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Mineral Composition and Antioxidant Status of Tomato with Application of Selenium

Willian Alfredo Narváez-Ortiz [1], Armando Ariel Becvort-Azcurra [1], Laura Olivia Fuentes-Lara [2], Adalberto Benavides-Mendoza [1,3], Jesús Rodolfo Valenzuela-García [4] and José Antonio González-Fuentes [1,3,*]

1 Departamento de Horticultura, Universidad Autónoma Agraria Antonio Narro (UAAAN), Calzada, Antonio Narro # 1923, Saltillo 25315, Coahuila, Mexico; williamnarvaezo@gmail.com (W.A.N.-O.); abecvort7@gmail.com (A.A.B.-A.); abenmen@gmail.com (A.B.-M.)
2 Departamento de Nutrición Animal, Universidad Autónoma Agraria Antonio Narro (UAAAN), Calzada, Antonio Narro # 1923, Saltillo 25315, Coahuila, Mexico; loflara@gmail.com
3 Doctorado en Agricultura Protegida, Universidad Autónoma Agraria Antonio Narro (UAAAN), Calzada, Antonio Narro # 1923, Saltillo 25315, Coahuila, Mexico
4 Departamento de Maquinaria Agrícola, Universidad Autónoma Agraria Antonio Narro (UAAAN), Calzada, Antonio Narro # 1923, Saltillo 25315, Coahuila, Mexico; j_valenzuela1@yahoo.com.mx
* Correspondence: jagf@uaaan.mx; Tel.: +52-844-160-7863

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Abstract: This experiment was carried out in a greenhouse to evaluate the effects of selenium application (as Na_2SeO_3) on mineral concentration (as N, P, K, Ca and Se), biomass, yield and total antioxidant status (TAS) of tomato fruit. The study consisted of two experiments: an irrigation experiment with the application of selenium at 0, 2.5 and 5 mg L^{-1} on the fertilizer solution in soil and perlite; and the foliar application experiment with selenium application at 0, 10, and 20 mg L^{-1} in foliar spray every 20 days. Results showed that mineral content (as K, Ca, Mg and P) was not modified by selenium application. However, N decreased due to the Se applied in fertilizer solution 5 mg L^{-1}, and a negative correlation was found between the selenium applied in foliar form and the nitrogen concentration. The Se concentration, TAS, and biomass increased in plants in all Se treatments. However, the best response in TAS and Se in fruits was observed with foliar spray every 20 days at concentrations of 10 mg L^{-1}, without negative responses in biomass or mineral content.

Keywords: tomato (*Lycopersicon esculentum* Mill.); sodium selenite; macronutrients; redox status; antioxidants

1. Introduction

Selenium (Se) is an essential element for humans, with intake values ranging from 60 to 75 µg per day according to the UK reference nutrient intake data [1]. However, these values are well below the levels of recommended consumption, which indicate that up to 300 µg of Se is required each day to reduce the risk of cancer [2]. Se in human diet comes from foods of vegetable and animal origin, with a Se content that reflects Se availability in soils [3]. Therefore, the daily intake can vary from 10 µg in areas with low Se content in the soil to up to 5000 µg in areas with selenic soils [2]. The risk of a low concentration of Se in the diet can occur in some regions (e.g., Denmark, Finland, New Zealand, eastern and central Siberia (Russia) and a long belt extending from northeast to south-central China and Inner Mongolia) [4]. On the other hand, particular attention has been given to the content of Se in fruits, seeds, and vegetables, because in most industrialized countries, the decrease in the ingestion of flours and meats [2] and climatic change [5] can be associated with lower intake or the availability in soil of this element , respectively.

Se is not considered essential for plants; however, its application in horticultural species is usually beneficial for growth and tolerance to stress [6]. Most of the fruits and vegetables grown in agricultural soils show Se concentrations lower than 0.01 μg g^{-1} [3]. This low concentration in plants is because, in a considerable number of soils of the world, the concentration of Se is low, ranging from 0.01 to 2.0 μg g^{-1} with an average of 0.4 μg g^{-1}, although concentrations ≥1200 μg Se g^{-1} can be found in soils known as seleniferous [7]. Therefore, in some countries in which soils are deficient in Se, this element is added to the fertilizers used for agricultural production [2]. Almost all vegetable crops, including tomato, are considered non-accumulative Se types, i.e., plants for which more than 25 μg Se g^{-1} dry weight in roots and leaves can produce toxicity [8]. This toxicity can be manifested through oxidative stress considering the pro-oxidant ability of Se or the competitive substitution of sulfur in proteins [9].

Biofortification is a strategy to counteract the low Se consumption in humans and animals. Selenium biofortification has shown positive results in various crops, including: rice [10], tomato [11], strawberry [12], lettuce [13], radish [14], and potato [15].

As with sulfur, Se has several oxidation states: selenide (Se^{-2}), elemental Se (Se^{0}), selenite (Se^{+4}), and selenate (Se^{+6}). The oxidized forms of Se (Se^{+4} and Se^{+6}) are absorbed by plants because of their high solubility, whereas Se^{0} and Se^{-2} are insoluble, and therefore are hardly absorbed by plants [16].

Se is metabolized in plants through the sulfur assimilation pathway, and its distribution and accumulation depend on the chemical species, the concentration of Se supplied, and its application form (soil, irrigation or via foliar spray), in addition to the nature and concentration of other anionic substances (as sulfate and phosphate) present in the solution [17]. In the environment, Se can be released by natural or anthropogenic processes and incorporated into soil and water [7]. Regarding the chemical forms, Se is mobilized by different processes within plant cells; accordingly, selenate can be mobilized through a primary transport process coupled to an H^{+}-ATPase by means of a sulfate transporter [17] or silicon transporter [18], and when it is absorbed, it can be maintained in inorganic form or transformed into organic forms (as SeMet) [19]. The absorption of selenite occurs differently [17], through a phosphate transporter [18], and when absorbed, selenite is maintained in organic form [16], which is a more efficient inducer of glutathione peroxidase activity [20].

Different studies conducted with Se application in different crop species indicate effects on photosynthetic activity [21], stress tolerance [22] and productivity [23], among other effects. However, the effect of Se-selenite on the mineral composition of different plant organs, which in turn is a crucial determinant of plant nutritional quality, has received less attention. The objectives of this work were to determine the effects of Se application as sodium selenite (Na_2SeO_3) in the fertilizer solution and by foliar spray on the mineral composition of roots, shoots, and fruits of tomato; in addition, the association of these changes with fruit yield and antioxidant status of fruit was examined.

2. Materials and Methods

This study consisted of two experiments conducted in a tunnel-type greenhouse, with a rigid polycarbonate cover and active ventilation through extractors and a wet wall located in Saltillo, Mexico; the two experiments are described below.

Tomato (*Lycopersicon esculentum* Mill.) variety Rio Grande (from the EDENA Seed Company, El Centro, CA, USA) with a determined growth habit was used. The seeds were planted in two polystyrene trays of 200 cavities, using peat moss and perlite as growing medium (75:25 *v*:*v*). The seedlings were transplanted 31 days after sowing (DAS) in 20 L black polyethylene pots using the following growing media as treatments: coarse agricultural soil and perlite (Irrigation experiment) and peat moss (Foliar application experiment). According to a previous analysis, the basal Se concentration in the irrigation water was 0.018 μg g^{-1}, while in the substrates it was 1.484 μg g^{-1} (coarse agricultural soil), 0.175 μg g^{-1} (perlite) and 0.016 μg g^{-1} (peat moss).

2.1. Irrigation Experiment

Coarse agricultural soil and perlite were used as growing medium. The following Se treatments were applied to the plants in each growing medium: irrigation with a nutrient solution [24] to the control plants and irrigation with the same nutrient solution plus 2.5 and 5 mg L^{-1} Se using sodium selenite (Na_2SeO_3, Sigma-Aldrich, St. Louis, MO, USA) as the source.

The applications of Se were included within the irrigation from 15 to 120 days after transplant (DAT). The two types of growing medium, combined with the three concentrations of Se, resulted in six treatments. A completely randomized experimental design was used, with factorial arrangement A × B, being the factors substrates (A) and Se concentrations (B), with 15 replicates (plants) per treatment. The experimental unit was one plant in a pot. The irrigation systems for the application of each nutrient solution and Se treatment were entirely independent to avoid the possibility of contamination.

The concentration of the nutrient solution was adapted according to the stage of crop development: 30% between 5 and 30 DAT, 50% between 31 and 40 DAT, 75% from 41 to 70 DAT and 100% from 71 to 100 DAT. The pH of the solution was maintained at ~6.5, using sulfuric acid. Anthesis occurred at 95 DAT, and the fruit harvest ended at 120 DAT when the third cluster was developed.

The end of the experiment was at 120 DAT. The fresh weight was determined for the belowground (roots) and aboveground (stems and leaves) of 12 plants. The dry weight was obtained by drying both plant structures until constant weight was obtained, which occurred after 72 h in a drying oven at 60 °C. Fresh fruit weight was determined for each plant by collecting and weighing the fruits in the six-red stage [25]. To obtain the dry fruit weight, randomly selected fruits from 10 experimental units (10 plants) were dried in an oven at 60 °C until a constant weight was obtained.

The mineral content was determined for the different structures (roots, shoots, and fruits). Total nitrogen (N) was determined by the micro Kjeldahl method [26]. A portion 0.05 g of the dry matter was taken and subject to acid digestion with 4 mL of digester mixture (1 L of concentrated sulfuric acid + 25 g of potassium sulfate + 10 g of red mercury oxide + 25 mL of copper sulfate saturated solution); subsequently, the result of the digestion was subjected to a distillation process with 25 mL of 50% sodium hydroxide. The distillation was captured in 30 mL of 2.2% boric acid and three drops of bromocresol green and methyl red, then titrated with 0.025 N sulfuric acid. The phosphorus (P) was determined by a spectrophotometric method [26], with an Ammonium molybdate reagent and Aminonaphthol sulphonic acid solution. The reading was performed with a UV-Vis spectrophotometer model Helios Epsilon at a wavelength of 640 nm. The selenium (Se), potassium (K), calcium (Ca), and magnesium (Mg) were extracted using a wet digestion technique [27]. One gram of the dry matter 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 and quantified with an Inductively Coupled Plasma Optical Emission Spectrometer brand THERMO JARELL ASH, Model IRIS Advantage, following procedure 984.27 [28].

The Se concentration data and the dry weight of the different organs (root, shoot, and fruit) were used to calculate the extraction by organ, with the organ weight slater added to obtain the total extraction of Se per plant. The relative distribution of Se in the plant was obtained by transforming the total extraction into a percentage, and then the percentage allocation to each organ of the plant was calculated.

The total antioxidant capacity was determined in 10 fruits that were randomly collected in each treatment, 10 days before harvest in the stage of fruit maturity. The total antioxidant status (TAS) was determined using the Total Antioxidant Status Kit Assay of Calbiochem® (Sigma-Aldrich, St. Louis, MO, USA), which is based on the technique developed by Miller et al. [29] and consists of a phosphate buffer solution (pH 7.2), chromogen (metmyoglobin and ABTS® [radical cation 2,2-Azinobis-(3-ethylbenzthiazolin-6-sulfonate)]), substrate (stabilized hydrogen peroxide) and as the standard, TROLOX (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid).

2.2. Foliar Application Experiment

This was similar to the first experiment with regard to the agronomic procedures and determinations made. The variants were that Se was applied only by foliar spray and using peat moss as the only growing medium. The treatments consisted of spray applications every 20 days with distilled water as a control and solutions of 10 and 20 mg L^{-1} Se as Na_2SeO_3 treatment. The experiment was harvested at 140 DAT. The experimental design was completely randomized.

2.3. Statistical Analyses

Analysis of variance (ANOVA) and mean comparison tests (Fisher´s LSD, α = 0.05) were performed on the data obtained in the two experiments. Spearman correlation coefficients were obtained to determine the degree of correlation between the different variables evaluated in the plants against the Se concentrations of the different parts of the plant. The calculations were done with the language and statistical computing environment R version 3.1.1 [30].

3. Results

3.1. Plant Biomass

Table 1 shows the results obtained for the fresh and dry biomass of roots, shoots (stems and leaves), and fruits from tomato plants subjected to different concentrations of Se applied in irrigation and as a foliar spray grown on different growing media (soil, peat moss, and perlite).

Table 1. Effect of selenium application through the fertilizer solution (Irrigation experiment) in different growing medium and via foliar spray application (Foliar application experiment) on the accumulation of biomass in the roots, shoots (stems and leaves), and fruits.

Treatments Substrate + Se (mg L^{-1})	Fresh Weight of Roots	Root Dry Weight	Fresh Weight of Shoots	Shoot Dry Weight	Fruit Fresh Weight	Fruit Dry Weight
	g $plant^{-1}$		g $plant^{-1}$		g $plant^{-1}$	
Irrigation Experiment 0, 2.5, and 5 mg L^{-1} Selenium in Fertilizer Solution						
Soil + 0	416 b †	54.0 c	1964 a	279.9 a	2852 ab	175.0 a
Soil + 2.5	417 b	55.3 bc	2049 a	291.9 a	2744 ab	166.8 ab
Soil + 5	255 c	33.3 d	1405 b	187.5 b	2425 b	149.6 b
Perlite + 0	488 a	60.5 ab	1509 b	204.4 b	2930 a	185.7 a
Perlite + 2.5	489 a	61.4 a	1013 c	128.5 c	2872 a	167.2 ab
Perlite + 5	154 d	18.4 e	430 d	58.1 d	939 c	54.4 c
ANOVA significance						
Substrate (S)	ns	ns	**	**	**	**
Concentration (C)	**	**	**	**	**	**
S × C	**	**	**	**	**	**
Foliar Application Experiment 0, 10, and 20 mg L^{-1} Foliar Selenium						
Peat moss + 0	455 b	47.4 a	2126 c	294.4 b	2997 ab	172.3 b
Peat moss + 10	510 a	52.9 a	2885 a	402.1 a	3148 a	217.6 a
Peat moss + 20	535 a	55.0 a	2624 b	366.8 a	2840 b	178.1 b
ANOVA significance						
Treatment	*	ns	**	**	ns	**

† Different letters in the same column in each experiment indicate significant differences, according to LSD ($p \leq 0.05$).
* $p < 0.05$; ** $p < 0.01$; ns = not significantly different.

3.1.1. Irrigation Experiment

The treatment with 5 mg L^{-1} showed unfavorable effects compared to treatments with 2.5 mg L^{-1} Se and the control (Table 1). The soil showed the best results for most of the variables, except the fresh and dry root weights, for which no differences were found between soil and perlite. The highest root weight was observed in the perlite growing medium both with Se (2.5 mg L^{-1}) and without. For the

shoot weight (stem and leaves), the highest values were obtained when using soil with Se (2.5 mg L^{-1}) and without Se. For fruit weight, both substrates presented adequate conditions to fruit production per plant, both without adding Se in the irrigation solution and when the concentration was 2.5 mg L^{-1}.

3.1.2. Foliar Application Experiment

The foliar application of Se at both concentrations of 10 and 20 mg L^{-1} showed positive effects by increasing the fresh and dry weights of roots and shoots (stem and leaves) and the dry weight of fruit. For the fresh fruit weight, no significant differences were found associated with the treatments.

3.2. Macronutrient Content in the Different Organs of Tomato Plants

3.2.1. Irrigation Experiment

The macronutrients that showed significant differences ($p \leq 0.05$) in their results were: N, P, K and Mg in shoot and N and Mg in fruits (Table 2). In the roots, Mg and K did not show significant effects associated with the treatments. The Se treatments did not modify the P concentration in the roots; however, a higher level of P was recorded in the perlite substrate in comparison with the plants grown in soil. The Ca concentration was not modified in plants cultivated in soil by the effect of Se treatments; whereas in plants grown in perlite, treatments with Se reduced the Ca concentration. The application of 2.5 mg L^{-1} Se in the nutrient solution to the soil was associated with an increase in the N content of up to 32.1 mg L^{-1}. In the shoots, the application of 2.5 and 5 mg L^{-1} Se in perlite increased the P, whereas, for Mg, the same effect was observed but only for the concentration of 2.5 mg L^{-1} Se in perlite. The levels of K were up to 21 mg g^{-1} when the combinations were perlite without Se and soil plus 2.5 mg L^{-1} Se. The concentration of Ca did not vary significantly among the different treatments, except for the treatment with Se at 5 mg L^{-1} in perlite in which Ca levels were reduced to values of 12 mg g^{-1}. Irrigation of plants in soil with 2.5 mg L^{-1} Se increased the N content. In the fruits, the treatments with Se did not modify the P and Ca concentration. The N increase when applying 2.5 mg L^{-1} Se in soil treatment, while the application of 5 mg L^{-1} in perlite reduced it. The level of K was reduced in plants cultivated in soil by applying 5 mg L^{-1} Se. Mg concentration was negatively affected in plants grown in perlite with the application of 5 mg L^{-1}.

Table 2. Macronutrient concentrations in mg g^{-1} in the different organs of tomato with the application of selenium in the nutrient solution at concentrations of 0, 2.5, and 5 mg L^{-1}.

Irrigation Experiment 0, 2.5, and 5 mg L^{-1} Selenium in the Fertilizer Solution Substrate + Se (mg L^{-1})										
Organ	Minerals	S + 0	S + 2.5	S + 5	P + 0	P + 2.5	P + 5	*ANOVA significance*		
								SB	C	SB × C
Root	N	26.6 b †	32.1 a	26.2 b	24.5 b	25.6 b	21.1 c	**	**	ns
	P	1.9 b	2.0 b	1.8 b	4.0 a	4.0 a	4.0 a	**	ns	ns
	K	17 a	16 a	20 a	19 a	20 a	17 a	ns	ns	ns
	Ca	11 a	10 ab	9 ab	12 a	7 b	7 b	ns	*	ns
	Mg	0.9 ab	0.8 ab	0.8 ab	1.4 a	0.5 ab	0.3 b	ns	ns	ns
Shoot	N	26.8 b	32.2 a	26.3 b	24.7 b	25.3 b	21.2 c	**	**	*
	P	3.6 c	4.7 b	3.3 c	4.2 bc	7.5 a	8.1 a	**	**	**
	K	17 abc	21 a	20 ab	21 a	15.6 bc	15.2 c	ns	ns	*
	Ca	21 a	20 a	21 a	21 a	23 a	12 b	ns	ns	ns
	Mg	1.0 b	0.9 b	1.0 b	1.0 b	1.6 a	0.6 b	ns	*	*

Table 2. *Cont.*

		Irrigation Experiment 0, 2.5, and 5 mg L^{-1} Selenium in the Fertilizer Solution Substrate + Se (mg L^{-1})								
Fruit	N	26.9 b	32.4 a	26.4 b	24.8 b	25.4 b	21.3 c	**	**	*
	P	4.5 a	4.6 a	4.7 a	4.8 a	5.5 a	5.5 a	ns	ns	ns
	K	29 ab	29 ab	20 c	34 a	28 ab	22 bc	ns	**	ns
	Ca	2.8 b	2.7 b	2.6 b	3.3 ab	2.6 b	3.9 a	ns	ns	ns
	Mg	0.2 a	0.2 a	0.2 a	0.2 a	0.2 a	0.1 b	**	**	**

† Different letters in the same row indicate significant differences, according to LSD ($p \leq 0.05$). S = soil; *P* = perlite; 0 = no selenium; 2.5 = 2.5 mg L^{-1} Se; 5 = 5 mg L^{-1} Se; SB = Substrate; C = Concentration; * $p < 0.05$; ** $p < 0.01$; ns = not significantly different.

3.2.2. Foliar Application Experiment

The foliar application of Se did not cause significant changes ($p \leq 0.05$) among the treatments for the different minerals evaluated in the roots and fruits (Table 3). On the other hand, for the minerals evaluated in the shoots of tomato plants, significant differences were only found for Mg and P. The concentration of P increased in plants treated with 20 mg L^{-1} of Se, while Mg concentration showed no changes between the plants treated with selenium (10 and 20 mg L^{-1}) and the control plants. However, a higher Mg concentration was observed in plants treated with 10 mg L^{-1} Se in comparison with the plants treated with 20 mg L^{-1} Se.

Table 3. Macronutrient concentrations in mg g^{-1} in the different organs of tomato grown in peat moss with the application of foliar selenium at concentrations of 0, 10, and 20 mg L^{-1}.

		Foliar Application Experiment 0, 10, and 20 mg L^{-1} Foliar Selenium Substrate + Se (mg L^{-1})			
Organ	Minerals	PM + 0	PM + 10	PM + 20	*ANOVA significance*
Root	N	27 a †	26 a	24 a	ns
	P	2.4 a	2.6 a	2.4 a	ns
	K	18 a	23 a	22 a	ns
	Ca	25 a	27 a	25 a	ns
	Mg	0.7 a	0.7 a	0.7 a	ns
Shoot	N	29 a	25 a	27 a	ns
	P	3.3 b	2.9 b	4.0 a	**
	K	21 a	24 a	24 a	ns
	Ca	30 a	32 a	30 a	ns
	Mg	0.7 ab	1.0 a	0.6 b	*
Fruit	N	29 a	25 a	27 a	ns
	P	5.6 a	5.3 a	5.8 a	ns
	K	27 a	26 a	30 a	ns
	Ca	5.8 a	5.4 a	6.8 a	ns
	Mg	0.1 a	0.1 a	0.1 a	ns

† Different letters in the same row indicate significant differences, according to LSD ($p \leq 0.05$). PM = peat moss; 0 = no selenium; 10 = 10 mg L^{-1} Se; 20 = 20 mg L^{-1} Se. * $p < 0.05$; ** $p < 0.01$; ns = not significantly different.

3.3. Selenium Content in the Different Organs of Tomato Plants

The Se concentration in the different organs of tomato plants and the Se extraction by the plant (root + shoot + fruits) for the irrigation and foliar application experiment are shown in Table 4.

Table 4. The concentration of selenium in the different organs of tomato expressed in μg of selenium per g of dry weight and the total extraction of selenium (roots + shoot + fruits) based on plant dry weight in the different treatments.

Treatments Substrate + Se (mg L^{-1})	Root	Shoot	Fruit	Extraction by Plant
	μg g^{-1}	μg g^{-1}	μg g^{-1}	μg $plant^{-1}$
Irrigation Experiment (0, 2.5, and 5 mg L^{-1} Selenium in Fertilizer Solution)				
Soil + 0	8.30 d †	9.06 d	5.38 e	3939 bc
Soil + 2.5	66.56 c	15.39 b	10.50 d	9938 a
Soil + 5	93.18 b	18.30 a	17.44 b	9168 a
Perlite + 0	9.80 d	5.66 e	6.22 e	2934 c
Perlite + 2.5	101.77 b	12.36 c	14.11 c	10,180 a
Perlite + 5	148.90 a	9.48 d	20.74 a	4419 b
ANOVA significance				
Substrate	**	**	**	**
Concentration	**	**	**	**
S × C	**	**	ns	**
Foliar Application Experiment (0, 10, and 20 mg L^{-1} Foliar Selenium)				
Peat moss + 0	15.60 b	11.30 a	3.87 b	4725 b
Peat moss + 10	15.13 b	20.43 a	9.83 a	11,331 a
Peat moss + 20	26.90 a	20.33 a	11.33 a	10,964 a
ANOVA significance				
Treatment	*	ns	**	*

† Different letters in the same column in each experiment indicate significant differences according to LSD ($p \leq 0.05$). * $p < 0.05$; ** $p < 0.01$; ns = not significantly different.

In the Irrigation experiment, the Se levels in roots, shoots, and fruits increased for plants irrigated with 5 and 2.5 mg L^{-1} Se in comparison with the control plants in both substrates. The highest value of Se in root and fruits was obtained in plants grown in perlite, whereas the greatest Se accumulation in the shoots was found in plants cultivated in the soil.

The foliar application of Se in the Foliar application experiment gave rise to differences in comparison to control treatment. With the application of 20 mg L^{-1}, increased concentrations of Se in roots and fruits of tomato were obtained, whereas, for the shoots, no significant effects were observed.

3.4. Relative Distribution of Selenium in Different Organs of the Plant

The application of Se 2.5 and 5 mg L^{-1} in the nutrient solution resulted in higher relative Se accumulation in roots (Figure 1) compared with Se mobilized toward the shoot. The relative distribution in roots, shoot, and fruit, showed the same tendency in plants grown in soil and perlite. The plants grown in soil showed a higher total Se extraction per plant in comparison with the plants grown in perlite (Table 4).

The application of Se as a foliar spray induced a more substantial relative accumulation of Se in shoots (stem and leaves) in comparison with the other organs (Figure 1). As the concentration of Se increased from 10 to 20 mg L^{-1} in the foliar spray, the relative accumulation in the control plants was higher in the roots and lower in the fruits, contrary to what was observed in plants treated with selenium (10 and 20 mg L^{-1}). For the total accumulation per plant with the foliar application, a maximum concentration of 11,331 μg of Se was found (Table 4).

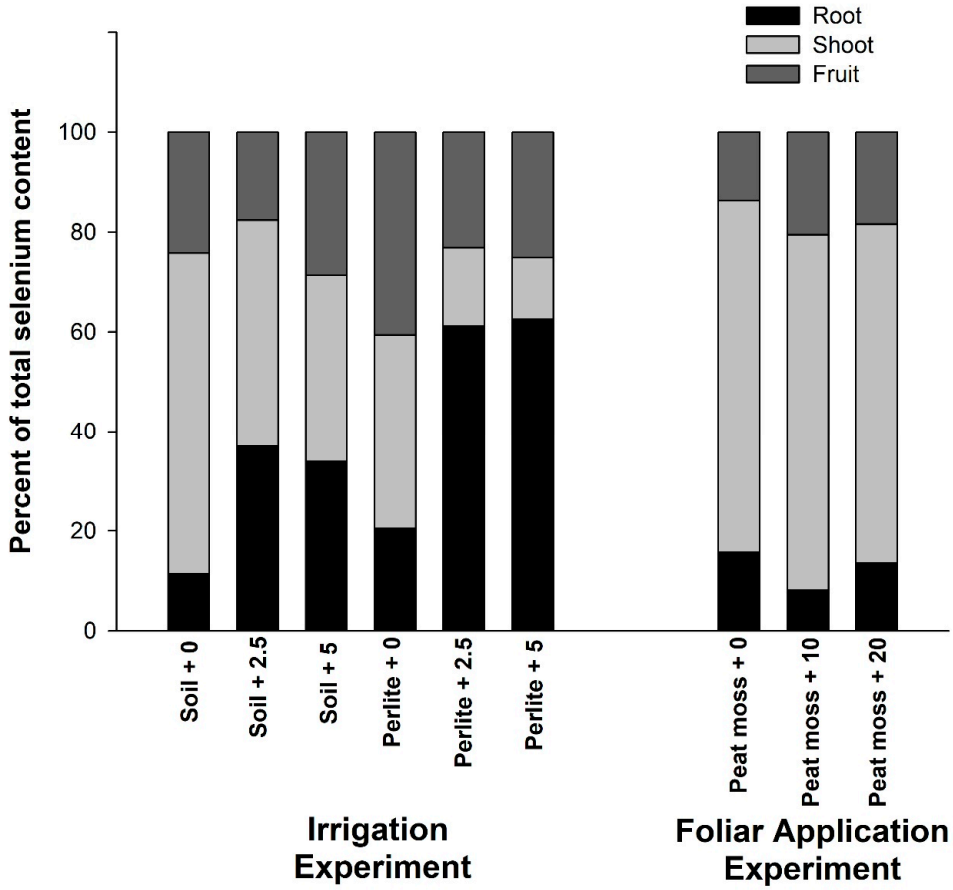

Figure 1. Relative selenium distribution in tomato plants with application of selenium in the fertilizer solution in different substrates (Irrigation experiment) and by foliar spraying (Foliar application experiment). The numbers in the treatments (2.5, 5, 10, and 20) indicate the selenium concentrations in mg per liter.

3.5. Total Antioxidant Capacity in Tomato Fruits

In the Irrigation experiment, the application of Se in concentrations of 5 and 2.5 mg L^{-1} to plants grown in soil caused an increase in the antioxidant status of the fruit. By contrast, in perlite, no differences were observed compared with the antioxidant status of the fruit in control plants. In the Foliar application experiment, applications of Se at concentrations of 20 and 10 mg L^{-1} resulted in the increased antioxidant status of the fruit (Table 5).

Table 5. Antioxidant status of tomato fruits with different concentrations and application forms of selenium.

Irrigation Experiment (0, 2.5, and 5 mg L^{-1} Selenium in Fertilizer Solution)		**Foliar Application Experiment (0, 10, and 20 mg L^{-1} Foliar Selenium)**	
Treatment Substrate + Se (mg L^{-1})	**Total Antioxidant Capacity (mM mg^{-1})**	**Treatment Substrate + Se (mg L^{-1})**	**Total Antioxidant Capacity (mM mg^{-1})**
Soil + 0	2.88 d †	Peat moss + 0	2.85 c
Soil + 2.5	3.68 ab	Peat moss + 10	3.26 b
Soil + 5	3.98 a	Peat moss + 20	3.86 a
Perlite + 0	3.25 c		
Perlite + 2.5	3.44 bc		
Perlite + 5	3.53 bc		

Table 5. *Cont.*

Irrigation Experiment (0, 2.5, and 5 mg L^{-1} Selenium in Fertilizer Solution)		Foliar Application Experiment (0, 10, and 20 mg L^{-1} Foliar Selenium)	
Treatment Substrate + Se (mg L^{-1})	**Total Antioxidant Capacity (mM mg^{-1})**	**Treatment Substrate + Se (mg L^{-1})**	**Total Antioxidant Capacity (mM mg^{-1})**
ANOVA significance			
Substrate (S)	ns		
Concentration (C)	**		
S × C	**	Treatment	**

† Different letters in the same column indicate significant differences, according to LSD ($p \leq 0.05$). ** $p < 0.01$; ns = not significantly different.

3.6. Correlation Matrix between the Concentrations of Selenium for the Different Organs of Tomato Plants against the Mineral Contents, Biomass, and Antioxidant Status of the Fruit

Table 6 shows the correlation matrix obtained from the values of Se concentration for the different organs of the plant against the content of other minerals, biomass and antioxidant status of the fruit for each experiment of the study. For purposes of presenting the results and discussion, only the Spearman correlation coefficients (ρ) with absolute values equal to or greater than 0.70 were considered.

Table 6. Correlation coefficients of the growth, mineral, and antioxidant variables against the concentration levels of selenium in the different organs of the plant.

		Irrigation Experiment (0, 2.5 and 5 mg L^{-1} Se in Fertilizer Solution)			Foliar Application Experiment (0, 10 and 20 mg L^{-1} Foliar Se)		
	Variables	**Se-Root**	**Se-Shoot**	**Se-Fruit**	**Se-Root**	**Se-Shoot**	**Se-Fruit**
Root	N	−0.44	0.40	−0.44	−0.22	−0.64	−0.77 *
	P	0.33	−0.47	0.19	−0.22	−0.08	0.28
	K	0.23	0.19	0.26	0.12	0.28	0.37
	Ca	−0.64	−0.05	−0.59	−0.55	0.04	−0.27
	Mg	−0.79 **	0.04	−0.64	0.08	−0.13	−0.43
	Se	1	0.33	0.91 **	1	0.13	0.55
Shoot	N	−0.44	0.39	−0.46	−0.22	−0.64	−0.77 *
	P	0.59	−0.22	0.52	0.55	0.39	0.47
	K	−0.41	0.12	−0.25	0.17	0.11	0.03
	Ca	−0.32	0.23	−0.31	−0.08	−0.13	−0.05
	Mg	−0.26	0.06	−0.33	−0.61	0.26	−0.02
	Se	0.33	1	0.40	0.13	1	0.74 *
Fruit	N	−0.41	0.43	−0.45	−0.15	−0.79 *	−0.78 *
	P	0.54	−0.07	0.36	0.43	−0.31	0.0
	K	−0.60	−0.33	−0.66	0.15	−0.13	0.35
	Ca	0.20	−0.50	0.07	0.51	−0.21	0.15
	Mg	−0.65	0.27	−0.65	0.09	0.83 **	0.69
	Se	0.91 **	0.40	1	0.55	0.74 *	1
	ANTIOX	0.62	0.75 **	0.70 **	0.67	0.54	0.88 *
Biomass	RFW	−0.48	−0.25	−0.56	0.58	0.21	0.70 *
	RDW	−0.50	−0.24	−0.56	0.58	0.15	0.72 *
	SFW	−0.77 **	0.12	−0.75 **	0.07	0.69	0.52
	SDW	−0.75 **	0.14	−0.74 **	0.03	0.71 *	0.58
	FFW	−0.67	−0.28	−0.69	−0.40	0.24	0.13
	FDW	−0.78 **	−0.30	−0.76 **	−0.23	0.33	0.22

* = significant ($p < 0.05$); ** significant ($p < 0.01$). RFW = Root fresh weight; RDW = Root dry weight; SFW = Shoot fresh weight; SDW = Shoot dry weight; FFW = Fruit fresh weight; FDW = Fruit dry weight.

In the Irrigation experiment, the increase in Se concentration in roots and fruits showed a negative relationship with the dry and fresh weights of the shoot, as well as against the dry weight of the fruit. Se in the roots was negatively associated with the Mg concentration in roots and Se in fruits. The antioxidant status of fruits was positively associated with Se levels in shoots and fruits.

In the Foliar application experiment, regarding the concentration of Se in roots, a correlation coefficient with a value equal to or higher than 0.70 was not obtained. The concentration of Se in leaves was negatively correlated with N in fruits, but was positively correlated with Mg and Se in fruits and with the dry weight of shoots. The amount of Se in fruits was negatively associated with N in roots, shoots, and fruits; however, the concentration of Se in fruits was also positively associated with fresh and dry weights of roots and with the antioxidant status of the fruits.

4. Discussion

Se application in the form of sodium selenite showed different effects on plants, depending on both the substrate in which the plants were grown and on the Se concentration and form of application. Se has three levels of biological activity, as follows: (i) trace concentrations are required for normal growth and development; (ii) moderate concentrations can be stored to maintain homeostatic functions; and (iii) high concentrations can result in toxic effects [31]. Trace and moderate concentrations can stimulate growth, which is in agreement with the results observed in the present study.

4.1. Biomass

The Se application at 2.5 mg L^{-1} in the fertilizer solution in plants grown in soil did not modify the biomass, on the other hand, the plants that were grown in perlite only reduced the weights of the shoot. The application of 10 mg L^{-1} by foliar spray increased the weights of the shoot, the fresh weight of root and the dry weight of fruit in comparison with the control plants; while the application of 5 mg L^{-1} in the nutrient solution reduced the plant biomass (Table 1). A recent study reported that Se inhibits root lengthening processes by decreasing auxin concentration [32], and at high concentrations in nutrient solutions, Se is concentrated primarily in roots [33], which might explain the reduction in root weight. Although the adverse effect of Se on growth remains not well understood, it may be related to the substitution of sulfur in proteins by Se, modifying the metabolism of sulfur in plants [34], which could trigger a decrease in biomass as the applied Se concentrations increase. A marked decrease in lettuce biomass at concentrations higher than 1.57 mg L^{-1} Se in the form of selenite in the nutrient solution, whereas at concentrations of 0.39 and 0.78 mg L^{-1}, no significant effects were observed [35]. However, Xue et al. [36] reported that Se at the concentration of 1 μg g^{-1} of soil reduced the biomass of lettuce. In contrast, foliar application of Se significantly promotes the growth of vegetables such as bulbs and leaves of onion [37], roots and leaves of carrot [38], flowers and leaves of radish [39], and garlic bulbs [40], as well as cereals such as rice [10] and wheat [41].

The effect of the type of fertilization with Se on biomass depends on the concentration of the element and the frequency of the application. When applying Se to the soil and/or substrate, plants can be challenged because of the variable concentrations of Se in soil and the factors that modify Se availability, such as soil type, redox potential, pH, microbiological activity, salinity, competition with other anions, adsorption in clay minerals, and metal oxides and hydroxides [42]. However, with the foliar application, soil chemistry and microbiological processes have less effect, ensuring greater efficiency of uptake, but other factors must be considered, such as the leaf area, structure of the leaf surface and differences in metabolism that occur in different groups of plants [43]. In a previous study, fertilization with Se (Na_2SeO_3), both via foliar spray and nutrient solution, for cultivation of buckwheat (*Fagopyrum esculentum* Moench) increased yield when applied at 5 g of Se per hectare as a foliar spray, and at 6 and 12 g of Se per hectare to the soil [23]. However, Schiavon et al. [14] reported an increase in radish biomass when applying 5 or 10 mg per plant via a single foliar application and ~0.79 mg L^{-1} in the nutrient solution. In these studies, the increased accumulation of root dry weight with the foliar application was highlighted, which can be accompanied by the higher growth of aerial

structures, which could partially explain the increase in biomass observed in the foliar application experiment (Table 1).

4.2. Mineral Concentrations

As a general result, Se applications at 5 mg L^{-1} in the Irrigation experiment reduced the concentration of N (root, shoot and fruits), K (shoots and fruits), Ca and Mg (root and shoot) in plants grown in perlite compared to control plants (perlite without Se). On the other hand, applications at 2.5 mg L^{-1} (Irrigation experiment) and Se applications in the Foliar experiment did not negatively affect the macro-nutrients concentrations. These results are consistent with those reported by Castillo-Godina et al. [44] when using sodium selenite in nutrient solution at concentrations of 2 and 5 mg L^{-1}, and also with those obtained by Smolen et al. [45], who reported a reduction in Ca and Mg levels in lettuce roots when applying Se and iodine by foliar spray, without observing differences in macronutrients when only Se was applied. The change in the mineral concentration observed in the present study (Table 2) was possibly due to a redox phenomenon on the cell membrane, and ultimately on the cellular transport and metabolism processes [46]. Selenite may change the permeability of membranes toward some cations and therefore affect transport in plant cells [47]. Effects have been reported in maize plants with applications of Se at ~4 and 7.9 mg L^{-1} that increased Ca and P; the same effect was observed for K but at lower concentrations (≈0.4 and 1.9 mg L^{-1}); in the case of Mg, Se treatments did not significantly modify the concentration [48]. Slight increases of Ca have been observed in hydroponic ferns (*Pterisvittata* L.) with Se concentrations in the form of selenite from 5 to 20 mg L^{-1}, whereas at concentrations lower than 2 mg L^{-1}, decreases in Ca were observed [49]. In our study, no significant differences were observed for calcium concentration in plant tissues (Tables 2 and 3); however, it has been reported that the increase in Ca concentration in plant tissues may be part of a mechanism used to increase tolerance to Se when plants are exposed to high concentrations [49]. Nawaz et al. [50] reported that the application of foliar Se at a concentration of 40 mg L^{-1} increased the transpiration rate and stomatal conductance in wheat. These physiological processes are related to the absorption and translocation of elements that move by mass flow [51], which could explain in part the positive correlation between the Se levels in the shoots and the concentrations of magnesium in fruits in the foliar application experiment (Table 6).

Although visual deficiency symptoms were not observed in tomato leaves for any treatment in the two experiments, the levels for K and Mg remained below the recommended values [52]. For tomato fruits, the mineral content remained above the recommended values for Ca (1.45–1.81 mg g^{-1}) and P (3.7–5 mg g^{-1}), whereas K and Mg contents were below the established recommendation of 36–48 mg g^{-1} and 1.16–2 mg g^{-1}, respectively; note that the content of mineral nutrients is provided per g dry weight [53].

The N concentration decrease in plant tissue, as observed in the treatments at 5 mg L^{-1} of Se in nutrient solution in plants grown in perlite (Table 2) and foliar spray (Table 6), has been described as a Se toxicity response [54], possibly due to the unspecific substitution of sulfur by Se in proteins and other compounds related to sulfur metabolism [16]. Another possible explanation is associated with the known antagonistic effects between molybdenum and selenite [55], which could translate into reduced nitrate reductase activity in roots. An antagonistic effect between Se and the anions in the solution could be another possible explanation for the decrease of N. However, this alternative was less likely because P, which is absorbed as anion, showed a positive response to Se in shoots of plants grown in perlite at concentrations of 2.5 and 5 mg L^{-1} (irrigation experiment) and in plant shoots with 20 mgL^{-1} Se (foliar experiment). Similar results about the concentration of P were found by Do Nascimento Da Silva et al. [55] with the application of selenite in hydroponic lettuce crops.

4.3. Selenium Concentration

When applying the 2.5 and 5 mg L^{-1} treatments of Se in the nutrient solution, the highest concentration of Se was obtained in roots (Table 4). Se applied in the form of selenite is absorbed

without difficulty by roots of plants and is rapidly transformed and accumulated in organo-selenium compounds in the roots [23], and the transport to leaves and fruits occur by the symplastic pathway [13]. Selenite, unlike selenate, has no restrictions on its absorption due to competition with sulfate [56], as observed by Asher et al. [57] in tomatoes, in which the absorption of selenite was little affected by the presence of sulfates. Se can be remobilized from leaves to fruits using the same high-affinity channels that transport sulfur, either in the form of organic compounds, such as selenomethionine (SetMet) and Se-methyl selenocysteine (MeSeCys) or to a lesser extent as selenite [58] or as selenate, product of the oxidation of selenite [57].

The Se concentrations in roots and fruits of plants cultivated in soil were lower than those in plants grown in perlite in Irrigation experiment (Table 4). This response was most likely because selenite can be strongly adsorbed by metal oxides, causing the selenite to have a lower availability for plants in the soil solution [59]. However, when using inert growing media such as perlite, the Se availability in solution is expected to be greater. The Se concentration in control plants was determined by the basal Se concentration in the growing media and the irrigation water.

The foliar application of Se facilitated the Se accumulation in roots and fruits (Table 4). Boldrin et al. [10] reported that the foliar Se applications facilitate the transport of Se in xylem and phloem. Poggi et al. [60] observed high mobility of Se in the phloem of potato plants from Se foliar applications. It is known that Se diffusion from epidermal cells to other cells occurs when Se is applied via foliar spray [61], although in high concentrations, Se can lead to toxicity and cause damage to the surface of the leaf [51].

4.4. Antioxidant Status in Tomato Fruits

The antioxidant role of Se depends primarily on the concentration, having dual effects. At high concentrations, Se is a pro-oxidant [9]. However, at low concentrations, Se stimulates the production of antioxidants in plants [62]. In our study, the production of antioxidants in fruits was induced in treatments with Se applications at 2.5 and 5 mg L^{-1} in the nutrient solution in plants grown in soil and at 10 and 20 mg L^{-1} via foliar spray (Table 5). Other authors describe a similar effect, possibly related to the increase by Se in the activity or concentration of enzymatic and non-enzymatic antioxidants [36,44]. These results are also consistent with those reported by several authors that indicate that Se at low concentrations protects against oxidative stress in higher plants [20,63]. Likewise, increased antioxidant capacity in lettuce is reported with Se application in soil [36], and with the foliar application of 1 mg L^{-1} Se (Na_2SeO_4) in tomato plants reduced the production of reactive oxygen species such as O^{2-} and H_2O_2 in fruits [64].

Our results suggest that the Se application in the fertilizing solution and by foliar spray is an adequate Se biofortification technique in tomato fruits, as well as contributing to the antioxidant state. If we consider an average fruit weight of 150 g with a water percentage of 95%, we find from the Irrigation experiment that each treatment would contribute the following Se amounts per fruit: soil + 0 (40.35 µg), perlite + 0 (46 µg), soil + 2.5 (78 µg), perlite + 2.5 (105.8 µg), soil + 5 (130.8 µg) and perlite + 5 (155.5 µg); meanwhile, for the Foliar experiment, the Se contribution per fruit would be: peat moss + 0 (29 µg), peat moss + 10 (73 µg) and peat moss + 20 (84.9 µg). Consequently, the concentrations evaluated in this research could be implemented in production systems with and without soil; preferably, the technique by foliar spray (10 mg L^{-1} Se), since it represents fewer applications and a lower soil or water contamination. On the other hand, Se applications in fertilizer solution (2.5 mg L^{-1} Se) can be more accessible through irrigation systems, either continuously, as in our study, or using intermittent applications, for example, in the seedling stage [65], every month during the plant growth period, or in the fruit set growing phase, as was reported for iodine biofortification in tomato [66].

5. Conclusions

Se application through fertilizing solution and by foliar spray is an adequate Se biofortification technique for tomato fruits; additionally, it promotes the antioxidant status of fruits.

According to the results of this study, Se biofortification is achieved by the technique of foliar spray (10 mg L^{-1} Se, every 20 days) with fewer applications than applications through the fertilizer solution (2.5 mg L^{-1} Se).

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