Article

Biofortification with selenium improves bioactive compounds and antioxidant activity in jalapeño pepper

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Abstract

Selenium (Se) is an essential oligo-element for human health and in plants is considered a beneficial element, as it is a growth promoter and a trigger for the antioxidant response in plants. Biofortification with Se aims to obtain foods rich in this oligo-element, of high nutritional quality that help combat the problems of malnutrition in the population. The present work aims to evaluate the ability of selenate (Na_2SeO_4) on yield, biosynthesis of bioactive compounds and their accumulation in chili fruits. For this, five treatments were applied via nutrient solution: 0, 1.5, 3, 4.5 and 6 mg L^{-1}. At harvest, nutraceutical quality, accumulation of Se in fruits, as well as crop yield were quantified. Biofortification with Se positively modified the biosynthesis of bioactive compounds and their concentration in fruit, without a decrease in yield. The incorporation of Se in the nutrient solution is an option to obtain functional foods with nutraceutical quality and with the possibility of improving public health after consumption.

Keywords: *Capsicum annuum* L., bioactive compounds, functional food.

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Introduction

Selenium (Se) is an important oligo-element in human nutrition, is essential to form proteins and a cofactor of antioxidant enzymes such as glutathione peroxidase (GPX), which protects the human body by catalyzing the reduction of reactive oxygen species (ROS) (Schiavon *et al*., 2020). In addition, in mammals, Se forms at least 25 selenoproteins that carry out antioxidant, catalytic, anti-inflammatory, antiviral and antitumor functions (Avery and Hoffmann, 2018).

A deficiency in this element causes health problems, including Keshan disease (an endemic cardiomyopathy), Kashin-Beck disease (endemic deforming osteoarthropathy) (Ulloa *et al*., 2021), acceleration of carcinogenic processes in the prostate (Sonkusre, 2020), fertility problems and weakening of the immune system, defense system against viral infectious diseases such as influenza, human immunodeficiency virus, muscular dystrophy and cystic fibrosis (Khurana *et al*., 2019). According to the World Health Organization, consumption of Se in the human diet should fluctuate between 55 and 200 μg day^{-1} in adults (Górska *et al*., 2021). The most common way by which the human organism acquires Se is through the consumption of foods, such as meat or fish (Sariñana-Navarrete *et al*., 2021), which contribute a high percentage of Se to the required daily intake.

In the world, there are about one billion people with Se deficiencies, mainly due to the consumption of plant-based diets (Błażewicz *et al*., 2020), which contain low concentrations of Se, since this element is present in soil in small amounts (López *et al*., 2021). On the other hand, this element is not considered essential for plants, but it could be considered a beneficial element because, in low concentrations, Se increases yield, antioxidant content and its concentration in the edible part (Preciado-Rangel *et al*., 2021).

One strategy to increase the content of Se in foods of plant origin is through biofortification, which consists of enhancing the bioactivity and content of Se in the edible parts of plants (Gaucin-Delgado *et al*., 2020). Foliar fertilization is the most practical way to incorporate Se into the food chain (Lyons, 2018). On the other hand, jalapeño pepper (*Capsicum annuum* L.), one of the most cultivated plants in the world, due to its importance in human nutrition as in the pharmaceutical industry (Espinosa-Palomeque *et al*., 2020), the fruits are a source of vitamins (A, E and C), carotenoids, capsaicinoids and phenolic compounds with antioxidant properties for the human diet (Natividad-Torres *et al*., 2021).

The application of micronutrients through the biofortification of crops is a very useful tool not only to increase the quantity of essential micronutrients but also to improve the biosynthesis of bioactive compounds (Gaucin-Delgado *et al*., 2020). The objective of this work was to determine the effect of foliar biofortification with Selenium on the yield, nutraceutical quality and antioxidant capacity of jalapeño pepper.

Materials and methods

Plant material and growing conditions

The study was conducted in a greenhouse with an automatic cooling system located at the facilities of the Universidad Autónoma Agraria Antonio Narro (UAAAN, for its acronym in Spanish) in the City of Torreón, Coahuila, Mexico (25° 33’ 26” north latitude,103° 22’ 31” west longitude, at an altitude of 1 230 m). The study crop was the jalapeño pepper cv. Hijo de Mitla, it germinated in polystyrene trays with 200 holes filled with peat (Premier®, Mexico) as a substrate. These plant trays were covered with black plastic for 72 h and watered every 24 h. The transplantation was carried out 45 days after sowing the seed, when the plants had an average height of 150 mm, each plant per pot. The pots were black polyethylene bags of 500 thick and 18 L capacity, filled with different proportions of sand: perlite (80:20 vv). The river sand was washed and disinfected with a solution of 5% sodium hypochlorite. The bags were placed in double row at 30 cm from center to center between bags and 1.6 m between rows, to obtain a plant density of 4.2 plants m^{-2}.

The water requirements of the crop were provided by manual irrigation to provide three irrigations per day, and each plant received 0.6 L in each irrigation, from transplantation to the beginning of flowering and 2.5 to 3.5 L from flowering to harvest. Pollination was carried out with an electric brush daily, from the beginning of flowering to setting. 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%.

Treatments and experimental design

A completely randomized experimental design was used, applying five doses: 0, 1.5, 3, 4.5 and 6 mg L^{-1} of Na_2SeO_3 (Sigma Aldrich) (León-Morales *et al*., 2019). Treatments with Se were applied every 15 days, with a total of six applications through foliar fertilization. It was watered with nutrient solution (Steiner, 1984), pH and electrical conductivity were maintained at 5.5 and 2 dS m^{-1} respectively. The yield, the nutraceutical quality of the fruit and the accumulation of selenium in fruits were determined. Ten plants per treatment were used.

Crop yield

In each experimental unit, yield was estimated by considering the number and weight of the individual fruit per plant, recording individual fruit traits such as weight with a scale (Ohaus 3729®, Mexico).

Nutraceutical quality

Preparation extract: for the determination of nutraceutical quality (phenolic compounds, flavonoids and antioxidant capacity), samples of 2 g of fresh fruit were mixed with 10 ml of ethanol in plastic tubes that were closed with screw cap. A ‘Stuart’-type stirrer was used to keep the mixture stirred for 24 h. After that, the tubes were centrifuged at 3 000 rpm for 5 minutes (Cardeño, 2007). The supernatants were extracted for physical-chemical analysis.

Total phenolic content

It was determined by a modification of the Folin-Ciocalteau method (Singleton, 1999). Fifty microliters of ethanolic extract were taken, diluted in 3 ml of mQ water, 250 µl of Folin-Ciocalteau (1N) was added, it was stirred and left to react for 3 min. Subsequently, 750 µl of Na_2CO_3 (20%) and 950 µl of mQ water were added. The solution was left to stand for 2 and the samples were measured in a UV-Vis spectrophotometer (CGoldenwall, wavelength range 340-1 000 nm and a spectral bandwidth: 5 to 760 nm). The standard solution was prepared with gallic acid. The results were expressed in mg 100 g^{-1} of fresh weight.

Total flavonoids

They were determined by spectrophotometry (Lamaison, 1990). Two hundred fifty microliters of ethanolic extract were taken and mixed with 1.25 ml of mQ water and 75 µl of $NaNO_2$ (5%), it was left 5 min and 150 µl of $AlCl_3$ (10%) was added. Subsequently, 500 µl of NaOH (1 M) and 275 µl of mQ water were added. It was vigorously stirred, and the samples were quantified in a UV-Vis spectrophotometer (CGoldenwall, wavelength range 340-1 000 nm and spectral bandwidth: 5 to 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 100 g^{-1} of fresh weight.

Total antioxidant capacity

It was measured by the DPPH + method *in vitro* (Brand-Williams, 1995). A solution of DPPH + (Aldrich) in ethanol was prepared, at a concentration of 0.025 mg ml^{-1}. Fifty microliters of the ethanolic extract were mixed with 1.95 µl of DPPH + solution, after 30 min the samples were quantified in a UV-Vis spectrophotometer (CGoldenwall, wavelength range at 340-1 000 nm and a spectral bandwidth: 5 to 517 nm). The results were expressed in equivalent to µM equiv Trolox 100 g m^{-1} fresh weight.

Capsaicin

Capsaicin content was measured by an adaptation of the method proposed by Cisneros-Pineda *et al*. (2007). The absorbance of the filtered extract was then obtained in a UV-Vis spectrophotometer (Goldenwall, wavelength range at 340-1000 nm) previously calibrated with acetonitrile as a blank at a wavelength of 273 nm. The capsaicin content was calculated by means of a standard curve using capsaicin (Sigma, St. Louis, Missouri, USA) as standard and the results are expressed in mg g^{-1} in fresh weight of capsaicin. The analyses were performed in triplicate.

Accumulation of selenium in the crop

The dried chili samples were ground in a porcelain mortar and digested with nitric and perchloric acid (3:1), using a hot plate at 100 °C. The solution was filtered and boiled to obtain 100 ml of working solution with deionized water. The concentration of selenium in tomato fruits was determined by atomic absorption spectrophotometry (Hsieh and Ganther, 1975), the results were expressed in µg kg^{-1} of dry weight of fruits.

Statistical analysis

The normality and homogeneity of the variances of the data obtained were verified using the Kolmogorov-Smirnov and Bartlett tests, respectively. Subsequently, simple classification analysis of variance and multiple comparison of means were performed using Tukey's test at a probability of 5% ($p \leq 0.05$), with the help of the statistical analysis package SAS v 9.0 (SAS Institute, 2004).

Results and discussion

Yield

The addition of Se did not significantly affect yield (Table 1), because Se is not considered an essential element for plant metabolism, supplementation with Se would not be expected to cause changes in crop growth and yield (Hernández-Hernández *et al*., 2019); however, it has been reported that applying high doses of Se reduces the yield of crops, because it causes oxidative stress in the plant, which can increase the production of free radicals by defending against the toxic effect caused by ROS (Hibaturrahman *et al*., 2020), on the contrary, using low doses increases yield (Rady *et al*., 2020) and prevents oxidation by regulating the uptake and redistribution of essential elements in antioxidant systems or maintaining the ion balance and structural integrity of the cell (Pannico *et al*., 2019), allowing the plant to adapt metabolically as physiologically as a response to the attack of oxidative stress caused by the increase of free radicals under stress conditions (Hasanuzzaman *et al*., 2020).

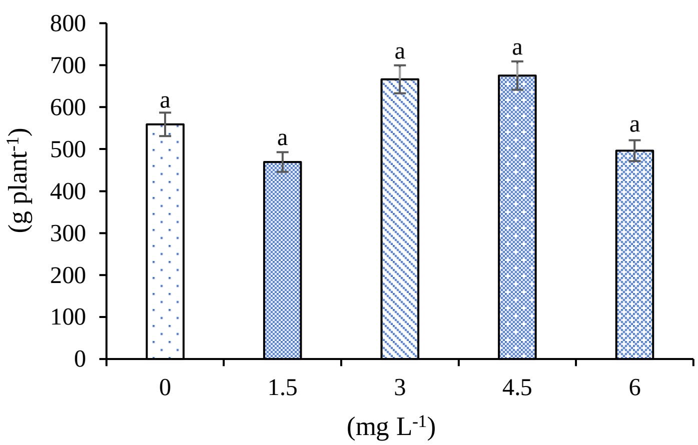

Figure 1. Effect of Se on the yield of chili fruits. Data are shown as mean ± standard deviation (SD) (n= 50). Columns with different letters are significantly different according to Tukey's test ($p < 0.05$).

The plant presents differential response to Se depending on the dose used, it has both a positive and negative impact on plants, since in low concentrations, Se accumulates as a stimulant by promoting growth, plant physiology, increasing yield (Gaucin-Delgado *et al*., 2020) and in high doses, negative effects are exerted on the growth and physiology of the plant (Silva *et al*., 2020) and even cell death (Shahid *et al*., 2018).

Bioactive compounds

The biosynthesis of phenolic compounds, flavonoids and antioxidant capacity was positively influenced by the application of Se (Figure 2a-2c), obtaining the highest values with 6 mg L^{-1}. Various studies have shown that the application of Se can increase the production of bioactive compounds (Motesharezadeh *et al*., 2020). Results like those of the present study were obtained in *Capsicum annuum* L., where the application of Se increased several bioactive compounds such as phenols, flavonoids and antioxidant capacity (Natividad-Torres *et al*., 2021).

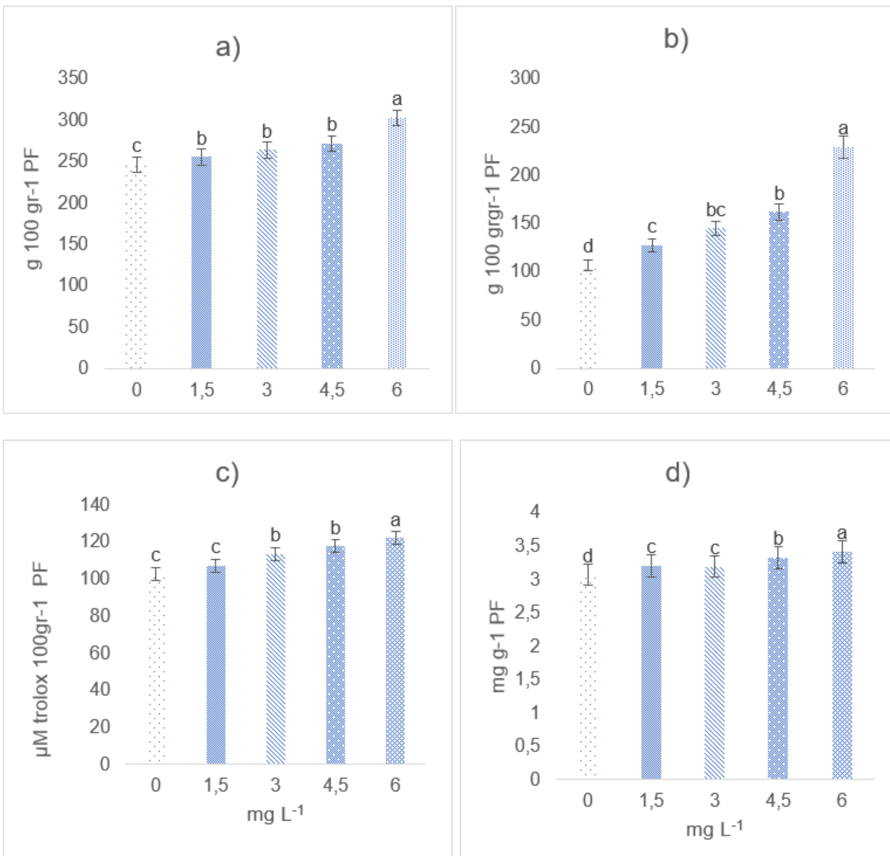

Figure 2. Effect of Se on the content of phenolics (a) total flavonoids (b); antioxidant capacity (c); and capsaicin (d) in chili fruits. Data are shown as mean ± standard deviation (SD) (n= 50). Columns with different letters are significantly different according to Tukey's test ($p< 0.05$).

This bioactive capacity is possibly attributed to the synergistic action that Se has, as it can act as a vital element by altering various physiological and biochemical processes (D'Amato *et al*., 2018), in addition, Se directly affects the antioxidant defense system by increasing the potential of plants to overcome the conditions of biotic and abiotic stress (Hachmann *et al*., 2019). The results obtained in our study are supported by Hibaturrahman *et al*. (2020), who found that supplementation in nutrient solution with Na_2SeO_4 increases antioxidant activity, thus protecting plants from oxidative stress.

The production of foods rich in bioactive compounds directly influences the cellular and physiological activities (Sabatino *et al*., 2019), obtaining, after their intake, a beneficial contribution to human health (Shahid *et al*., 2018) due to their various characteristics, protection in coronary diseases (Groth *et al*., 2020) and after avoiding cellular aging (Hernández-Hernández *et al*., 2019), in addition to being used as an alternative for cancer prevention (Vinceti *et al*., 2018).

Capsaicin

Capsaicin is an oleoresin, an active component of hot peppers (Friedman *et al*., 2019). Capsaicin was positively affected by the doses of Se evaluated (Figure 2d). The concentration of capsaicin was in accordance with the doses of Se used, the increase in the pungency of the chili improves its quality, since this characteristic is appreciated by consumers (Uarrota *et al*., 2021). The results indicate a response of the capsaicin content in the fruits of jalapeño pepper to Se, although the mechanism of action in this process has not been defined. Biosynthesis is strongly influenced by genotype-environment interactions in Capsicum fruits (Naves *et al*., 2019), phenylpropanoids (Aza-González *et al*., 2011) and ABC transporters, specifically the ABCC and ABCG subfamilies, which may be playing important roles in the transport of secondary metabolites such as capsaicin and dihydrocapsaicin to placental vacuoles, affecting their content in fruits (Lopez-Ortiz *et al*., 2019).

Probably to the interaction of Se in the plant by causing stressful effects (Avery and Hoffmann, 2018) that could be related to an increase in the PAL activity and capsaicin synthase, increasing the accumulation of capsaicinoids in chili (Ulloa *et al*., 2021), by negatively regulating peroxidase activity at appropriate levels (Hernández-Pérez *et al*., 2020).

Although biosynthesis is a controlled genetic trait, the environment also plays an important role depending on the genotype (Scossa *et al*., 2019), in addition, this feature is also regulated from the point of view of development and the environment (Naves *et al*., 2019). On the other hand, capsaicin provides the spicy oral sensation in most chili peppers (Scossa *et al*., 2019); however, its most important biological properties are its ability to act as antioxidants to reduce oxidative stress that leads to the prevention of several degenerative diseases (Friedman *et al*., 2019).

Selenium content in the crop

The addition of 6 mg L^{-1} increased the content of Se in chili fruits, there is a positive correlation between Selenium in fruits and its availability (r^2= 0.98) (Figure 3). The absorption of Se depends on the age of the plant, plant species and the chemical form of the element being applied, its concentration and the method of application (Yin *et al*., 2019). Vegetables accumulate higher amounts of Se (Dai *et al*., 2019).

Previous studies show that biofortification significantly increases the quantity of essential elements in the edible part of plants (Silva *et al*., 2020), which may increase in crops biofortified with Se up to 30% with respect to untreated crops (Zhu *et al*., 2017). Se in plants is metabolized along with sulfur within plant tissues, being transformed into selenoproteins that allow it to be stored as Se-Met (Zhang *et al*., 2019), accelerating transport, accumulation, volatilization and tolerance to Se (Raina *et al*., 2020).

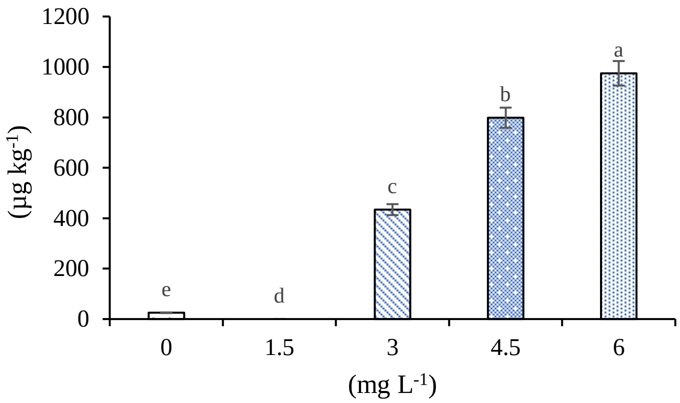

Figure 3. Concentration of Se in chili fruits. Data are shown as mean ± standard deviation (SD) (n= 50). Columns with different letters are significantly different according to Tukey's test (p< 0.05).

It acts as a potent antioxidant, by allowing lower levels of lipid peroxidation and increased activity of antioxidant enzymes, as well as better resistance to oxidative stress (Skrypnik *et al*., 2019). According to the dietary guide for Americans, most of the required nutrients should be obtained with food intake (Padilla-Samaniego *et al*., 2020). In this sense, the consumption of 0.74 g of jalapeño pepper biofortified with selenium supplies the daily requirements of this oligo-element, which is 55 mg per day in adults (Chomchan *et al*., 2017; León-Morales *et al*., 2019).

Conclusions

Agronomic biofortification with selenium improves nutraceutical quality and Se concentration, without affecting the yield of the jalapeño pepper crop, the use of Se is an alternative to obtain functional foods and increase the accumulation of oligo- element in jalapeño pepper fruits, with the possibility of protecting human health with its consumption.

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