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Effect of Selenium on Elemental Concentration and Antioxidant Enzymatic Activity of Tomato Plants

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ABSTRACT

Selenium is an essential element for humans, therefore, adding it to plants is convenient for biofortification. Thus, the aim of this work was to analyze experimentally the ability of sodium selenite to increase the concentration of Se and modify the antioxidant activity in tomato plants. We used plants of the Toro hybrid variety and applied three treatments: 0, 2 and 5 mg L^{-1} of selenium as sodium selenite (Na_2SeO_3) using an irrigation system. Three samplings 40, 80, and 120 days after transplantation and a quantification of the accumulation of selenium and macronutrients in leaves, stems, and fruits were carried out. Plant height, stem diameter, firmness, and total solids of fruits and total dry matter were measured. The enzyme activity of catalase, glutathione peroxidase, and superoxide dismutase was quantified. The results indicated positive effects of Se on agronomic variables of the plants and fruit quality; however, fruit production showed no significant differences. Se had no effect on the concentration of the macro and micronutrients. The addition of 5 mg L^{-1} of Se resulted in significant increases in the concentration of selenium on a dry basis, reaching 20.4 µg g^{-1} in leaves, 52.3 µg g^{-1} in stems, and 35.8 µg g^{-1} in fruits. The increase in enzyme activity in the fruits was enhanced by the application of 5 mg L^{-1} of Se: There was up to 352.7% more catalase activity, 312.2% more glutathione peroxidase activity, and 200.8% more superoxide dismutase activity compared with the control.

Keywords: Biofortification, Catalase, Glutathione peroxidase, Sodium selenite, Superoxide dismutase.

INTRODUCTION

Eating healthy foods high in antioxidant nutrients contributes to the protection of cells from oxidative damage and to the prevention of various diseases (Broadley *et al.*, 2006). Free radicals cause oxidative chain reactions that are eliminated by the action of defensive antioxidant systems, including enzymes such as SuperOxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GSH-Px) according to Sahnoun *et al.* (1997). Antioxidant enzymes generally use trace elements such as Selenium (Se) as cofactors, as in the case of GSH-Px (Arthur, 2003). Selenium is thought to be associated with antioxidant metabolism (Lin *et al.*, 2012; Feng *et al.*, 2013) through its role as a cofactor of selenoenzymes (Combs, 2001); its deficiency could induce changes in cellular redox balance. In humans, the average reference intake of Se is 60 to 75 µg per day, according to 1980 data from the U.S. Food and Nutrition Board (Broadley *et al.*, 2006); however, these values are well below the consumption levels cited by Combs (2001) and Broadley *et al.* (2006), indicating up to 300 µg per day for reducing the risk of cancer. Generally, cultivated plants that grow in non-seleniferous soils have low concentrations of Se, ranging from 0.01 to 1 mg kg^{-1} dry weight. Thus, in some countries

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where soils are poor in selenium, it is added to the fertilizers used for agricultural production (Broadley *et al.*, 2006).

Selenium is metabolized in plants in the sulfur assimilation pathway, and its distribution and accumulation depends on the chemical species and the concentration of the element supplied by irrigation and through the leaves and on the nature and concentration of other substances in soil solution (Terry *et al.*, 2000). In the environment, selenium can be released by natural processes or induced by human activity and become incorporated into the soil and water (White *et al.*, 2004). Regarding its chemical form, selenium is mobilized by various processes within plant cells. Thus, selenate (Se^{+6}) can be mobilized through a primary transport process coupled with a H^{+}-ATPase with the help of a sulfate (Terry *et al.*, 2000) or silicon transporter (Zhao *et al.*, 2010); once absorbed by plants, it remains in inorganic form (De Souza *et al.*, 1998; Cartes *et al.*, 2006). In contrast, the absorption of selenite (Se^{+4}) occurs differently (Terry *et al.*, 2000), that is, through a phosphate transporter (Zhao *et al.*, 2010). Once absorbed, selenite remains in organic form (De Souza *et al.*, 1998; Cartes *et al.*, 2006), and it has been shown to be a more efficient inducer of glutathione peroxidase (Cartes *et al.*, 2005).

Based on the above, the enrichment of food crops with selenium has been proposed as a strategy for improving the intake of selenium. The objectives of this study were: (1) To document changes in plant growth and the concentration of mineral elements in different organs in response to the application of selenium (as sodium selenite), and (2) To analyze changes in certain antioxidant metabolites of the tomato plant *Lycopersicon esculentum* L. (Mill). This species was used as a biological model because it was characterized as a non-accumulator of selenium (White *et al.*, 2004).

MATERIALS AND METHODS

The experiment was conducted in 2012 at the Agrarian University "Antonio Narro," located in Saltillo, México. Tomato seeds of the hybrid saladette type "Toro" with determinate growth were seeded. The seedlings were grown in polystyrene trays with 200 cavities, and we monitored the growth and care of the seedlings for a period of 40 days. Subsequently, the seedlings were transplanted into 12 L pots, using peat moss and perlite as substrates in a ratio of 70:30. Plants were grown in a shade house structure with a black shade polypropylene net that had a shade factor of 25%. Maximum and minimum temperature and relative humidity were 25 and 18ºC and 50 and 65 %, respectively. Crop nutrition was addressed by means of Steiner's universal nutrient solution (Steiner, 1961) adjusted to a pH value of 5.5 to 6.5 with sulfuric acid to ensure the availability of mineral elements in the nutrient solution and keep the selenite ion in its protonated form (Preciado-Rangel *et al.*, 2006). The concentration of the solution applied was chosen according to the phenological stage: 25% in the initial stages and reaching 75% after flowering. The following treatments were applied to the plants: irrigation with a nutrient solution as a control (Steiner, 1961) and irrigation with the same nutrient solution plus 5 and 10 mg L^{-1} of selenium, using reagent-grade anhydrous sodium selenite as a source (Na_2SeO_3, Sigma-Aldrich). The pruning of lateral buds and plant tutoring were performed every 8 days; pests and diseases were controlled by preventive applications of phytosanitary products, which do not contain selenium as indicated by the manufacturer. The variables assessed were obtained from three samplings taken 40, 80, and 120 days after transplantation. Leaf, stem, and fruit tissues were taken at each sampling.

We used a completely randomized experimental design. An analysis of variance was carried out on agronomic and fruit variables and on mineral concentration and

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enzyme activity using the Statistical Analysis System (SAS) 9.1.3 software. A Tukey mean comparison test ($P \leq 0.05$) was performed to identify differences between treatments. The mean tests were applied independently to each plant organ. In addition, a rank correlation test (Spearman R) was carried out to verify the statistical association between the concentrations of Se and the mineral elements in leaves, stems, and fruits.

Fruit Production

The number of Fruits Per Plant (FPP) was obtained by harvesting the ripe fruits from 8 plants that were chosen completely at random from each treatment. The Weight of the fruits was measured to obtain the production of Fruits Per Plant (FWPP) in g $plant^{-1}$.

Agronomic Variables

At each of the three samplings, plant height was measured as the Stem Length (SL) from the base of the stem to the last leaf in the aerial part of the plant using a tape measure; Stem Diameter (SD) was measured at the base of the plant by means of a vernier. The Polar and Equatorial Diameters (PFD and EFD) of each fruit were measured using a simple vernier, firmness was measured using a manual penetrometer (QA Supplies, model FT011), and the amount of total soluble solids was measured using a hand refractometer (Atago Master Refractometer). To obtain the percentage of Total Dry Matter (TDM), 6 plants were randomly selected from each treatment, and two physiologically mature leaves, stems, and fruits were cut from them. The structures were weighed to obtain fresh weight; the stems were cut into pieces and the fruits into thin slices. They were then placed in a drying oven at a temperature of 60°C for 48 hours. After that time, they were weighed again, and the percentage of total dry matter was calculated.

Determination of Selenium and Mineral Nutrients

From the samples obtained for estimating TDM, a portion of 5 g was macerated in a porcelain mortar. One gram of the mash was taken and subjected to acid digestion with nitric and perchloric acids in a ratio of 3:1 using a hot plate at 100°C. Subsequently, the solution was filtered with Whatman filter paper (No. 42 ashless), and a working solution of 100 mL was prepared with the addition of deionized water. The reading was performed using an Induction Plasma Spectrometer (ICP), Thermo Jarell Ash brand, IRIS Advantage model, following the 984.27 method (A.O.A.C., 2000). The quantified nutrients were K, Mg, and Ca, in addition to the corresponding quantification of selenium. Nitrogen was quantified by the Kjeldahl method (AOAC, 1980), and P was quantified by the colorimetric method with an aminonaphthol sulfonic acid reagent. The reading was performed with a UV-Vis spectrophotometer (Genesys 10S Thermo Scientific) at an absorbance of 640 nm (Harris and Popat, 1954).

Activity of Antioxidant Proteins

For the preparation of the extracts of leaves, stems, and fruits, we macerated 1 g of fresh tissue at each sampling in a mortar with liquid nitrogen; from the resulting mash, we took 0.3 g and transferred them to a 2.0 mL centrifuge microtube, adding 1.5 mL of phosphate buffer (pH 7) that had previously been stored at 4°C. The tube containing the sample was subjected to a vortex (Genie 1 Touch Mixer Model SI-0136) to homogenize the sample; it was then sonicated (Branson Sonifier 1510 model 1510R-DTH) on ice for 10 minutes and then centrifuged in a refrigerated centrifuge (Prism Labnet International Inc.) at 12,000 rpm for 10

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minutes at 2°C. The supernatant was separated and stored at -80°C.

Total soluble proteins were quantified to determine specific enzyme activity. To perform this quantification, we used the extract mentioned above and placed 5 µL of the extract together with 250 µL of Bradford reagent (Bradford, 1976) into each of the wells of an ELISA multiplate; the reading was performed in a spectrophotometer (Thermo Scientific Genesys 10S UV-Vis) at an absorbance of 630 nm.

Catalase Enzyme Activity

Catalase activity was analyzed according to the modified method of Lubinsky and Bewley (1979) using UV-visible spectrophotometry at a wavelength of 275 nm. An amount of 20 µL was taken from the plant tissue extracts, and a 1:20 dilution was made with 380 µL of phosphate buffer (pH 7). A blank was prepared with 400 µL of phosphate buffer (pH 7), 150 µL of H_2SO_4 (2%) and 2 µL of H_2O_2 (30%). A time 0 was determined for the sample diluted 1:20 with 150 µL of H_2SO_4 (2%) and 2 µL of H_2O_2 (30%); it was called time 0 because the possible reaction was stopped previously with H_2SO_4. At time 1, 2 µL of H_2O_2 (30%) were added to the sample diluted 1:20, and it was allowed to react for 1 min with vigorous stirring. Another 150 µL of H_2SO_4 (2%) was added to stop the reaction. This was done for each sample, and 275 nm was used for the readings (spectrophotometer UV-Vis Genesys 10S Thermo Scientific). A standard curve of H_2O_2 was made with concentrations of 0, 15, 20, 25, 30, 35, 40 and 100 mM. One unit of catalase is defined as 1 µmol of H_2O_2 mL^{-1} min^{-1}.

Glutathione Peroxidase Enzyme Activity

The activity of glutathione peroxidase was measured using a modified version of the method of Xue *et al.* (2001). We used the same plant tissue extract used for previous quantifications. An amount of 0.2 mL was taken from the supernatant and placed in a 1.5 mL Eppendorf tube, adding 0.4 mL of GSH (0.1 mmol) and 0.2 mL of sodium and potassium buffer (0.067M). After pre-heating the mixtures in a water bath at 25°C for 5 minutes, 0.2 mL of H_2O_2 (1.3 mmol) was added to initiate the reaction. The reaction was held for 10 minutes and stopped by adding 1 mL of trichloroacetic acid (1%); the mixture was placed in an ice bath for 30 minutes and then centrifuged for 10 minutes at 3,000 rpm. Then, 0.48 mL was taken from the supernatant and placed into a beaker, to which was added 2.2 mL Na_2HPO_4 (0.32 M) and 0.32 mL of 1.0 mmol 5,5-DiThiobis(2-NitroBenzoic acid) (DTNB) for color development. Absorbance was measured at a wavelength of 412 nm with a UV-Vis spectrophotometer (Thermo Scientific Genesys 10S) after being left in repose for 5 min. We generated a standard curve for the pure enzyme obtained from a commercial Glutathione Peroxidase kit (GPx, Assay Science Cell Research Laboratories). A unit of glutathione peroxidase is defined as 1 mU of GSH-Px mL^{-1} min^{-1}.

Superoxide Dismutase Enzyme Activity

The quantification was done using an SOD kit (SOD assay kit Sigma-Aldrich 19160). A standard curve was generated as indicated by the SOD kit, and enzyme activity was calculated from the percentage of inhibition rate by incubating the mixtures at 37°C for 20 minutes and reading the absorbance at 450 nm in an ELISA microplate reader (model LEX-808 IU). A unit of superoxide dismutase is defined as 50% inhibition of formazan at 450 nm.

RESULTS AND DISCUSSION

Fruit Production

The number of Fruits Per Plant (FPP) and Fruit Weight Per Plant (FWPP) showed no significant differences ($P \leq 0.05$) between the

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Table 1. Comparison of means of Total Dry Matter (TDM), number of Fruits Per Plant (FPP) and Fruit Weight Per Plant (FWPP), evaluated on three sampling dates in tomato plants with applications of selenium in nutrient solution at three different concentrations: 0, 2 and 5 mg L^{-1}.[a]

Treatments	TDM (%)			FPP	FWPP
	Leaf	Stem	Fruit	(No)	(g)
Control	43.7 b	26.6 a	32.8 a	10.8 a	860.1 a
2 mg L^{-1}	48.1 ab	28.3 a	28.9 a	13.0 a	1049.3 a
5 mg L^{-1}	50.9 a	29.9 a	30.0 a	13.9 a	1147.1 a

[a] Mean values with different superscript letters in the same column differ significantly according to Tukey´s test (P≤ 0.05).

treatments (Table 1). These results agreed with those of Becvort-Azcurra *et al.* (2012), who applied 2.5 and 5 mg L^{-1} of selenium to tomato plants grown in perlite substrates and soil and found no significant differences in fruit production, and those of Yang *et al*. (2003), who recorded a non-significant effect of selenium application on soybean yield. There are reports, however, about the positive effects of the addition of selenium to horticultural crop species, such as that by Germ *et al.* (2005), who found this effect on the biomass of *Cucurbita pepo* fruits. Meanwhile, Nancy *et al*. (2014) showed that tomato fruit yield and quality were higher with increasing concentrations of selenium in soil, seed, or foliar application (until a maximum of 10 mg L^{-1}). Other authors have also reported that Se increases the yield of rice (Wang *et al*., 2013), lettuce (Xue *et al*., 2001) and soybean (Djanaguiraman, 2004). Conversely, Yao *et al*. (2013) reported an increase in wheat yield by Se foliar application. The impact of selenium applications in the improvement of yield and quality of crops depends on several factors, such as soil texture, the physico-chemical characteristics, and the method and time of Se application (Lyons *et al*., 2003). With respect to Total Dry Matter (TDM) content, there was a significant difference (P≤ 0.05) between treatments in leaves but not in stems and fruits (Table 1). A similar behavior was reported by Becvort-Azcurra *et al.* (2012), who found no difference in the TDM of leaves, stems, and fruits after the addition of Se to tomato plants.

Agronomic Variables

The results for the agronomic variables are shown in Table 2. It can be observed that there were significant differences (P≤ 0.05) between treatments associated with the application of Se, with a significant increase in the values of Stem Length (SL), Stem Diameter (SD), fruit total soluble solids, firmness, and Diameter of Fruits (PFD and EFD). The positive responses observed in

Table 2. Comparison of means of the agronomic variables evaluated on three sampling dates in tomato plants with applications of selenium in nutrient solution at three different concentrations: 0, 2 and 5 mg L^{-1}.[a]

Treatments	SL cm	SD mm	PDF cm	EDF cm	Fruit firmness kg cm^{-2}	Total Soluble Solids of Fruits °Bx
Control	61.1 b*	11.8 b	5.49b	5.42b	2.9 c	4.3 b
2 mg L^{-1}	67.5 a	13.4 a	6.24a	5.93a	4.3 b	5.1 a
5 mg L^{-1}	65.2 a	13.3 a	6.38a	5.96a	4.5 a	4.9 a

[a] Mean values with different superscript letters in the same column differ significantly according to Tukey´s test (P≤ 0.05). SL= Stem Length; SD = Stem Diameter; PFD = Polar Diameter of Fruits, EFD= Equatorial Diameter of Fruits.

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the plants are consistent with the report by Hartikainen *et al.* (2000) when the Se concentration in *Lolium perenne* leaves was less than 10 mg kg^{-1}. They are also consistent with the reports by other authors about a positive effect of the addition of Se on species such as *Lolium perenne* (Hartikainen *et al.*, 2000), *Lactuca sativa* (Xue *et al.*, 2001) and *Solanum tuberosum* (Turakainen *et al.*, 2004).

Mineral Nutrients

The concentration of selenium in leaves, stems, and fruits, expressed in µg g^{-1}, is shown in Figure 1. In the leaves and stems, both Se treatments were significantly different from the control ($P \leq 0.05$); however, in the fruits, the only difference was between 5 mg L^{-1} of Se and the control. The peak value in fruits was 35.8 µg g^{-1} when treated with 5 mg L^{-1}, resulting in 53.1% more selenium over the control plants, which is higher than that reported by Nancy *et al*. (2014) in tomato fruits (29.5 µg g^{-1}) developed with 10 mg L^{-1} sodium selenate through soil application and a rate accumulation of 52.5% over the control plants. Other reports have confirmed Se accumulation in wheat grains (Nawaz *et al*., 2014) and rice grains (Boldrin *et al*., 2013) obtained by Se fertigation and Se foliar application, respectively.

The effect of Se on plants depends mainly on its concentration. According to Hamilton (2004), Se has three levels of biological activity: (1) Trace concentrations are required for normal growth and development; (2) Moderate concentrations can be stored to maintain homeostatic functions; and (3) High concentrations may result in toxic effects. Studies on *Lolium perenne* and *Lactuca sativa* have shown that although Se is harmful to plants in high concentrations (>10 and 1.0 mg kg^{-1}, respectively), it could exert beneficial effects at low concentrations (Hartikainen *et al.*, 2000; Xue *et al.*, 2001). In this regard, Becvort-Azcurra *et al.* (2012) found toxic effects in tomato plants when applying 10 and 20 mg L^{-1} in the nutrient solution. Moreover, the values of the concentration of Se in leaves, stems, and fruits under all the treatments applied in this study were higher than those reported by Becvort-Azcurra *et al.* (2012). It is possible that this difference is due to the use of soil and perlite as substrates by these authors, which most likely modified the availability of selenium. Furthermore, the values of Se in fruits of the control plants were high compared with those reported by Eurola *et al.* (1989) and by Becvort-Azcurra *et al.* (2012), who added Se to soil. As for the distribution of selenium in

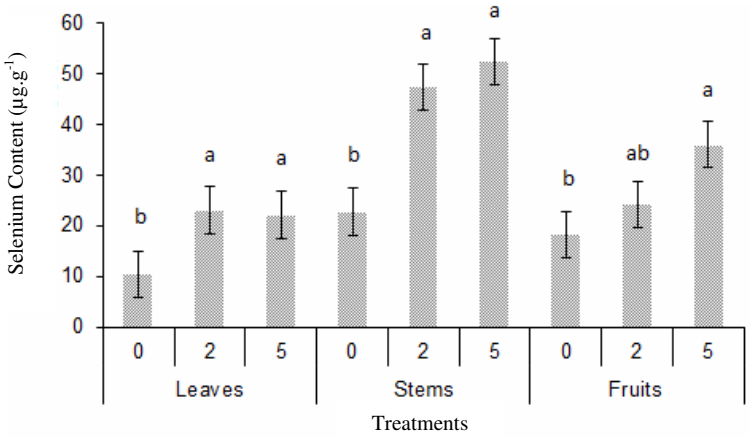

Figure 1. Selenium concentration in tomato leaves, stems and fruits with applications of selenium in nutrient solution; the concentrations used are indicated on the x axis (0, 2 and 5 mg L^{-1}). Means with different label letter differ significantly according to Tukey's test ($P \leq 0.05$). The mean tests were applied independently to each plant organ.

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the plants, Kabata-Pendias and Pendias (2001) reported an uneven accumulation among the different organs; actively growing tissues usually contain higher amounts of Se, and many species of plants accumulate higher amounts of selenium in stems and leaves than in root tissues. Our results do not agree with the above because we obtained more selenium in the stems and lower concentrations in fruits and leaves (Figure 1). A similar result was reported by Arvy (1993) and Becvort-Azcurra *et al.* (2012), who found the highest concentrations of selenium in stems, followed by leaves and fruits. If only leaves and fruits are compared, a higher accumulation in fruits was obtained than in the control, whereas Nancy *et al*. (2014) reported higher values in the leaves.

The concentrations of macronutrient elements in the plant tissues are shown in Table 3. The macronutrients that showed significant differences ($P \leq 0.05$) between treatments were Mg in leaves and K in stems and fruits. Despite the above results, the Spearman coefficient showed no significant correlation ($P \leq 0.05$) between the concentration of Se and the rest of the mineral elements. This fact suggests that the presence of Se in the concentrations used in this study did not interfere with the absorption of other elements. This does not agree with Kabata-Pendias and Pendias (2001), who noted that higher levels of selenium in plants can suppress the concentration of N in tissues and can inhibit the absorption of some metals such as Mg. Meanwhile, Smoleń *et al.* (2014) reported a reduction in the levels of Ca and Mg in lettuce roots with the foliar application of Se and I, although they did not observe any difference in the content of macronutrients in leaves when applying Se individually. As for the correlation between Se concentrations in different plant tissues, they were all positive and significant (Leaf Se/Stem Se, R= 0.50; Leaf Se/Fruit Se, R= 0.68 fruit; Stem Se/Fruit Se, R= 0.59), which suggests absence of competition for Se between the different organs and an accumulation of Se by the organs in direct proportion to its availability.

Activity of Antioxidant Proteins

The results for the activities of catalase and glutathione peroxidase are shown in Figures 2 and 3, respectively. Both cases show a similar behavior in which enzyme activity shows a tendency to grow in treatments with Se, but the only significant change is in enzyme activity of fruits when 5 mg L^{-1} of Se was applied. Regarding the

Table 3. Concentration of macronutrients elements and selenium in the different organs of tomato with selenium application in nutrient solution at three different concentrations 0, 2 and 5 mg L^{-1}. Concentrations of N, P, K, Ca and Mg are given in percentage, the selenium in µg g^{-1}. [a]

Plant organs	Se (mg L^{-1})	N	P	K	Ca	Mg	Se
Leaves	0	3.36a*	0.76a	3.57a	5.16a	0.58c	9.90b
	2	3.24a	0.82a	4.42a	4.72a	0.68b	20.9a
	5	3.09a	0.91a	3.47a	3.54a	0.71a	20.4a
Stems	0	2.36a	0.70a	3.84ab	3.30a	0.49a	21.7b
	2	2.50a	0.77a	4.47a	3.90a	0.31a	45.6a
	5	2.49a	0.72a	3.40b	2.82a	0.37a	52.3a
Fruits	0	2.20a	0.59a	2.95ab	0.86a	0.40a	16.8b
	2	1.87a	0.47a	3.44a	1.26a	0.34a	24.5ab
	5	2.11a	0.56a	2.90b	2.82a	0.33a	35.8a

[a] Mean values with different superscript letters in the same column differ significantly according to Tukey´s test ($P \leq 0.05$).

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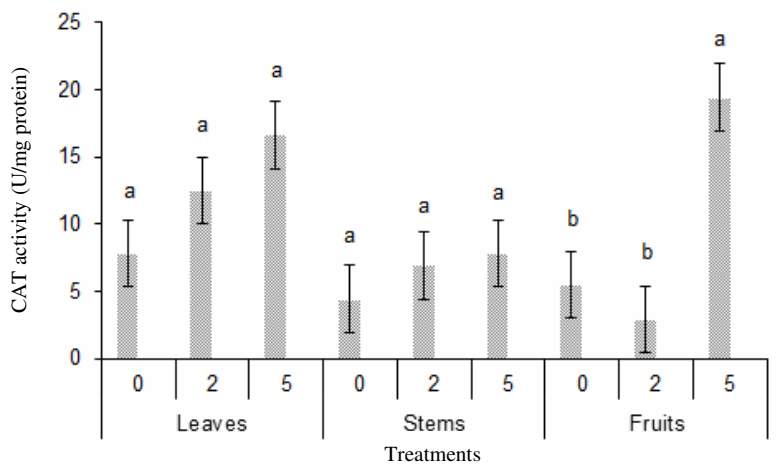

Figure 2. Catalase enzyme activity in tomato leaves, stems and fruits with application of 0, 2 and 5 mg L^{-1} of selenium. Means with different label letter differ significantly according to Tukey's test ($P \leq 0.05$). The mean tests were applied independently to each plant organ.

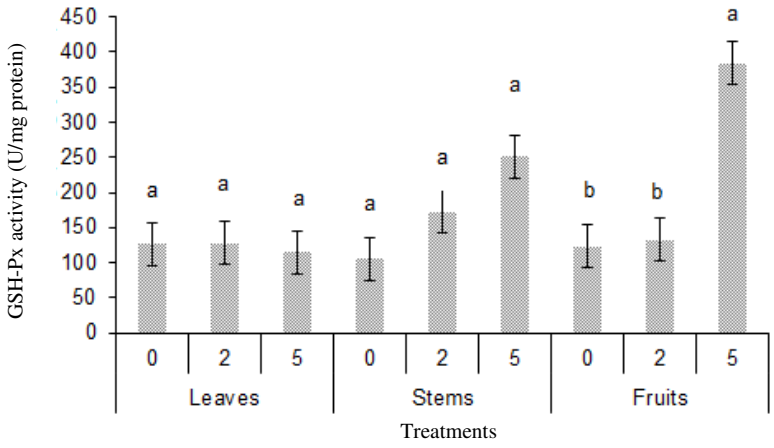

Figure 3. Glutathione peroxidase enzyme activity in tomato leaves, stems and fruits with application of 0, 2 and 5 mg L^{-1} of selenium. Means with different label letter differ significantly according to Tukey's test ($P \leq 0.05$). The mean tests were applied independently to each plant organ.

antioxidant activity, recent investigations have shown that selenium not only promotes plant growth and development but also decreases senescence, increases antioxidant enzyme activity (Xue *et al.*, 2001; Djanaguiraman *et al.*, 2005; Saidi *et al.*, 2014, Nawaz *et al.*, 2014) and increases resistance and antioxidant capacity under various types of stress (Peng *et al.*, 2002; Djanaguiraman *et al.*, 2005; Lin *et al.*, 2012; Feng *et al.*, 2013).

The results for Catalase (CAT) activity are shown in Figure 2. Similarly, Nowak *et al.* (2004) found significantly enhanced activity in redox enzymes, particularly in catalase, in response to several concentrations of Se added to wheat plants, and Saidi *et al.* (2014) demonstrated a positive change in the activities of catalase in response to the addition of selenium in sunflower.

The Glutathione Peroxidase (GSH-Px) enzyme showed a positive behavior similar to CAT (Figure 3). Some studies have reported the decrease in H_2O_2 levels due to the reactivation of antioxidants by proper doses of selenium, particularly of H_2O_2-

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quenchers such as GSH-Px (Filek *et al*., 2009; Kumar *et al*., 2012). Hartikainen *et al.* (2000) showed that cellular antioxidant activity is associated with an increase in GSH-Px activity and that the latter is positively related to the concentration of Se in plant tissue. Cartes *et al.* (2005) demonstrated that selenite was more efficient than selenate as an inducer of GSH-Px activity in *Lolium perenne*, and in *Senecio scadens* L. both selenite and selenate induced an increase in GSH-Px activity (Paciolla *et al.,* 2011).

The percentage of SuperOxide Dismutase (SOD) activity is shown in Figure 4. Despite an increasing trend observed in SOD activity in leaves and fruits after applying Se, only one significant result was obtained in fruits. Xue *et al.* (2001) observed an increase in SOD activity in senescent lettuce plants when adding Se; however, Saidi *et al.* (2014) observed decreased activity of SOD when applying selenium as pre-soaked in sunflower seeds. The difference with our results might indicate different responses to selenium, depending on the application form or the organ under study: fruits, leaves or stems.

It can be said that the applied doses of selenium were appropriate for generating an increase in enzyme activity, but this fact did not cause any stress conditions for the plant. In other cases, the increase in the activity of antioxidant enzymes responds to a stimulus stress, as occurred with those reported by Valizadeh *et al*. (2013), who noted a significant increase in activity of SOD and different POX isoenzymes under salt stress on alfalfa (*Medicago sativa* L.), and Abbasi *et al*. (2014) found that CAT and GPX activity increased in some genotypes of common vetch (*Vicia sativa* L.) under drought stress.

CONCLUSIONS

The variables of growth, plant height, stem diameter, fruit firmness, and total solids of fruits responded positively to the application of selenium, whereas this application caused no interference with the absorption of N, P, K, Ca, and Mg. The addition of selenium in the nutrient solution significantly increased the concentration of this element in plants; the 5 mg L^{-1} treatment allowed the doubling of the concentration of this element in fruits compared with the control treatment. This concentration of selenium in fruits was positively correlated with the concentration

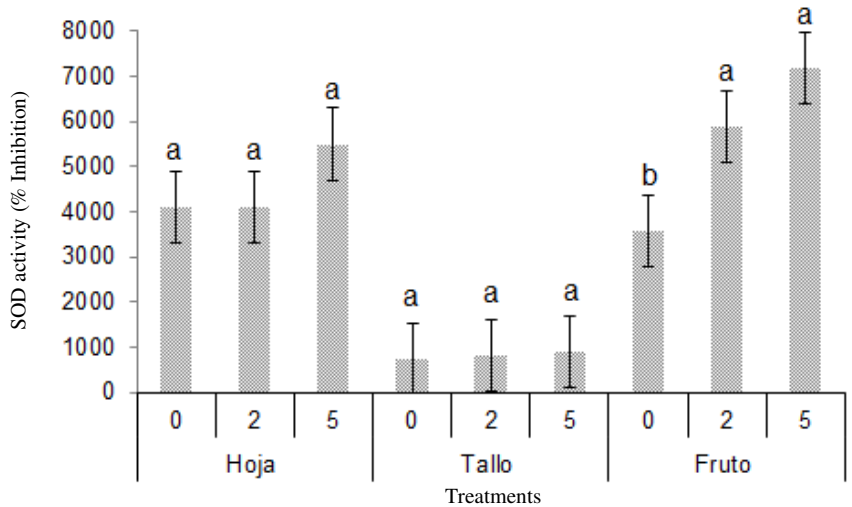

Figure 4. Superoxide dismutase enzyme activity in tomato leaves, stems and fruits with application of 0, 2 and 5 mg L^{-1} of selenium. Means with different label letter differ significantly according to Tukey's test ($P \leq 0.05$). The mean tests were applied independently to each plant organ.

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in leaves and stems. Moreover, Se resulted in an increased enzyme activity of catalase, glutathione peroxidase and superoxide dismutase in fruits, although analyses of the markers of oxidative stress such as hydrogen peroxide and/or lipid peroxidation are necessary for better understanding of this antioxidative picture.

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اثر سلنیوم روی غلظت این عنصر و فعالیت آنتی اکسیدانی گیاه گوجه فرنگی

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چکیده

سلنیوم عنصری ضروری برای انسان است، از این رو، افزودن آن به گیاهان روش ساده ای برای غنی سازی زیستی است. بر این اساس، هدف پژوهش حاضر تجزیه تحلیل آزمایشی توان سلنیت سدیم برای افزایش غلظت Seو تغییر فعالیت آنتی اکسیدانی در گوجه فرنگی بود. به این منظور از رقم هیبرید گوجه فرنگی به نام تورو (Toro) استفاده شد و سه تیمار شامل ۰ ، ۲ ، و ۵mg L^{-1} سلنیوم به صورت سلنیت سدیم (Na_2SeO_3) از طریق سامانه آبیاری اعمال شد.سپس، برای اندازه گیری تجمع سلنیوم و عناصر غذایی پر مصرف دربرگ ها، ساقه ها، و میوه ها، سه نوبت نمونه برداری در ۴۰ روز،۸۰ روز و ۱۲۰ روز بعد از نشا کاری انجام شد. نیز، بلندی گیاه، سفتی، و کل مواد جامد میوه و کل ماده خشک اندازه گیری شد. همچنین، فعالیت آنزیم های کاتالیز، گلوتاتیون پراکسیداز، و superoxide dismutase به صورت کمّی اندازه گیری شد. نتایج حاکی از اثر مثبت Se روی متغییر های زراعی و کیفیت گیاه و میوه های آن بود، هرچند تاثیر متفاوت معنی داری روی تولید میوه دیده نشد. گفتنی است که Se هیچ تاثیری روی غلظت عناصر کم مصرف و پر مصرف نداشت. افزودن ۵mg L^{-1} از سلنیوم منجر به افزایش معنی دار غلظت سلنیوم بر حسب ماده خشک شد که مقدار آن به 20.4 µg g^{-1} در برگها، 52.3 µg g^{-1}در ساقه ها، و 35.8 µg g^{-1}در میوه ها می رسید. نیز، فعالیت آنزیم در میوه ها با اضافه کردن ۵mg L^{-1} سلنیوم فزونی گرفت: در مقایسه با تیمار شاهد، فعالیت کاتالیز ۳۵۲/۷٪ زیادتر شد، فعالیت گلوتاتیون پراکسیداز۳۱۲/۲٪، وفعالیت superoxide dismutase ۲۰۰/۸٪ افزایش یافت.

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