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REGULAR ARTICLE

Sodium selenite treatment of vegetable seeds and seedlings and the effect on antioxidant status

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ABSTRACT

Selenium treatment of plants improves the response to stress and increases their edible contents. The aim of this study was to treat the seeds and seedlings of tomato, lettuce and melon with selenium to verify the effects on growth, antioxidant status, and vitamin C concentration in the photosynthetic tissues of seedlings. The experiment consisted of two phases: in the first phase, selenium was applied at concentrations of 0, 0.1 and 1 mg L^{-1} using immersion pre-treatment of the seeds, and fresh and dry weight, leaf area, oxidation-reduction potential (ORP) and concentration of vitamin C were then determined in seedling leaves. In the second phase, selenium was applied to the seedlings at concentrations of 0 and 2 mg L^{-1} in the nutrient solution and at concentrations of 0 and 5 mg L^{-1} in a foliar spray at 15 and 30 days after seeding, and the same response variables were measured. Both types of selenium applications were shown to positively affect the antioxidant status and the vitamin C concentration of the three vegetables. Therefore, selenium applied to the seeds positively changes the antioxidant status and the concentration of vitamin C in the three crops. Additionally, selenium significantly modified melon growth. The application of selenium by irrigation and leaf spraying in seedlings significantly improved the antioxidant status and the vitamin C concentration in the three species, with only minor changes in biomass and leaf area.

Keywords: Antioxidants, Lettuce; Melon; Selenium; Tomato; Vitamin C

INTRODUCTION

Selenium (Se) is an essential trace element for animals and humans but not for plants; however, it accumulates in different organs of plants, which absorb inorganic Se in the soil and water as selenate (Se^{6+}) and selenite (Se^{4+}) (Broadley et al., 2006). Selenate is absorbed through a transport process coupled to a H^+-ATPase, with the help of a sulfate (Terry et al., 2000) or silicon transporter (Zhao et al., 2010); once absorbed by the plants, maintains the inorganic form (De Souza et al., 1998; Cartes et al., 2006). In contrast, the absorption of selenite occurs in a different way (Terry et al., 2000), 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 has been shown to be a more efficient inducer of glutathione peroxidase (Cartes et al., 2005). Selenium is thought to be associated with antioxidant metabolism (Lin et al., 2012; Feng et al., 2013) through its function as a cofactor of selenoenzymes (Combs, 2001); its deficiency could provoke changes in the cellular redox balance.

Se content in soils varies considerably, and its availability in agricultural soils is usually low; therefore, Se is often used in fertilizers for crops (Fordyce, 2013). Several researchers have described Se application to plants (Bittman et al., 2000; Xue et al., 2001; Rayman, 2008; Becvort-Azcurra et al., 2012; Castillo-Godina et al., 2016), observing a positive effect on antioxidant activity, productivity and yield, and biofortification of leaves and fruits. However, negative consequences have also been described in the literature, usually caused by high concentration of selenium. For that reason, it is important to select the most appropriate method to apply selenium and induce antioxidants and other positive responses in plants (Businelli et al., 2015). The application of Se in seeds may be an alternative; however, information

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regarding Se application to seeds (Jisha et al., 2013; Nawaz et al., 2013) and seedlings (Nawaz et al., 2014; Businelli et al., 2015), and its effect on growth and antioxidants is limited. The aim of this study was to apply Se in the form of Se^{4+} to seeds and seedlings of tomato (*Solanum lycopersicum* L.), lettuce (*Lactuca sativa* L.) and melon (*Cucumis melo* L.) and study the effects on growth, antioxidant status and the concentration of vitamin C in the photosynthetic tissues of the seedlings. We hypothesized that the application of Se^{4+} to both the seeds and seedlings via a nutrient solution and foliar spraying will modify the cellular redox balance, increasing the antioxidant capacity of photosynthetic tissues.

MATERIALS AND METHODS

This work was carried out at the Antonio Narro Agricultural University in Saltillo, Mexico. Three species were evaluated: tomato cv. Rio Grande, lettuce cv. Great Lakes, and melon cv. Top Mark. The experimental procedure had two phases. In phase 1, selenium was applied as sodium selenite (Se^{4+}) (Sigma-Aldrich, USA) to seeds by dip solutions with concentrations of 0, 0.1 and 1 mg L^{-1} for absorption times of 8 h for tomato, 2 h for lettuce, and 12 h for melon. Imbibition times were based on the maximum water uptake by the seeds obtained in a preliminary test. Subsequently, seeds treated with Se^{4+} were planted in polyethylene pots with 1 kg substrate consisting of peat moss and perlite (70:30 v:v) using a random treatment distribution. In the seedlings, the following variables were determined for six replicates per treatment: fresh and dry biomass of the whole seedlings with an Adventurer Pro analytical balance (Ohaus, Inc., USA); leaf area with a LI-3100C leaf area meter (LI-COR, Inc., USA); vitamin C concentration in the leaves by the titration method (Padayatti et al., 2001); pH and redox potential (ORP) of fresh extracts of stems and leaves with a HI98185-01 potentiometer (HANNA, Inc., USA) using the technique described by Benavides-Mendoza et al. (2002).

In phase 2, the vegetable seeds of the three species were sown without Se^{4+} treatment in polyethylene pots with 2 kg substrate consisting of peat moss and perlite (70:30 v:v). After emerging, the seedlings were treated with Se^{4+} at concentrations of 0 and 2 mg L^{-1} in the nutrient solution using an irrigation system, which was based on the method of Steiner (Steiner, 1961) with 50% of the concentration at a pH of 6.5 from 15 to 30 days after sowing. The seedlings were irrigated with 200 mL day^{-1} per plant. The second type of treatment was Se^{4+} at concentrations of 0 and 5 mg L^{-1} by foliar spray at 15 and 30 days after sowing with no surfactant or adherent. The same analyses were performed 30 days after sowing. The statistical design was randomized, with complete blocks receiving treatments with six replicates. Data analysis was performed with the R program (R Core Team, 2015).

RESULTS

Leaf area, fresh weight and dry weight

The seed treatment with Se^{4+} resulted in no significant differences in leaf area, fresh weight, and dry weight in tomato and lettuce. For melon, the leaf area was significantly different in the group that received 1 mg L^{-1} compared to the control treatment. Fresh weight showed significant differences in the three concentrations. Dry weight was significantly increased by treatment with 0.1 mg L^{-1}, but by increasing the concentration to 1 mg L^{-1}, there were no significant differences (Table 1). Following Se^{4+} application to the seedlings via the nutrient solution and foliar spray, the three vegetables showed no significant differences between concentrations and types of application except for the dry weight of the tomato.

Oxidation-reduction potential (ORP)

After the Se^{4+} application to the seeds, the ORP values showed significant differences with a concentration of 1 mg L^{-1} in

Table 1: Average values of leaf area and biomass of tomato, lettuce and melon seedlings for Se^{4+} treatment of the seeds and seedlings by nutrient solution and foliar spray

Treatment mg L^{-1}	Tomato			Lettuce			Melon		
	LA	FW	DW	LA	FW	DW	LA	FW	DW
	cm^2	g	g	cm^2	g	g	cm^2	g	g
Se^{4+} applied to seeds									
0	571.47a	57.56a	7.88a	1884.3a	109.22a	10.35a	854.07b	46.88c	5.18b
0.1	512.54a	49.40a	6.91a	1686.8a	120.78a	11.26a	877.95ab	65.43a	8.12a
1.0	587.62a	58.10a	8.25a	1772.1a	101.59a	8.51a	922.56a	57.62b	8.97a
Se^{4+} applied to seedlings in the nutrient solution									
0	2559.0a	66.12a	7.45a	2693.4a	221.28a	14.43a	3580.4a	234.44a	31.33a
2	2560.5a	58.69a	6.34b	3386.1a	246.87a	17.11a	3150.9a	205.37a	38.50a
Se^{4+} applied to seedlings by foliar spray									
0	2760.0a	69.09a	7.22a	3224.5a	252.68a	16.75a	2888.9a	213.30a	37.42a
5	2460.2a	60.05a	7.55a	2904.4a	217.99a	14.45a	3223.1a	229.26a	39.31a

LA=Leaf area, FW=Fresh weight, DW=Dry weight. Values followed by identical letters in columns showed no statistically significant differences among treatments according to Tukey's test (P>0.05) tomato and 0.1 and 1 mg L^{-1} in lettuce and melon compared to the corresponding control treatments. By increasing the Se^{4+} dose from 0.1 to 1 mg L^{-1}, the ORP increased 18.6% in lettuce and decreased 35.9 and 21.2% in tomato and melon, respectively. The ORP values of the three vegetables showed significant differences following the Se^{4+} application to the seedlings in both the 2 mg L^{-1} nutrient solution and the 5 mg L^{-1} foliar spray treatments compared to the control treatments, with improvements in antioxidant capacity, except for the foliar spray application to tomato (Fig. 1).

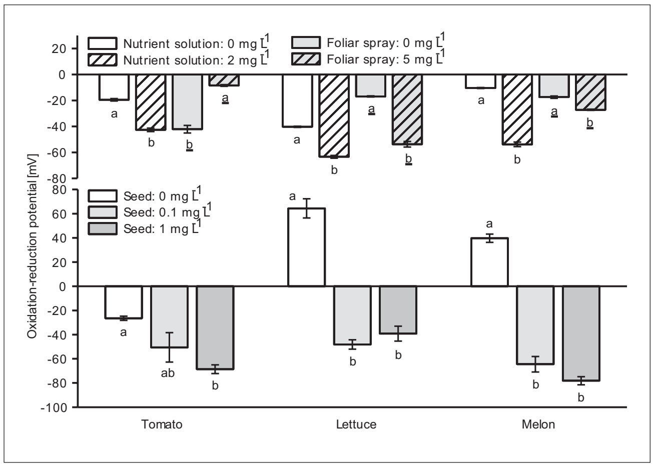

Fig 1. Average values and standard error of the oxidation-reduction potential from fresh extracts of tomato, lettuce and melon seedlings for Se^{4+} treatment of the seeds and seedlings by nutrient solution and foliar spray. Identical letters indicate no statistically significant differences among treatments and species using Tukey's test ($P > 0.05$).

Vitamin C

Vitamin C concentration showed significant differences only in tomato following Se^{4+} application to the seed. The 0.1 mg L^{-1} dose of Se^{4+} applied to the seed increased the vitamin C concentration in the tomato by 44.9% (Fig. 2).

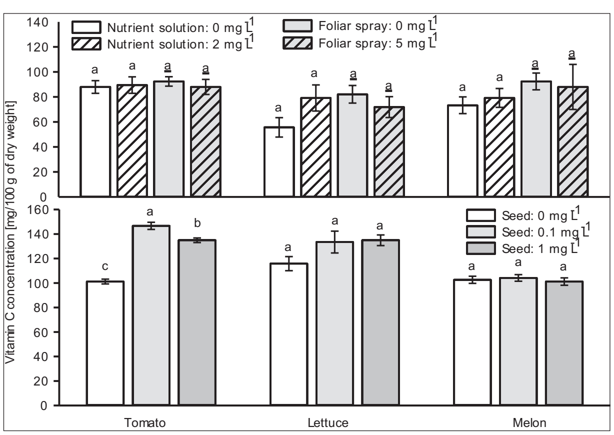

Fig 2. Average values and standard error of the vitamin C concentrations in the leaves of tomato, lettuce and melon seedlings for Se^{4+} treatment of the seeds and seedlings by nutrient solution and foliar spray. Identical letters indicate no statistically significant differences among treatments and species using Tukey's test ($P > 0.05$).

Se^{4+} treatment of the seeds had a positive effect on the concentration of vitamin C and the antioxidant capacity in the tomato, lettuce, and melon seedlings. The ORP showed a tendency to decrease, and the vitamin C concentration showed a tendency to increase. The adjusted linear regression models for the relationships between these variables are presented in Table 2. The regression coefficient was significant ($P \leq 0.05$), but because of the wide dispersion of the data, the R^2 value was very low. However, when Se^{4+} was applied to the the seedlings in the nutrient solution and foliar spray, the ORP and vitamin C values were proportional, which indicated a different response than that observed with application of Se^{4+} to the seeds (Table 2).

Table 2: Relationship among oxidation-reduction potential and the vitamin C concentration in the fresh leaf extracts of tomato, lettuce and melon seedlings for different Se^{4+} application methods

Se^{4+} application method	Regression model	Coefficient of determination
Seed	Vit C=-0.0854*ORP+116.8769	R^2=0.0448
Nutrient solution	Vit C=0.0161*ORP+78.1051	R^2=0.0002
Foliar spray	Vit C=0.2329*ORP+92.2322	R^2=0.0280

pH

The pH values of the fresh leaf extract in tomato showed no statistically significant differences after Se^{4+} applications. Significant differences in pH values were observed in lettuce with the Se^{4+} application to the seeds, in the melon with Se^{4+} application in the nutrient solution, and in lettuce and melon for Se^{4+} application via foliar spray. The pH values of the vegetable seedlings increased between 0.3 and 0.7% when Se was applied via the nutrient solution and decreased between 1.7 and 2.5% when Se was applied via foliar spray (Fig. 3).

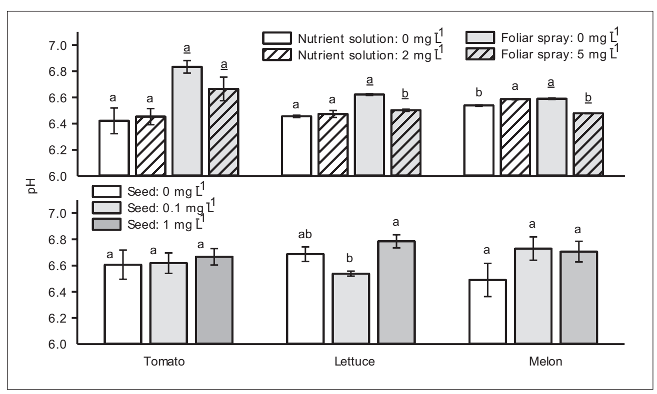

Fig 3. Average values and standard error of the pH values of the fresh leaf extract of tomato, lettuce and melon seedlings, for Se^{4+} treatment of the seeds and seedlings by nutrient solution and foliar spray. Identical letters indicate no statistically significant differences among treatments and species using Tukey's test ($P > 0.05$).

DISCUSSION

Becvort-Azcurra et al. (2012) reported that Se applications up to 2.5 mg L^{-1} in fertilizer solution in the soil and perlite substrate for tomato had positive effects on plant growth. Xue et al. (2001) also reported that Se concentrations up to 0.1 mg kg^{-1} in the soil had positive effects on growth in lettuce. In the case of lettuce, it was reported that 1 mg kg^{-1} of Se was toxic (Xue et al., 2001). In this study, Se^{4+} application at 1 mg L^{-1} to the seeds caused no toxicity (Table 1).

The quantities of Se^{4+} applied in the nutrient solution and with the foliar spray are high but did not exceed those used by Becvort-Azcurra et al. (2012) and Xue et al. (2001), who, along with Ramos et al. (2010), reported a positive effect of Se on vegetable growth (Table 1).

Se^{4+} treatment in tomato, lettuce and melon improved the antioxidant capacity (Fig. 1), which is reflected by the reduction in the oxidation-reduction potential (ORP) of the fresh extract from the seedlings (Benavides-Mendoza et al., 2002). ORP values indicate the antioxidant capacity, i.e., the ability of the system under analysis to donate electrons compared to hydrogen electrode (Benavides-Mendoza et al., 2002); lower ORP values indicate a greater capacity to donate electrons and act as an antioxidant. In lettuce plants, a low ORP in leaf extracts was linked to increased catalase activity (López-Gutiérrez et al., 2015). In this study, the relationship between ORP and vitamin C concentration was significant but showed variability. An association between ORP and vitamin C is expected, as the ORP is an indicator of the concentration of antioxidant molecules present in plant cells. However, there was a very low R^2 value possibly due to other antioxidants that also contribute to the ORP. The ability to donate electrons to the system is higher when the ORP value is lower. The increase in electron delivery may be related to increased activity of antioxidant enzymes, which was found in the presence of specific Se concentrations (Freeman et al., 2010).

The increase in the antioxidant capacity of the seedlings treated with Se^{4+} supports the use of this element in crop fertilizer. Se^{4+} treatments of 0.1, 2 and 5 mg L^{-1} in the seeds and seedlings by nutrient solution and foliar spray, respectively, showed the better performances to increase the antioxidant capacity (Fig. 1).

Antioxidant capacity and vitamin C concentration showed proportional increases in response to Se^{4+} application, except for the foliar spray method in lettuce and melon, where the vitamin C concentration decreased (Figs. 1 and 2, Table 2). The increase in vitamin C concentration following Se^{4+} treatment of the seeds indicates that this method may induce stress tolerance in seedlings (Fig. 2).

The positive effects of Se^{4+} on plant antioxidants (Figs. 1 and 2) was described by other authors, such as Xu et al. (2003), Hajiboland and Amjad (2007), Ramos et al. (2010) and Becvort-Azcurra et al. (2012) (Fig. 1). In *Stanleya pinnata,* a selenium hyperaccumulator plant, Freeman et al. (2010) found that the presence of Se in the form of Se^{6+} induced higher vitamin C contents, possibly as a response to oxidative stress caused by the high concentration of Se.

In this study, the Se concentrations applied as Se^{4+} were not high, and the presence of this nutrient in low concentrations in both the seeds and seedlings had an antioxidant effect and increased the vitamin C concentration in plant cells (Djanaguiraman et al., 2010) (Figs. 1 and 2). Similarly, Ramírez et al. (2010) found that other tolerance-inducing compounds and even environmental factors (Munné-Bosch et al., 2013) could increase the vitamin C concentration (Fig. 2). The pH of the fresh leaf extracts of the three crops showed normal values between 6.4 and 6.8. It was expected that the pH would be related to the ORP or vitamin C, considering that the pH is an indicator of protonation and the activity of molecules (Fig. 3). The results, however, indicated no association between the variables. In other studies, the pH has been linked to the quality of the storage organs (Benavides-Mendoza et al., 2002).

CONCLUSIONS

Se^{4+} application in tomato, lettuce and melon, both in the seeds and seedlings via nutrient solution and foliar spray, improved the antioxidant status and vitamin C concentration and also increased the leaf area and the fresh and dry weights of the seedlings in some cases. Based on these results, Se^{4+} applications of 1 mg L^{-1} in tomato seeds and 0.1 mg L^{-1} in melon seeds are recommended to increase the leaf area and the fresh and dry weight, which can improve fruit yields. To improve the antioxidant capacity and vitamin C concentration, Se^{4+} applications of 0.1 mg L^{-1} in the seeds for all three vegetables are recommended. Increased doses could possibly be used in vegetables, except for lettuce, and in the seedlings at 2 mg L^{-1} in a nutrient solution for the three vegetables and at 5 mg L^{-1} by foliar spray in lettuce and melon.

Authors contributions

All authors contributed extensively to the work presented in this paper. MESV performed the field experimentation, data analysis, and the first draft of the manuscript; ABM planned and designed the study conceptualization, data curation, results interpretation, and critically revised the paper; NART participated in the laboratory experimentation; MCF participated in the field experimentation; AMM participated in data curation, data interpretation, analytical tools and manuscript writing. Also all five authors were involved in the review and editing the final manuscript.

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