Trickle irrigation was used providing 0.6 L watering per plant thrice a day from transplant to flowering and 2.5-3.5 L from flowering to harvest. The minimum and maximum temperature inside the greenhouse fluctuated between 17.7 and 31.6 °C, respectively, while the minimum and maximum relative humidity ranged between 30 and 70%.

Experimental design and treatments

Sodium selenite was applied in treatments of 2, 4, 6, and 8 mg L⁻¹ of Na_2SeO_4 (Sigma Aldrich, U.S.A.) per plant, and one lot with nutritional solution only (without Se) as control group (Puccinelli *et al.*, 2017). The treatments were applied every 15 days adding Steiner (1984) nutritional solution, which contained the following elements in mol_c m⁻³: NO₃ 12.0; H₂PO₄ 1.0; SO₄ 7.0.0; K 7.0; Ca 9.0.0 and Mg 4.0 with the following micronutrients (in mg L⁻¹): Mn 1.6; Cu 0.11; B 0.865; Zn 0.023; Mo 0.048; and Fe 5; pH, and electrical conductivity was maintained at 5.5-2.0 dS m⁻¹ respectively. Plants were maintained inside a greenhouse for 120 days. Fruit yield, weight loss percentage, and nutraceutical quality were quantified. Ten replicates were used per treatment and the experiment was performed twice.

Yield

From each treatment, all fruit per plant per replicate were harvested from the first to the fifth tomato cluster when the fruit showed an intense red color.

Total dry material

At the end of the experiment, the plants of each treatment were sectioned in their different organs (root, stem, and leaves) and introduced in a stove with air-forced circulation at 70 °C until they reached constant weight. Each sample weight was reported in grams.

Fruit quality

Fruit quality was evaluated in three samples taken at random from each cluster corresponding to each treatment replicate, measuring average fruit firmness and total soluble solids (TSS).

Fruit firmness was determined with a penetrometer (Fruit Hardness Tester FHT200), with an 8-mm diameter strut; readings were taken on the opposite sides of the fruit to obtain an average; the results were expressed in Newton units (N).

Total soluble solids were evaluated in °Brix; for this purpose, a drop of fruit juice was obtained, and the reading was determined with a manual refractometer from 0 to 32% (Master 2311).

Nutraceutical fruit quality

Total phenolic content was determined using a modification of Folin-Ciocalteu method (Singleton *et al.*, 1999); 50 µL of ethanolic extract were taken, diluted in 3 mL of Milli-Q (MQ, Darmstadt, DE) water; 250 µL of Folin-Ciocalteu reagent (1N) was added, stirred and left in reaction for 3 min. Subsequently 750 µL of Na₂CO₃ (20%) and 950 µL of MQ water were added. The solution was allowed to stand for 2 h, and the samples were quantified in an ultraviolet (UV)-Vis spectrophotometer at 760 nm. The standard was prepared with gallic acid. The results were expressed in mg GAE/100 g⁻¹ fresh weight.

Total flavonoids were determined by colorimetry (Moretti *et al.*, 2013); 250 µL of ethanolic extract were taken, mixed with 1.25 mL of MQ water and 75 µL of NaNO₂ (5%). After 5-min rest, 150 µL AlCl₃ (aluminum chloride-1-Ethyl-3-methylimidazolium chloride, Sigma-Aldrich, St. Louis, MO, U.S.A.) were added. Subsequently, 500 µL of NaOH (1M) and 275 µL of MQ water were added vigorously stirred, and samples were quantified in a UV-Vis spectrophotometer at 510 nm. The standard was prepared with quercetin dissolved in absolute ethanol ($y = 0.0122x - 0.0067$; $r^2 = 0.965$). The results were expressed in mg QE/100 g⁻¹ fresh weight.

Total antioxidant capacity was measured by the in-vitro 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH⁺) method (Brand-Williams *et al.*, 1995). A DPPH⁺ solution (Sigma-Aldrich, St. Louis, MO, U.S.A.) was prepared in ethanol at 0.025 mg mL⁻¹ concentration; 50 µL of ethanolic extract were mixed with 1950 µL of DPPH⁺ solution; after 30 min the samples were quantified in an UV-Vis spectrophotometer at 517 nm. The results were expressed in µM equivalent in Trolox/100 g⁻¹ fresh weight.

Lycopene extraction

It was performed by the fluorescence in situ hybridization (FISH) method (Fernandez *et al.*, 2007), with some modifications. Approximately 1 g of sample was placed in test tubes covered with 50 mL polytetrafluoroethylene (PTFE) aluminum while on ice. Lycopene extraction solution (39 mL) consisting of hexane 0.05% (w/v) butylated hydroxytoluene (BHT) in acetone and 95% ethanol in a 1:1:1 ratio were added to the tubes and stirred at 180 rpm for 10 min. Six mL of cold distilled water were added to each tube and stirred for an additional five min for better separation of polar and nonpolar compounds. The tubes were then removed from stirring and left for 15 min at room temperature for separation into polar and non-polar layers. The supernatant was placed in new 15 mL aluminum-covered test tubes and kept at -80 °C for other experiments. Absorbance of the supernatant (hexane layer) containing lycopene was read three times using the spectrophotometer at a wavelength of 503 nm. Absolute hexane was used as a blank. Lycopene amounts in the tissues were estimated using the following formula:

$$\text{Lycopene (mg / kg)} = (x / y) \times A_{503} \times 3.12 \quad (1)$$

Where x is the amount of hexane (mL) and sample weight is A₅₀₃; absorbance was read at 503 nm and 3.12 the extinction coefficient.

Vitamin C

It was obtained by means of the titration method (Ordóñez-Santos *et al.*, 2013). Fresh fruit samples of 10 g were used, crushed with 10 mL at 2% of hydrochloric acid, and filtered; then, they were placed in

Erlenmeyer flasks adjusted to 100 mL using distilled water. Subsequently, samples were titrated with 10 mL of the dilution, using 2,6 dichlorophenolindophenol (1×10^{-3} N) to determine vitamin C content using the formula:

$$\text{Vit C (mg} \cdot 100\text{g PF)} = \frac{(\text{mL of 2.6 dichlorophenolindophenol}) (0.088)(\text{total volume})(100)}{(\text{aliquote volume})(\text{sample weight})} \quad (2)$$

Selenium accumulation in fruit

Dried tomato samples were ground in a porcelain mortar and digested with nitric and perchloric acid (3: 1) using a plate and heating at 100 °C. The solution was filtered and boiled to 100 ml working solution with deionized water. Selenium concentration in tomato fruit was determined by atomic absorption spectrophotometry (AOAC, 1990). The results were expressed in $\mu\text{g kg}^{-1}$ of dry fruit weight.

Statistical analysis

Data were processed by a one-way analysis of variance (ANOVA) and Tukey's test with a significance level of 5%, using STATISTICA software (version 8.0.360.0 StatSoft Inc., Tulsa, OK, U.S.A.) for Windows.

Results and Discussion

Yield

The Se addition of 2 mg L^{-1} in the nutritional solution increased yield 23.9% with respect to the control treatment (Figure 1a). Selenium has been reported to improve yield in low dosage (Narváez-Ortiz *et al.*, 2018; Rady *et al.*, 2020) and act as antioxidant when plant capacity increases to resist oxidative stress caused by ROS under stress conditions. This effect is due to a decrease in lipid peroxidation, H_2O_2 , and superoxide radicals, as well as an increase in peroxidase enzymes and polyphenol oxidase (Boldrin *et al.*, 2016; Babalar *et al.*, 2019). On the contrary, high dose decreases crop yield since it acts as a lipid pro-oxidant and increases free radical production causing oxidative stress Zieba *et al.*, 2020). In general, plant response to Selenium differs according to the applied concentration (Puccinelli *et al.*, 2017), species sensitivity (Lyons, 2018), chemical species used and form of application, which is why the optimum dose for each crop should be determined (Ramos *et al.*, 2010; Oliveira *et al.*, 2018). In hydroponic tomato, the recommended dose for Se is 1.27 mg L^{-1} to avoid phytotoxicity (Edelstein, 2016). Nonetheless, this dose depends on the variety used (Zhao *et al.*, 2017). Tomato is not a species that accumulates Se, and a concentration higher than $25 \mu\text{g}$ of selenium g^{-1} of dry root and leaf weight is toxic (Edelstein *et al.*, 2016). This phytotoxicity may show through oxidative stress, considering the pro-oxidant ability of selenium or by sulfur competitive substitution in proteins (Das *et al.*, 2019), causing also nutritional unbalance, reduced photosynthetic activity, oxidative stress, growth and yield reduction (Da Cruz Ferreira *et al.*, 2020). No visual damage was observed in leaves in this study but less plant vigor was confirmed by a drastic decrease in total dry matter (Figure 1b).

Fruit quality

Selenium addition in the nutritional solution modified tomato fruit quality, especially firmness and TSS, which increased 27 and 17.58% with the greatest Se dose compared with the control group (Figure 1c-d); the greatest fruit firmness was probably due to a greater lignification of the fruit pericarp cell walls. Selenium has demonstrated to increase peroxidase enzymes (Garduño-Zepeda *et al.*, 2018; Hibaturrahman *et al.*, 2020), which participate in different functions, such as lignification, suberization, and reticulation of cell wall structural proteins (Hiraga *et al.*, 2001). Peroxidases catalyse reticulation of the cell wall components, such as extensins, phenolics, and polysaccharides. Cell wall reinforcement through these crossed links may act as a mechanical barrier for pathogen penetration (Brisson *et al.*, 1994). With respect to TTS in fruit, they increased 21.33% with the highest Se dose compared with fruit of the control treatment (Figure 1d). Similar results were also reported (Schiavon *et al.*, 2013; Castillo-Godina *et al.*, 2016; Puccinelli *et al.*, 2017), indicating an increase

in TSS in tomato fruit by using Se in nutritional solution. The use of Se has been reported to affect positively starch accumulation (Lidon *et al.*, 2018; Ramadan *et al.*, 2020) and at the same time show a predominant effect in soluble solid accumulation in ripe fruit (Vallarino *et al.*, 2017). The results obtained suggest that Se addition in the nutritional solution may increase useful fruit life and modify positively TSS, aspects that impact positively in consumer preferences.

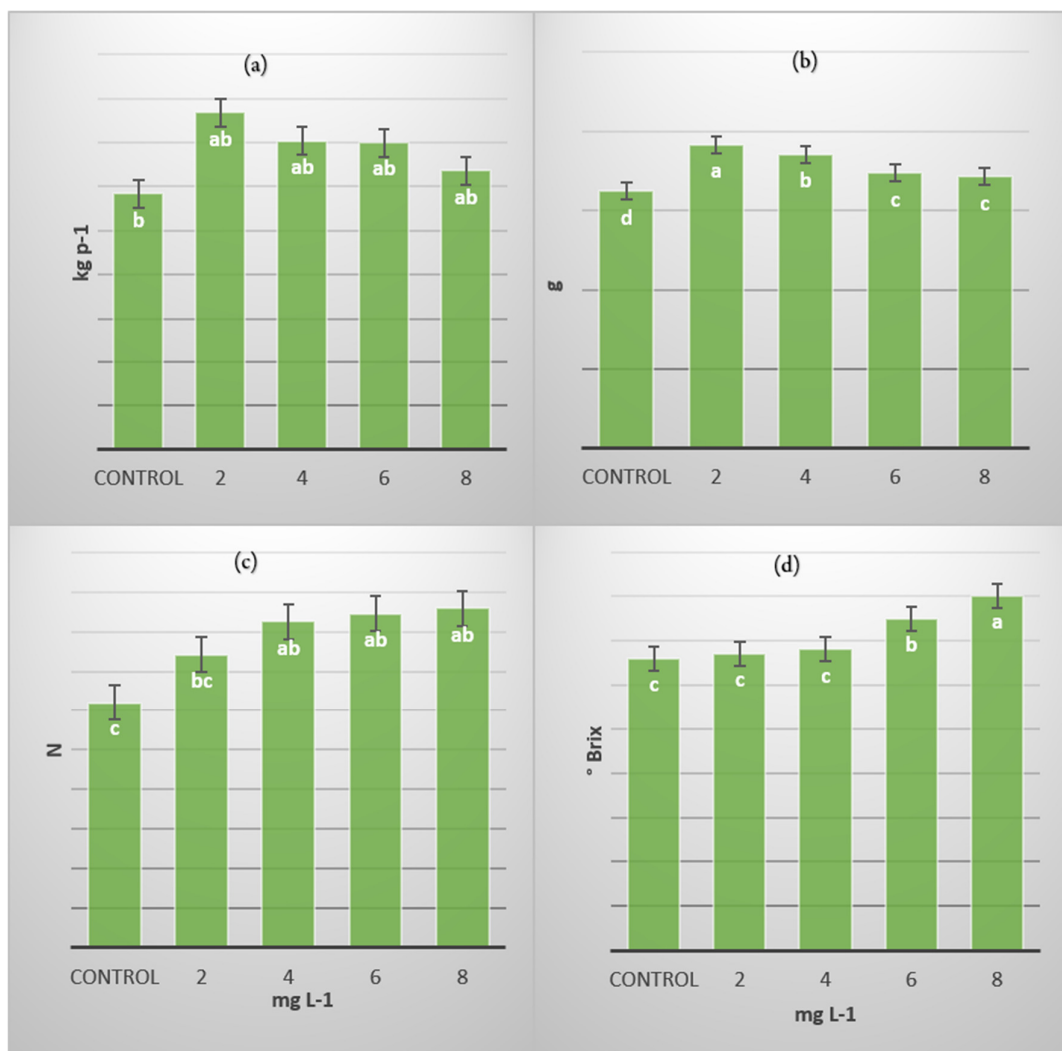


Figure 1. Effect of selenium concentration in the nutritional solution on yield content per tomato plant (a); dry matter per plant (b); firmness (c); and total soluble solids (TSS) (d) in tomato fruit

* Average values in columns with different letters differ statistically among them (Tukey's $p \leq 0.05$)

Fruit nutraceutical quality

Biosynthesis of phytochemical compounds (phenolic, flavonoid, and antioxidant capacity) was affected by the Se doses used, obtaining higher values with 2 mg L⁻¹, compared with the control treatment and higher doses. Selenium has demonstrated to stimulate phytochemical compound production in plants at lower concentrations compared with the control treatment and higher doses. Selenium has also shown to stimulate phytochemical compound production in plants exerting an antioxidant action in cellular biochemistry by increasing electron delivery; this result may be related to a greater antioxidant enzyme activity in peroxidase glutathione activity (Hawrylak-Nowak *et al.*, 2018) and a decrease in lipid peroxidation (Astaneh *et al.*, 2018).

The adequate Se doses provide an increase in total phenolic compound concentration and flavonoids (Golubkina *et al.*, 2019; Hachmann *et al.*, 2019), since Se increases secondary metabolite synthesis activating protection mechanisms that can decrease oxidative stress in chloroplasts (Hu *et al.*, 2019; Dall'Acqua *et al.*, 2019). Moreover, Se is an essential constituent of selenoenzymes, some of which have antioxidant functions improving the nutraceutical quality of the edible part of the crop (Garduño-Zepeda *et al.*, 2018; Silva *et al.*, 2020) and reducing ROS production, such as, O₂⁻ and H₂O₂ (Thavarajah *et al.*, 2017; D'Amato *et al.*, 2018). However, Se in high concentrations acts as pro-oxidant causing oxidative stress in plants (Astaneh *et al.*, 2018; Skrypnik *et al.*, 2019), which is possibly related to an increase in enzymatic and non-enzymatic antioxidants (Pannico *et al.*, 2019; Pérez *et al.*, 2019). This result causes failure in the protein structure when non-specific cysteine and methionine in proteins are substituted by SeCys and SeMet in the plant (Chomchan *et al.*, 2017; Garduño-Zepeda *et al.*, 2018), which leads to replacing cysteine with SeCys protein preventing the formation of disulfide bridges. These bridges are essential for protein structure and function and the replacement of cysteine with SeCys in the active site of the enzymes that deteriorate catalytic activity (Hu *et al.*, 2019). The stress caused by high Se doses unchains an oxidant disequilibrium in antioxidants (Rady *et al.*, 2020), causing structural damage in different macromolecules, lipids, proteins and on deoxyribonucleic acid (DNA) (Garduño-Zepeda *et al.*, 2018), and likely provoking apoptosis (Qui-Zapata *et al.*, 2010) and accumulation of degrading metabolites, such as malondialdehyde (MDA), a widely used aldehyde as oxidative stress biomarker (Hassan *et al.*, 2016).

A diet rich in phytochemical compounds is associated with a lower risk of cancer diseases and prevention of many others (Narváez-Ortiz *et al.*, 2018), thus the importance of increasing biosynthesis of these compounds in fruit before harvest and subsequent consumption.

Lycopene

Lycopene content in tomato fruit increased significantly with the dose of 2 mg L⁻¹, compared with the control treatment and higher Se doses. When the tomato fruit turns from green to yellow, the enzymatic antioxidant system protects the fruit from oxidative harm (Mondal *et al.*, 2004). As the fruit gets red, ascorbate and glutathione decrease as fruit ripening advances (Jiménez *et al.*, 2002). This decrease is due to the presence of carotenoids (lycopene and β-carotene), mainly switched on in fruit maturity stage (Del Giudice *et al.*, 2015). In this study, carotenoids (lycopene) decreased with high Se doses, which could have been related with Se since it increases ascorbate and glutathione concentration (Hassan *et al.*, 2016), delaying fruit maturity by reducing ethylene biosynthesis (Puccinelli *et al.*, 2017). Ethylene is a crucial hormone that controls fruit maturity period. Given that Se addition forms a methionine and converts to Se-Met that accumulates as organic Se in plant tissues, high doses may reduce free methionine levels – an important substrate of ethylene biosynthesis – and finally decrease ethylene production (Hachmann *et al.*, 2019; Oliveira *et al.*, 2019). Because lycopene is responsible for tomato fruit color characteristics and one of the main antioxidants consumed by humans in a regular diet, it is also an important visual characteristic for consumers.

Vitamin C

Due to the inability of humans to synthesize vitamin C, the main source are fruits (Pullar *et al.*, 2017). The most important function of ascorbate or vitamin C in plant cells is electron donation (ascorbate-glutathione cycle), where the ascorbate peroxidase (APX) enzyme uses two ascorbate molecules to reduce H₂O₂ into water and monodehydroascorbate (MDA) (Foyer *et al.*, 2011). At the same time, it can be recycled through the dehydroascorbate (DHA) reductase enzyme (Chen *et al.*, 2003). Tomato fruit are rich in carotenoids, vitamins A and C, of which the latter is an antioxidant that protects tissue damage caused by ROS (Nepal *et al.*, 2019). Vitamin C content in tomato fruit increased significantly with the 2 mg L⁻¹ dose, compared with the control treatment and higher Se doses. The use of Se has been reported to increase vitamin C in fruit (Bastías *et al.*, 2016). Vitamin C participates as an endogenous antioxidant in plants and exerts a protective action against free radicals in addition to an enzymatic cofactor that acts involved in photosynthesis (Palencia

et al., 2016). Se functions as a peroxidase deglutition cofactor, which may increase ascorbic acid content through a stimulating effect on glutathione (GSH), reducing DHA chemically to ascorbic acid by the ascorbate-glutathione cycle (Golubkina *et al.*, 2017).

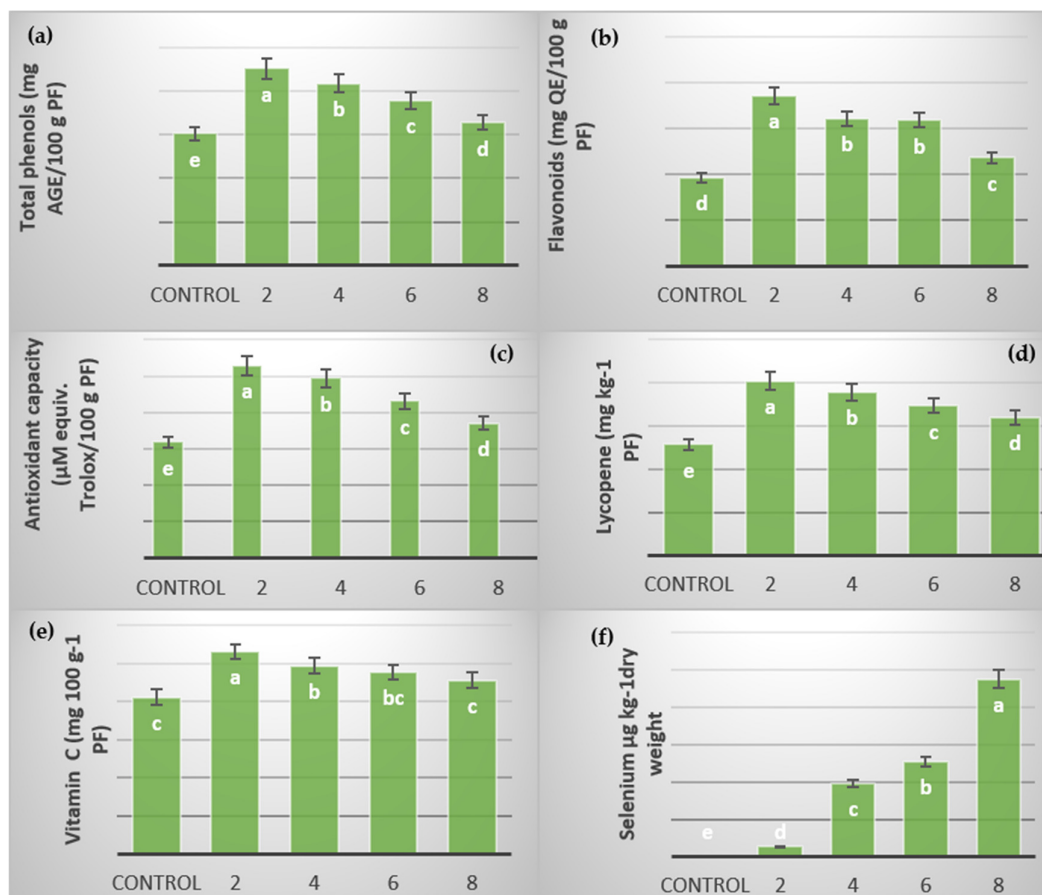


Figure 2. Effect of selenium doses on phenolic (a), total flavonoids (b), antioxidant capacity (c), lycopene (d), vitamin C (e), and selenium concentration (f) contents in tomato fruit.

* Average values in the columns with different letters differ statistically among them (Tukey's $p \leq 0.05$).

Selenium

Selenium content in tomato fruit increased 58% at higher Se doses compared with those in the control treatment (Figure 2f). The greatest absorption of this element might have taken place because it is chemically similar to sulfur. Thus, the roots easily absorb and metabolize them through high affinity sulfate transporters in the plasmatic membrane (Chomchan *et al.*, 2017) and incorporate routes with plant proteins to form seleno-cysteine (SeCys) and seleno-methionine (Se-Met) (White, 2016); then, they move without chemical modification through the xylem up to the leaves before reducing to other compounds (Jiang *et al.*, 2018; Alves *et al.*, 2020). Similar results were reported (Andrade *et al.*, 2018) when increased Se content was found in fruit as a result of adding it in the nutritional solution. Selenium absorption and distribution in plants depend on the chemical species used, concentration and application method (Sabatino *et al.*, 2019; Rady *et al.*, 2020). Presumably, the constant exposure of the plant root system to the Se-enriched solution and the lack of Se-soil interactions acted jointly to make soilless cultivations particularly efficient (Ryant *et al.*, 2020). The smallest Se dose may satisfy the daily consumption requirements of this element (55-200 mg/day per adult) proposed by WHO (Stefani *et al.*, 2020).

Conclusions

Agronomic biofortification with selenium applied in the nutritional solution improved yield, nutraceutical quality, and Se concentration in tomato fruit. The optimum dose that maximized yield and nutraceutical quality, as well as the recommended Se consumption concentration in tomato fruit in this study was 2 mg L⁻¹ of Se (Na₂SeO₄) since higher doses decrease yield and bioactive compound biosynthesis in tomato fruit. The use of selenium is a viable alternative to increase yield and obtain functional food.

Authors' Contributions

Conceptualization: JMGD, PPR; Methodology: JMGD, HOO; Validation: JJRP; Formal analysis: MFH; Investigation: JMGD; Data curation: PPR, LGHM; Funding acquisition: PPR, ESC; Project administration: PPR. Writing: JMGD, PPR; Review and editing: LGHM, PPR. All authors read and approved the final manuscript.

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Conflict of Interests

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

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