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Selenium Fertilization Alters the Chemical Composition and Antioxidant Constituents of Tomato (*Solanum lycopersicon* L.)

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Supporting Information

ABSTRACT: Although selenium (Se) is a known anticarcinogen, little is known regarding how Se affects other nutritional qualities in crops. Tomato (*Solanum lycopersicon*) was supplied with 0−50 μM selenate and analyzed for elemental composition and antioxidant compounds. When supplied at low doses (5 and 10 μM) via the roots, Se stimulated the synthesis of phenolic compounds in leaves and reduced the levels of Mo, Fe, Mn, and Cu in roots. At higher doses (25 and 50 μM Se) leaf glutathione levels were 3−5-fold enhanced. Supply of selenate via foliar spray (0, 2, or 20 mg Se plant^{-1}) resulted in Se-biofortified tomato fruits, with Se levels low enough not to pose a health risk. The Se-biofortified fruits showed enhanced levels of the antioxidant flavonoids naringenin chalcone and kaempferol and a concomitant decrease of cinnamic acid derivatives. Thus, tomato fruits can be safely enriched with Se, and Se biofortification may enhance levels of other neutraceutical compounds.

KEYWORDS: *Solanum lycopersicon L., selenium, sulfur, antioxidants, biofortification*

INTRODUCTION

Selenium (Se) is a ubiquitous element in the environment; its concentration in soils is generally low (<2 ppm),[1] although higher concentrations (>10 ppm) can occur in seleniferous areas.[2]

Se enters the food chain through plants, which take it up from the soil mainly in the form of selenate. Due to the chemical similarity of Se to sulfur (S), selenate is readily transported across cell membranes via sulfate transporters and metabolized to Se-amino acids (selenocysteine and selenomethionine) via the sulfate assimilatory pathway.[3−5]

The essentiality of Se for higher plants has not been established to date, although there have been reports of beneficial effects of Se on plant growth of hyper-accumulators and, at low doses, of non-hyper-accumulator plants.[6] However, this element has been recognized as an essential micronutrient for animals and humans for decades.[7] Indeed, Se is a key component of 25 human enzymes, including glutathione peroxidase, selenoprotein P, and tetraiodothyronine 5′-deiodinase, which are involved in several major regulative and protective redox mechanisms.[8−10]

In recent years, the importance of selenium (Se) in the human diet has received great attention worldwide because the effects of Se deficiency on human health have become a topic of interest in public health systems.[11] Selenium deficiency occurs in several parts of the world, especially in China, the United Kingdom, Australia, New Zealand, Africa, central Siberia, parts of India, Bangladesh, and Eastern Europe,[10,12−14] where the presence of Se in most soils is low and only trace amounts of this element can be accumulated in crop-derived foods.[15,16] Worldwide, it is estimated that between 0.5 and 1 billion people suffer from Se deficiency.[17]

A recommended dietary allowance (RDA) of 50−70 μg Se day^{-1} has been established for regular adults.[18,19] Habitually lower dietary intake of Se leads to reduced Se status, which may cause health disorders such as oxidative stress-related conditions, hypothyroidism, weakened immune system, cardiovascular disease, reduced male fertility, and increased risk of cancer.[10,20−24] In contrast, adequate Se intake in the human diet has long-term health benefits besides meeting basic nutritional requirements. In support of this, some organic forms of Se such as methyl-selenocysteine (MSeC) have been reported to exhibit anticarcinogenic activity against different types of cancer.[25] In fact, several studies have recommended a dietary Se supplement of 55−200 μg day^{-1} to reduce the incidence of lung and prostate cancers[25−27] and to fortify resistance against viral infections such as HIV.[28]

In the context of these health effects, low or diminishing Se status in some areas of the world is giving cause for concern. Because Se is scarcely available in most soils and that plants are the main dietary source of this element to humans and animals, in recent years several studies have addressed different ways to increase the Se content in crops.[11,13,14,29] The main approaches include the application of Se fertilizers to plants, genetic selection of varieties that accumulate more Se, and genetic engineering approaches to enhance Se uptake.[29]

Among vegetables, tomato (*Solanum lycopersicon* L.) is one of the most consumed worldwide, and in terms of plant production, it is second only to potato.[30] It is regarded as an

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important component of the human diet, being low in fat and calories, free of cholesterol, and rich in vitamin C, vitamin E, folic acid, and potassium.[31] Furthermore, tomato and tomato-based products are excellent sources of secondary metabolites (e.g., β-carotene, lycopene, and phenolic compounds), a number of which play a role in the prevention of cancer[32] and cardiovascular diseases.[33] The synthesis of these phytochemicals may be affected in response to various environmental conditions, including Se fertilization, as reported in various plant species.[34−36]

To date, the possibility to enhance the nutritional value of tomato plants through Se fertilization has been scarcely investigated.[37]

The main aim of the current study was to determine the potential for increasing Se and antioxidant content in *S. lycopersicon* fruits through foliar supplementation of selenate. Furthermore, as tomato plants can routinely be cultivated in soilless growing systems, we tested a different fertilization approach by adding selenate to the nutrient solution used to grow tomato plants hydroponically. In this experiment, the effects of Se were evaluated in plant tissues.

MATERIALS AND METHODS

Hydroponic Experiment. *Plant Material.* To estimate the effects of Se on tomato plants, a hydroponic experiment was realized. Seeds of tomato (*S. lycopersicon* cv. Margoble) were surface-sterilized by rinsing in 70% (v/v) ethanol for 30−60 s, then in 5% (v/v) sodium hypochlorite (NaClO) for 30 min while rocking on a platform, and washed in distilled water for 5 × 10 min. The seeds were allowed to germinate and grow for 8 days in half-strength MS[38] agar medium inside a chamber with a 14 h light/10 h dark cycle, air temperature of 26/21 °C, and relative humidity of 70/85% and at a photon flux density (PFD) of 280 mol m^{-2} s^{-1}. Germinated seedlings were transferred to 3 L pots (density = 7 plants per pot) and cultivated in a thoroughly aerated nutrient solution with the following composition (μM): KH_2PO_4 (80), $Ca(NO_3)_2$ (1000), KNO_3 (250), $MgSO_4$ (1000), FeNaEDTA (20), B (4.6), Cl (1.1), Mn (0.9), Zn (0.09), and Mo (0.01). The nutrient solution in each pot was renewed every 3 days to ensure a constant supply of macro- and microelements to plants. At 40 days since the transplant, Se in the form of selenate (Na_2SeO_4) was added to the nutrient solution at the following concentrations: 5, 10, 25, 50, or 100 μM. A group of plants was not exposed to selenate and used as the control.

The experimental design for seedling growth in hydroponic was randomized. As we previously evaluated plant responses to Se at very short time,[39] tomato seedlings were grown for 24 h and 5 days in the presence or absence of selenate. Per each treatment at each time point three pots (each corresponding to a biological replicate) were selected. The entire experiment was replicated two times (n = 6). After harvest, plants were carefully washed with distilled water and dried with blotting paper. For fresh and dry weight measurements, six plants per treatment were divided into roots and shoots and weighed separately. The samples were next placed in a drying oven for 2 days at 70 °C and then allowed to cool for 2 h inside a closed bell jar before the dry weight was measured. Samples from the remaining plants were immediately frozen with liquid nitrogen and kept at −80 °C for further analyses.

Determination of Total Se and Macro- and Microelements in Plants. Dried foliar and root tissues of hydroponically grown tomato plants were digested in nitric acid as described by Zarcinas et al.[40] Inductively coupled plasma atomic emission spectroscopy (ICP-AES) was used as described by Fassel[41] to determine each digest's elemental concentrations (Se, S, Mo, Mn, Mg, Ca, Fe, Cu). The linear range, limit of detection (LOD), and limit of quantitation (LOQ) of the considered elements are reported in Table 1 S (Supporting Information).

For each experimental treatment, data obtained were the means of three measurements from three plants each and were expressed as milligrams of element per kilogram of dry weight (dw).

Analysis of Sulfate and Selenate Content in Plants. Frozen foliar and root tissues (500 mg) were ground in liquid nitrogen, and then 10 mL of distilled water was added. The samples were incubated for 2 h in a heating block at 85 °C. The obtained extracts were filtered (0.45 μm, Millipore) and analyzed for sulfate and selenate concentrations by HPLC using a Dionex IonPac AS11 4 mm column, coupled to an AG 14 guard column and a CD20 conductivity detector. The column was eluted over a period of 18 min with 3.5 mM Na_2CO_3/1 mM $NaHCO_3$ in H_2O, at a flow rate of 0.9 mL min^{-1} and at 1400 psi of pressure.

Sulfate and selenate contents were expressed in milligrams of anion per kilogram of fresh weight (fw); for each treatment the data shown are the means of three measurements with three plants in each replicate.

Determination of Low Molecular Weight Thiol Compounds. Frozen leaf samples (250 mg) from five biological replicates were ground with a mortar and pestle to extract soluble antioxidants with 0.1 N HCl and 1 mM EDTA. Following centrifugation at 10000g for 10 min, extracts were tested for low-molecular-weight (LMW) thiol levels. Prepared extracts (50 μL) were derivatized with SBD-F fluorophore (Sigma-Aldrich, St. Louis, MO, USA). Low-molecular-weight thiols were separated by isocratic HPLC using the method described in Masi et al.[42] The mobile phase was 3% methanol in 75 mM NH_4^+ formate, pH 2.9.

Soil Experiment. An additional experiment was performed in *S. lycopersicum* plants cultivated in soil to estimate the capacity of plants to accumulate Se in fruits following selenate application to tomato leaves. The effects of Se on plant production and on the antioxidant properties of fruits were also evaluated.

For this experiment (May−July 2012), seeds of tomato were allowed to germinate in vermiculite for 10 days inside the greenhouse. Germinated seedlings were then transferred to pots (one plant per pot) containing peat, soil, and Perlite in the ratio 60:30:10. Pots were divided over three groups containing seven plants each and were watered twice a day.

One month after transplanting, before fruit appearance, a unique foliar application of selenate (Na_2SeO_4) to two plant groups was performed. Each plant of the first group was sprayed with the dose of 2 mg Se per plant, whereas each plant of the other group was sprayed with 20 mg Se per plant. The concentration of Se in the treating solutions was adjusted to spray the same volume of solution on each plant. Plants of the remaining group served as control and were sprayed with a volume of water equal to that used for Se treatment. During foliar Se treatment, pots were covered to avoid Se contamination of soil. Between 20 and 40 days after Se treatment, ripe fruits were harvested daily, weighed, and kept at −80 °C for further analyses.

Determination of Total Se, C, and N in Plants. Total Se was determined in leaves, roots, and fruits via ICP-AES as previously described. The quantification of carbon (C) and nitrogen (N) was performed using an elemental analyzer (Vario MACRO CNS, Hanau, Germany).

For each experimental treatment, data obtained were the means of three measurements from three plants each and were expressed as milligrams of element per kilogram dry weight.

Determination of Total Se and S in Soil. Samples of soil were collected at the beginning and end of the experiment from each pot. For the analysis by ICP-AES, samples were dried at room temperature, and 2 g of each was digested with a solution of 65% (v/v) HNO_3/37% (v/v) HCl (ratio HNO_3/HCl 1:3 v/v) and warmed until boiling for 30 min under agitation, according to the manufacturer's instructions. The solution was then passed through a 0.45 μm filter (Millipore), and the quantification of Se and S was performed as previously described for elemental analysis in plants.

Analysis of Secondary Metabolites. The identification and quantification of polyphenols was performed in leaves and roots of plants cultivated in hydroponics, whereas the identification and

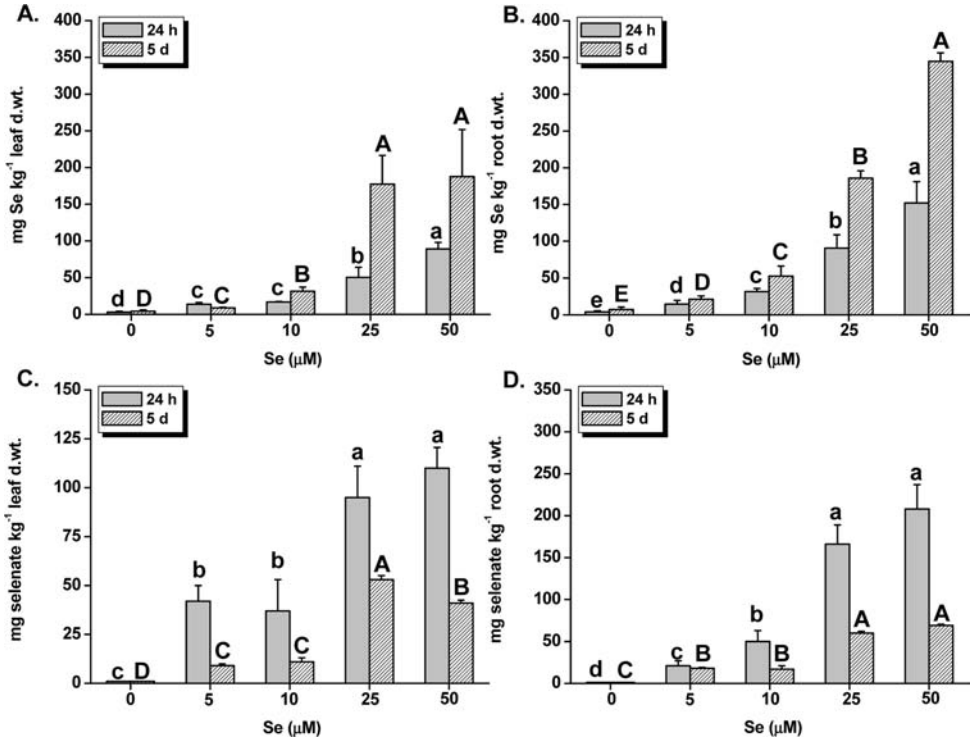

Figure 1. Concentrations of selenium (Se) and selenate in leaves (A and C, respectively) and roots (B and D, respectively) of *Solanum lycopersicon* plants cultivated in the presence of Se doses ranging from 0 (control) to 50 μM. Letters above bars indicate significant differences of the means ($P < 0.05$, ± SD). Lower case letters compare light gray bars, whereas capital letters compare dark gray bars.

quantification of both polyphenols and carotenoids was realized on tomato fruits obtained from plants cultivated in soil.

Extraction of Polyphenols. The extraction of polyphenols from fresh leaves, roots, peels, and fleshes of fruits were performed using a methanol/water (1:1, v/v) solution inside an ultrasonic bath for 15 min. Samples of leaves (800 mg), roots (800 mg), and peels (50 mg) were extracted with 4 mL of solvent and then centrifuged (3000 rpm × 10 min), and the solid matter was re-extracted with a further 4 mL of solvent. After centrifugation, the solid residue was re-extracted with 2 mL of solvent. Extracts were collected in a volumetric flask, and the volume was adjusted to 15 mL. Flesh samples (2 g) were extracted with 6 mL of solvent. The extracts were collected in a round-bottom flask, and solvent was removed under vacuum at 35 °C. The semisolid residue was then redissolved with 2 mL of extracting solution in a volumetric flask. The extracts were then filtered (0.45 μm, Millipore) before HPLC analysis.

For the analyses of phenolic compounds in hydroponically grown plants, three biological replicates of leaves and roots were used for each experimental condition (each sample of leaf and root derived from an individual plant) at both times (24 h and 5 days). For the analyses performed on tomato fruits, five biological replicates per treatment were used, with each fruit derived from an individual plant. For each biological replicate three measurements were realized. All of the fruits used for the analysis were harvested at the same day.

Qualitative and quantitative analyses of polyphenols were performed via HPLC-MS and HPLC-DAD, respectively. HPLC-DAD analyses were performed on an Agilent 1260 chromatograph equipped with an autosampler and a diode array detector 1260 series, whereas HPLC-MS analyses were obtained on a Varian 212 chromatograph equipped with a Prostar 430 autosampler and MS500 ion trap (Varian) mass spectrometer. For the separation of polyphenols, a ZORBAX Eclipse XDB C-8 column (3.5 μm × 2.1 mm × 150 mm, Agilent) was used in both HPLC systems. The eluents were a water solution of formic acid (1%, v/v, eluent A) and methanol (100%, eluent B), at a flow rate of 200 μL min^{-1}. The gradient was as follows: starting with 90% A/10% B, then in 16 min to 100% B, and isocratic until 20 min. Re-equilibration time to initial conditions was from 21 to 28 min. The column was thermostated at 35 °C.

For the HPLC-MS analysis, electrospray ionization (ESI) in negative ion mode (50−2000 uma) was used. The identification of the main polyphenol compounds in the samples was achieved via ion trap mass spectrometry (Varian 500 MS) coupled to the HPLC system, by comparison with appropriate standards (chlorogenic acid for phenols and rutin for flavonoids) and analysis of the fragmentation patterns of spectra through the Turbo Detection Data Scanning (TDDS) function. For the analysis, the ESI as a source in the full scan negative ion-mode (50−3500 uma) was used.

Quantitative analysis of polyphenols was obtained using the diode array detector. The selected wavelengths for quantitative purposes were 330 and 350 nm for chlorogenic acid and flavonoids, respectively. Chlorogenic acid and rutin were used as reference compounds. The experimental conditions for the identification of polyphenols are reported in Table 2 S of the Supporting Information.

Carotenoids. Carotenoids from peel samples (100 mg) were extracted with 4 mL of a cyclohexane/ethyl acetate (1:1, v/v) solution inside an ultrasonic bath for 15 min. Extraction was repeated three times with the same amount of solvent. Extracts were filtered and collected in a flask, and then 2 mL of deionized water was added to collect all of the water contained in the organic phase. Subsequently, the two phases were separated using a separator funnel. The organic phase was anhydrificated with natrium sulfate (Na_2SO_4), extracting solution was added to 5 mL final volume, and the mixture was filtered (0.45 μm, Millipore) before HPLC analysis.

The analyses were performed on five biological replicates per treatment, with each replicate (fruit) derived from an individual plant. For each biological replicate three measurements were performed. All of the fruits used for the analysis were harvested on the same day.

Carotenoid analysis was performed on a YMC Carotenoid C-30 column (5 μm × 4.6 mm × 250 mm), a specific chromatographic column for the analysis of carotenoids. The mobile phase consisted of methanol (100%, v/v, solvent A), and a mixture of methyl *tert*-butyl ether/methanol (90:10) was solvent B. The gradient was as follows: starting with 100% A, then in 12 min to 10% A/90% B, isocratic until

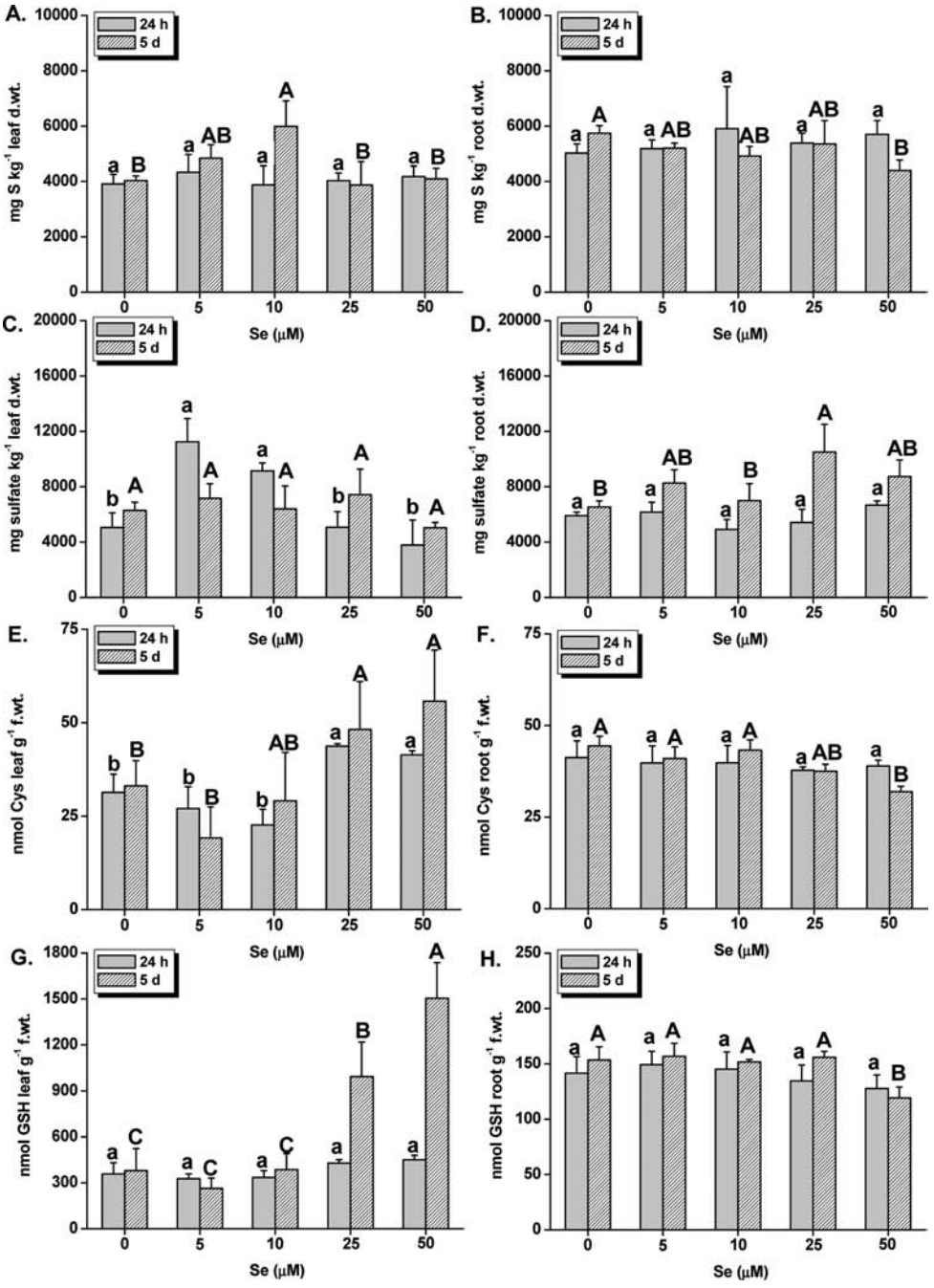

Figure 2. Concentration of sulfur (S), sulfate, cysteine (Cys), and glutathione (GSH) in leaves (A, C, and E, respectively) and roots (B, D, and F, respectively) of *Solanum lycopersicon* plants cultivated in the presence of Se doses ranging from 0 (control) to 50 μM. Letters above bars indicate significant differences of the means ($P < 0.05$, ± SD). Lower case letters compare light gray bars, whereas capital letters compare dark gray bars.

12 min. Re-equilibration time to initial conditions was from 13 to 20 min. The flow rates were 800 μL min^{-1} for HPLC-MS and 1.3 mL min^{-1} for HPLC-DAD. The column was thermostated at 16 °C.

The identification of the main carotenoid compounds in the samples was achieved via ion trap mass spectrometry (Varian 500 MS) coupled to the HPLC system, by comparison with appropriate standards (chlorogenic acid for phenols and rutin for flavonoids) and analysis of the fragmentation patterns of spectra through the TDDS function. The analysis was carried out using atmospheric pressure chemical ionization (APCI) as a source, in the positive ion mode, and the mass range considered was 500−605 uma.

Quantitative analysis of carotenoids was obtained using the diode array detector. The selected wavelength for quantitative purposes was 475 nm. Lycopene and β-carotene were quantified using relative standards. The experimental conditions for the identification of carotenoids are reported in Table 2 S of the Supporting Information.

Method Validation. The extraction and the chromatographic method for the quantitative analysis of secondary metabolites were validated by verifying the recovery percentage of chlorogenic acid, rutin, and β-carotene in replicates of leaf, root, peel, and flesh samples. The linear range, calibration curve, LOD, LOQ, and R^2 of the antioxidant constituents analyzed by HPLC-DAD are reported in Table 3 S of the Supporting Information. The identified compounds and the relative fragmentation values and retention times are reported in Tables 4 S and 5 S, whereas the recovery percentages of antioxidants are shown in Table 6 S (Supporting Information).

Statistical Analysis. For each plant and soil parameter determined in this study, the analysis of variance (ANOVA) was performed

Table 1. Content and Profile of Phenolic Acids in Leaves of Tomato Plants Cultivated Hydroponically in the Presence of Se Concentrations Ranging from 0 (Control) to 50 μM for 24 h and 5 Days[a]

	control	5 μM Se	10 μM Se	25 μM Se	50 μM Se
		Leaves (24 h, mg kg^{-1} fw)			
4-*O*-caffeoylquinic acid	23.1 ± 0.7b	34.9 ± 3.2a	35.0 ± 2.7a	23.4 ± 4.87b	24.0 ± 7.5b
quinic acid derivative 1	13.2 ± 0.9c	29.7 ± 2.3b	55.0 ± 2.3a	33.7 ± 5.43b	31.0 ± 2.3b
5-*O*-feruloylquinic acid	6.2 ± 1.3c	12.4 ± 3.7b	34.5 ± 2.9a	18.2 ± 4.71b	4.8 ± 2.0c
caffeic acid hexose 1	13.1 ± 3.5b	9.1 ± 1.0bc	39.4 ± 2.1a	13.9 ± 2.82b	4.8 ± 1.3c
4-*O*-feruloylquinic acid	10.9 ± 0.8b	11.3 ± 0.7b	31.9 ± 0.8a	13.3 ± 2.09b	46.8 ± 18.3a
quinic acid derivative 2	13.2 ± 1.2c	8.8 ± 1.57c	73.3 ± 1.2a	45.5 ± 6.23b	42.5 ± 29.1ab
caffeic acid hexose 2	12.7 ± 3.0c	18.1 ± 2.6bc	37.2 ± 6.2a	23.5 ± 4.03b	24.0 ± 13.4ab
5-*O*-caffeoylquinic acid	17.6 ± 2.4c	8.9 ± 5.6c	54.2 ± 31.8ab	65.5 ± 5.15a	35.2 ± 8.2b
chlorogenic acid	16.8 ± 1.5b	4.4 ± 1.6c	10.7 ± 1.4b	56.0 ± 2.44a	54.3 ± 17.2a
		Leaves (5 Days, mg kg^{-1} fw)			
4-*O*-caffeoylquinic acid	31.6 ± 8.3b	47.5 ± 2.7a	32.7 ± 3.0b	32.1 ± 5.9b	29.79 ± 1.7b
quinic acid derivative 1	55.7 ± 5.8b	69.4 ± 4.9a	46.2 ± 5.9b	47.7 ± 10.2b	44.33 ± 5.4b
5-*O*-feruloylquinic acid	11.7 ± 1.6b	25.6 ± 9.0a	15.4 ± 8.9ab	19.6 ± 5.4ab	27.03 ± 4.4a
caffeic acid hexose 1	18.5 ± 0.9c	66.6 ± 22.9a	24.9 ± 13.3bc	28.9 ± 5.9b	34.01 ± 0.2b
4-*O*-feruloylquinic acid	27.1 ± 3.5b	43.5 ± 6.0a	25.1 ± 5.8b	26.9 ± 3.5b	28.60 ± 1.3b
quinic acid derivative 2	48.0 ± 0.2b	89.1 ± 11.1a	50.6 ± 7.1b	56.3 ± 12.3b	59.73 ± 3.7b
caffeic acid hexose 2	30.0 ± 3.0b	49.3 ± 7.1a	36.5 ± 2.6ab	35.3 ± 8.0ab	30.40 ± 2.6b
5-*O*-caffeoylquinic acid	44.0 ± 4.0a	42.0 ± 7.0ab	51.5 ± 13.5a	47.5 ± 6.8a	33.02 ± 4.2b
chlorogenic acid	66.0 ± 10.1ab	42.3 ± 15.8b	81.2 ± 22.2a	73.6 ± 16.0a	47.66 ± 9.7b

[a]Different letters across rows indicate statistical differences between treatments ($P < 0.05$).

followed by pairwise post hoc analyses to determine significant differences ($P < 0.05$, ± SD) among the experimental conditions, as reported by Schiavon et al.[39]

RESULTS

Hydroponic Experiment. *Plant Growth.* Treating tomato plants with Se for 24 h did not alter the fresh weight of leaves, except when plants were cultivated in the presence of 10 μM Se (Figure 1A; Supporting Information). In this case, the leaf biomass production was higher than the control. At the end of the experimental period (5 days), the values of leaf biomass of plants supplied with 5 and 10 μM Se for 5 days were higher than that of plants grown without Se. The percentage increase of leaf fresh weight was 2-fold higher for plants treated with 5 μM Se (+40%) than for the control plants; the other Se treatments resulted in a percentage increase similar to that of the control.

The root fresh weight was increased when plants were treated for 24 h with doses of Se ranging from 5 to 25 μM, relative to the control without Se (Figure 1B; Supporting Information). After 5 days, plants grown in the presence of 10 μM Se displayed the maximum degree of root biomass production (40% more than control, $P < 0.05$), whereas the root fresh weight of plants exposed to higher Se concentrations was somewhat lower, albeit not significantly, than that of control plants. Plants that were cultivated in the presence of higher Se concentrations displayed symptoms of toxicity (e.g., necrosis, dark leaves).

Se and Selenate Accumulation. The Se concentration in leaves of tomato plants supplied with different levels of Se increased with time (24 h versus 5 days) and with the dosage of Se supplied, up to 100 mg kg^{-1} dw at 24 h and 175 mg kg^{-1} dw at 5 days for the 50 μM treatment (Figure 1A). The leaf Se concentration for the 5 and 10 μM treatments, which showed evidence of enhanced growth, was 20−30 mg kg^{-1} dw (Figure 1A). In roots, similar patterns of Se accumulation were observed as in leaves, increasing with Se supply and with time (Figure 1B). Generally, Se concentration in root tissues was higher than that measured in leaves, with some exceptions.

Plants accumulated around 2-fold more selenate when cultivated in the presence of the highest doses of Se (25 and 50 μM) than when treated with 5 or 10 μM (Figure 2C,D). In foliar tissues a significant reduction in selenate accumulation occurred after 5 days of Se treatment, to the extent of 70−80% in plants exposed to 5 and 10 μM Se and 45 and 65% in plants grown with 25 and 50 μM Se, respectively (Figure 1C).

In roots, selenate accumulation after 24 h increased with increasing dose of Se supplied between 5 and 25 μM Se and then saturated (Figure 1D). As observed in foliar tissues, the level of selenate decreased in roots after 5 days of plant exposure to Se, relative to the 24 h time point, especially in plants cultivated with 25 and 50 μM Se.

Effect of Se on Sulfur and Sulfate Accumulation. Treating plants with Se for 24 h did not affect the content of sulfur (S) in leaves (Figure 2A) and roots (Figure 2B). However, after 5 days of Se treatment S accumulated more in leaves of plants exposed to 10 μM Se and decreased in roots of plants supplied with the maximum Se dose of 50 μM.

The foliar level of sulfate increased in plants treated for 24 h with low Se (5 and 10 μM), whereas in plants exposed to 25 and 50 μM Se the levels of sulfate were similar to those in control plants (Figure 2C). After 5 days of Se treatment, sulfate accumulation did not change among the experimental conditions.

In roots, no significant variation in sulfate content was observed in plants following a 24 h time period of Se exposure (Figure 2D). However, the amount of sulfate was elevated in roots of plants exposed to 25 μM Se for 5 days.

Effect of Se on Cysteine and Glutathione Contents. In plants supplied with 25 and 50 μM Se a significantly higher cysteine (Cys) concentration was observed at both 24 h and at 5 days of Se treatment than in control plants and in plants exposed to lower Se concentration (5 and 10 μM) (Figure 2E). The leaf content of glutathione (GSH) also markedly increased

Table 2. Content and Profile of Flavonoids in Leaves of Tomato Plants Cultivated Hydroponically in the Presence of Se Concentrations Ranging from 0 (Control) to 50 μM for 24 h and 5 Days[a]

flavonoid	control	5 μM Se	10 μM Se	25 μM Se	50 μM Se
			Leaves (24 h, mg kg^{-1}fw)		
quercetin-hexose-deoxyhexose pentose	182.4 ± 74.7a	182.8 ± 71.5a	162.0 ± 54.7a	171.9 ± 70.4a	83.0 ± 56.0a
kaempferol	43.2 ± 12.4b	68.9 ± 8.9b	380.9 ± 118.2a	18.7 ± 7.7c	41.1 ± 16.8bc
kaempferol-3,7-di-*O*-glucoside	107.8 ± 56.5b	299.4 ± 41.4a	286.2 ± 117.2ab	209.3 ± 137.4ab	134.5 ± 90.4b
kaempferol hexose pentose	2190.1 ± 1294.4a	58.2 ± 14.8b	29.4 ± 12.1bc	32.7 ± 13.4b	12.8 ± 5.2c
rutin	1705.6 ± 1068.4c	5179.7 ± 734.1a	6156.2 ± 1138.2a	3476.0 ± 1009.8b	3297.8 ± 361.9b
kaempferol glucose rhamnose	0.0 ± 0.0b	0.0 ± 0.0b	2.5 ± 1.4a	0.0 ± 0.0b	0.0 ± 0.0b
p-coumaric acid conjugate of rutin	163.5 ± 93.0a	76.2 ± 22.0a	59.7 ± 17.0a	48.0 ± 20.8a	30.4 ± 12.5b
quercetin-deoxyhexose-hexose	100.6 ± 52.1b	229.8 ± 35.3a	287.6 ± 22.5a	186.4 ± 96.2ab	156.3 ± 38.2b
kaempferol 3-*O*-glucoside 7-*O*-rhamnoside	0.0 ± 0.0c	0.0 ± 0.0c	33.8 ± 16.9a	38.0 ± 26.9a	1.2 ± 0.7b
			Leaves (5 Days, mg kg^{-1} fw)		
quercetin-hexose-deoxyhexose pentose	179.0 ± 71.8a	204.0 ± 128.5ab	112.5 ± 67.1ab	85.9 ± 18.7b	36.8 ± 18.2c
kaempferol	53.9 ± 26.9a	104.7 ± 42.9a	84.7 ± 37.8a	86.0 ± 25.7a	78.2 ± 32.0a
kaempferol-3,7-di-*O*-glucoside	450.2 ± 114.1a	272.0 ± 170.2ab	542.2 ± 147.7a	297.5 ± 20.4b	422.6 ± 78.3a
kaempferol hexose pentose	2012.1 ± 1050.1a	53.8 ± 22.0b	55.8 ± 22.8b	10.9 ± 2.0c	16.2 ± 5.6c
rutin	7376.6 ± 1762.41ab	6815.9 ± 2498.9ab	8849.4 ± 124.3a	6173.5 ± 905.2b	4906.7 ± 775.7b
kaempferol glucose rhamnose	0.0 ± 0.0b	0.0 ± 0.0b	1.8 ± 0.7a	0.0 ± 0.0b	0.0 ± 0.0b
p-coumaric acid conjugate of rutin	193.0 ± 79.0a	49.5 ± 30.6bc	102.6 ± 42.0a	23.7 ± 9.7c	80.8 ± 331ab
quercetin-deoxyhexose-hexose	372.2 ± 155.4a	477.5 ± 318.5a	397.9 ± 117.1a	230.8 ± 33.7a	245.8 ± 35.3a
kaempferol 3-*O*-glucoside 7-*O*-rhamnoside	0.0 ± 0.0b	87.0 ± 35.6a	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b

[a]Different letters along rows indicate statistical differences among treatments ($P < 0.05$).

Table 3. Content and Profile of Phenolic Acids and Flavonoids in Roots of Tomato Plants Cultivated Hydroponically in the Presence of Se Concentrations Ranging from 0 (Control) to 50 μM for 24 h and 5 Days[a]

	control	5 μM Se	10 μM Se	25 μM Se	50 μM Se
phenolic acid			**Roots (24 h, mg kg^{-1} fw)**		
4-*O*-caffeoylquinic acid	13.2 ± 1.6a	9.1 ± 5.0ab	6.7 ± 0.2b	5.7 ± 0.8b	6.9 ± 0.6b
5-*O*-dimethoxycinnamoylquinic acid	38.0 ± 22.0a	19.6 ± 11.1a	19.8 ± 3.6a	17.6 ± 13.7ab	7.6 ± 2.7b
5-*O*-caffeoylquinic acid	5.3 ± 3.1b	14.7 ± 2.6a	10.7 ± 2.5ab	7.1 ± 4.8ab	14.2 ± 5.3a
chlorogenic acid	8.0 ± 4.4b	22.3 ± 3.8a	16.6 ± 8.0ab	11.3 ± 7.2ab	22.7 ± 8.2a
phenolic acid			**Roots (5 Days, mg kg^{-1} fw)**		
4-*O*-caffeoylquinic acid	8.6 ± 2.0a	7.1 ± 1.2a	8.7 ± 0.4a	7.3 ± 0.5a	6.8 ± 0.4a
5-*O*-dimethoxycinnamoylquinic acid	57.5 ± 2.14a	66.9 ± 10.8a	26.9 ± 21.9bc	15.3 ± 7.1bc	12.6 ± 3.4c
5-*O*-caffeoylquinic acid	17.7 ± 8.65b	32.9 ± 5.9a	31.9 ± 5.9ab	20.9 ± 6.3ab	25.4 ± 10.4ab
chlorogenic acid	28.3 ± 8.88a	51.5 ± 19.6a	40.3 ± 18.0a	32.3 ± 9.6a	38.0 ± 13.4a
flavonoid			**Roots (24 h, mg kg^{-1} fw)**		
rutin	87.3 ± 43.7a	130.4 ± 42.0a	70.3 ± 21.4a	17.4 ± 7.7b	28.9 ± 17.7b
flavonoid			**Roots (5 Days, mg kg^{-1} fw)**		
rutin	34.7 ± 24.2bc	15.5 ± 4.4c	30.4 ± 21.1bc	209.9 ± 114.7a	64.3 ± 27.0ab

[a]Different letters across rows indicate statistical differences between treatments ($P < 0.05$).

in plants supplied with 25 and 50 μM Se, but only at 5 days (Figure 2G).

The root levels of Cys (Figure 2F) and GSH (Figure 2H) did not change in plants after Se addition, except for plants treated with 50 μM Se, for which a decrease of these compounds was detected at 5 days.

Effect of Se on Phenol and Flavonoid Accumulation. In leaves, nine main phenolic compounds were identified (Table 1). At the 24 h time point, these compounds were more synthesized in plants supplied with 10 μM Se than in the control, with the exception of chlorogenic acid, which was markedly accumulated only in plants treated with the highest doses of Se (25 and 50 μM). The contents of 5-*O*-caffeoylquinic acid and caffeoylquinic acid hydroxide isomer were substantially higher in plants supplied with Se concentrations ranging from 10 to 50 μM compared to the control. The leaf accumulation of quinic acid derivative 1 was higher in Se-treated plants than in control plants. A similar accumulation trend was found for 5-*O*-feruloylquinic acid, except when plants were exposed to 50 μM Se, in which case the values were comparable to those of the control plants. With respect to the other phenolic compounds, 4-*O*-caffeoylquinic acid was mainly produced in leaves of plants treated with low Se doses (5 and 10 μM), whereas the content of caffeic acid hexoses 1 and 2 was distinctly elevated only in plants treated with 10 μM Se. For 4-*O*-feruloylquinic acid, a trend of accumulation similar to the caffeic acid hexose isomers was evident, except for the high content measured in plants cultivated in the presence of 50 μM Se.

After 5 days of Se treatment, the synthesis of most phenolics (4-*O*-caffeoylquinic acid, caffeoylquinic acid, caffeic acid hexose, 4-*O*-feruloylquinic acid, quinic acid derivatives 1 and 2) was more pronounced in plants supplied with the lowest dose of Se (5 μM). Only the levels of 5-*O*-feruloylquinic acid and caffeic

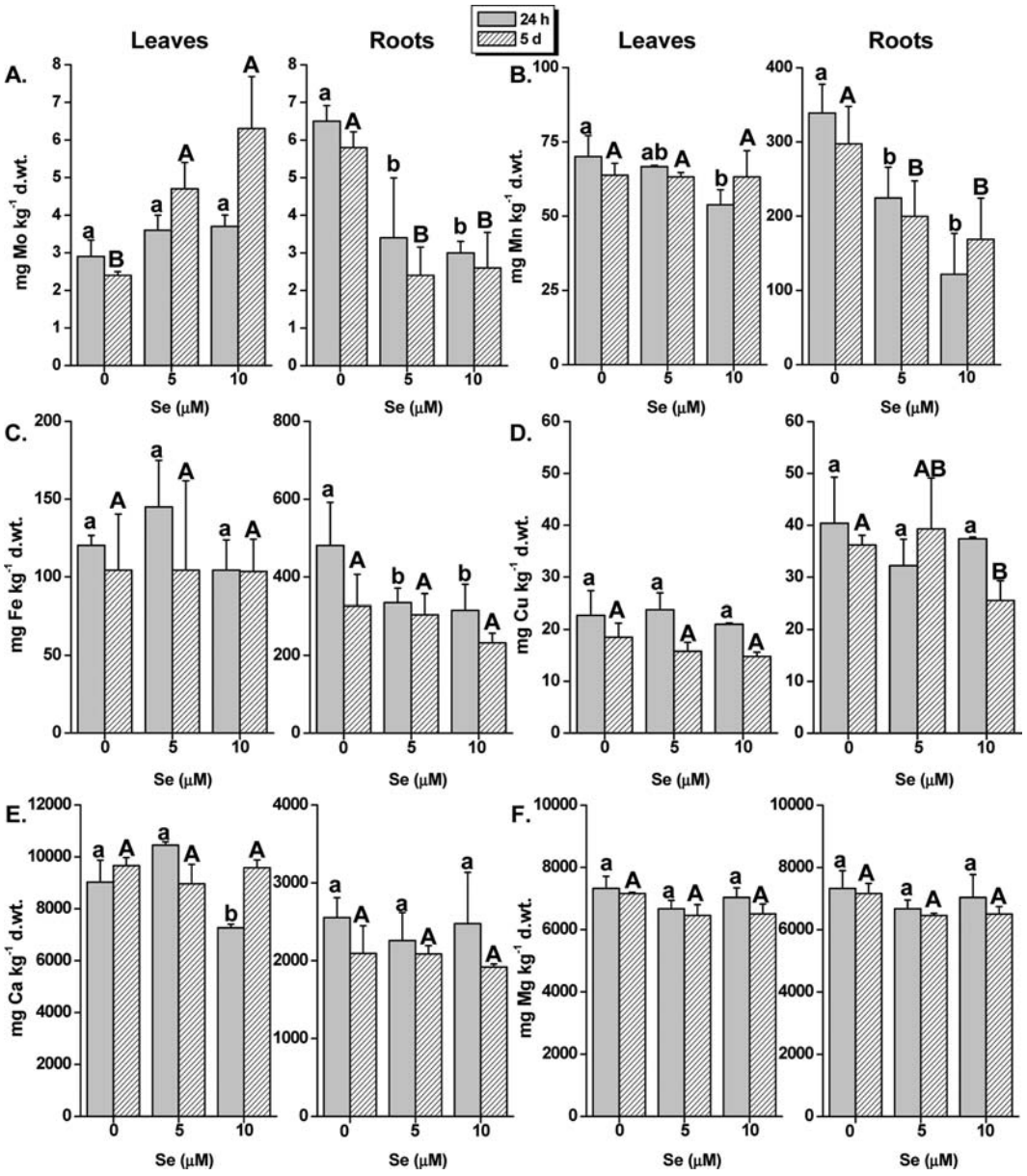

Figure 3. Elemental composition of leaves and roots of *Solanum lycopersicon* plants cultivated in the presence of Se doses ranging from 0 (control) to 10 μM. The analyzed elements included Mo (A), Mn (B), Fe (C), Cu (D), Ca (E), and Mg (F). Letters above bars indicate significant differences of the means ($P < 0.05$, ± SD). Lower case letters compare light gray bars, whereas capital letters compare dark gray bars.

acid hexose 1 were higher in plants grown with 50 μM Se than in the control, whereas 5-*O*-caffeoylquinic acid was lower. Chlorogenic acid did not show a clear Se-related pattern of accumulation.

Nine flavonoid compounds were identified in leaves of *S. lycopersicon* (Table 2). The synthesis of most flavonoids was enhanced in leaves of plants cultivated with Se for 24 h, with the exception of quercetin-hexose-deoxyhexose pentose. Maximum accumulation of some of these compounds, such as kaempferol, rutin, and kaempferol glucose rhamnose, was observed in plants treated with 10 μM Se. In the case of rutin, high values of this compound were also measured in plants treated with other doses of Se. An increase in content was also observed for quercetin-deoxyhexose-hexose and kaempferol-3,7-di-*O*-glucoside when plants were grown in the presence of low Se doses, whereas the presence of kaempferol 3-*O*-glucoside 7-*O*-rhamnoside was detected in plants to which Se was furnished at doses >10 μM.

A different trend of accumulation was evident for kaempferol hexose pentose and *p*-coumaric acid conjugate of rutin, which decreased in leaves after 24 h of plant exposure to Se. In the case of *p*-coumaric acid conjugate of rutin, such a reduction was observed only in plants supplied with 50 μM Se.

The exposure of plants to Se for 5 days determined a variation in the content of individual flavonoids compared to the 24 h treatments, with the exception of kaempferol glucose rhamnose and kaempferol hexose pentose, which showed a steady pattern of accumulation (Table 2). The levels of kaempferol, kaempferol-3,7-di-*O*-glucoside, and quercetin-deoxyhexose-hexose were almost comparable between control plants and plants supplied with Se. Interestingly, rutin decreased in plants treated with 25 and 50 mM Se, as well as quercetin-hexose-deoxyhexose pentose. Kaempferol 3-*O*-gluco-

side 7-*O*-rhamnoside was detected only in plants grown with 5 μM Se, whereas the accumulation pattern of *p*-coumaric acid conjugate of rutin was variable.

In roots, only four main phenolic acids were identified (Table 3). After 24 h, the contents of 4-*O*-caffeoylquinic and 5-*O*-dimethoxycinnamoylquinic acids decreased in plants cultivated with 10 and 50 μM Se, respectively. At 5 days, the level of 5-*O*-dimethoxycinnamoylquinic declined also in plants exposed to 10 and 25 μM Se, whereas no variation was observed for 4-*O*-caffeoylquinic acid between control plants and plants treated with Se.

The amounts of 5-*O*-caffeoylquinic and chlorogenic acids were generally higher in plants from the Se-treated categories. Rutin was the only flavonoid detected in roots (Table 3). At 24 h its content in plants supplied with the highest levels of selenate (25 and 50 μM) was lower than in the control. After 5 days of Se treatment, a strong increase in rutin level was observed in plants treated with 25 μM Se, whereas no differences were evident among the other experimental conditions.

Effect of Se on Plant Elemental Composition. The quantification of some micro- and macronutrients was performed only in control plants and in plants treated with 5 and 10 μM Se. This was because high plant biomass production and most of the variation in phenolic compounds were observed at low Se doses.

In leaves, a significant increase of molybdenum (Mo) content in plants supplied with Se was observed, especially after 5 days (Figure 3A). In the case of manganese (Mn), a weak reduction of the amount of this element was evidenced at 24 h when plants were exposed to 10 μM Se, whereas no variation between control plants and plants cultivated with Se was observed at 5 days (Figure 3B). The content of iron (Fe) was almost unchanged during the experimental period (Figure 3C), whereas the levels of copper (Cu) decreased in plants treated with Se from 24 h to 5 days of Se exposure but were similar to those measured in the control (Figure 3D).

The content of all micronutrients appreciably decreased in roots of plants supplied with Se. Mo (Figure 3A), Mn (Figure 3B), and Fe (Figure 3C) levels, in particular, were reduced after 24 h of Se treatment, whereas the content of Cu was reduced in roots by 10 μM Se treatment at 5 days (Figure 4D).

With respect to macronutrient accumulation, calcium (Ca) content diminished in leaves of plants cultivated with 10 μM Se for 24 h, but at 5 days it was comparable to that of control plants (Figure 3E). Magnesium (Mg) foliar content was apparently unaffected by Se treatment (Figure 3F). The root content of Ca and Mg did not change in response to Se application (Figure 3E,F).

Soil Experiment. *Se and Macronutrient Accumulation in Plants.* When tomato plants were grown in soil pots and sprayed with 20 mg of Se per plant, selenium accumulated at high levels in foliar tissues (Figure 5). Spraying a dose of 2 mg of Se per plant resulted in a 10-fold lower Se concentration in leaves.

In roots, Se accumulation was not detectable in plants treated with 2 mg of Se per plant, whereas the amount of Se was very low (approximately 1.2 mg kg^{-1}) when plants were exposed to Se at the highest dose (20 mg per plant).

Plants were able to accumulate Se in fruits at both doses of sprayed selenate, with maximum values (around 4 mg Se kg^{-1} dw) measured under the 20 mg of Se per plant treatment. Plant yield in terms of total number of fruits produced was reduced

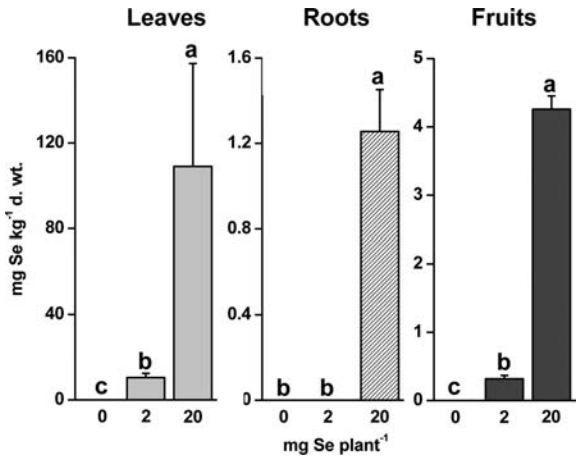

Figure 4. Concentration of Se in leaves, roots, and fruits of *Solanum lycopersicon* grown in soil and sprayed with selenate doses ranging from 0 (control) to 20 mg Se plant^{-1}. Leaves and roots were analyzed at the end of the experimental period, whereas fruits were those harvested the earlier week, when most plants produced ripened ones. Letters above bars indicate significant differences of the means ($P < 0.05$, ± SD).

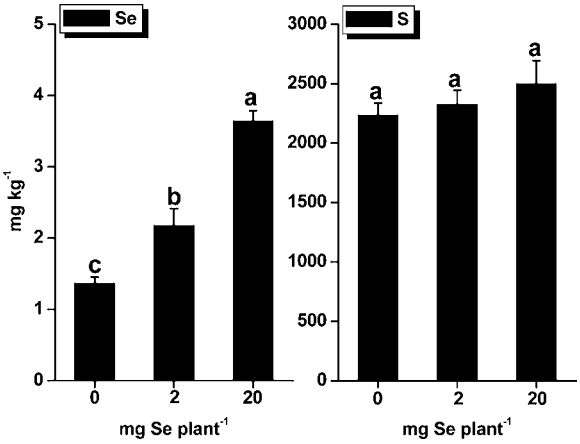

Figure 5. Concentrations of Se and S in soil where *Solanum lycopersicon* plants were cultivated and treated at the foliar level with selenate doses ranging from 0 (control) to 20 mg Se plant^{-1}. Letters above bars indicate significant differences of the means ($P < 0.05$, ± SD).

by Se treatment by about −14 to −17%, but the average weight of fruits was comparable among plants of the three experimental conditions (Table 4).

The relative content of carbon in tomato plants was not affected by the Se application to plants, whereas the percentages of nitrogen and sulfur in root tissues were lower in plants exposed to Se at the end of the experimental period (Figure 2; Supporting Information).

Quantification of Secondary Compounds in Tomato Fruits. The analysis of secondary metabolites in the fruit peel of tomato plants allowed the identification of six main phenolic acids, nine flavonoids, and two carotenoid compounds (Table 4).

The total content of phenolic acids substantially decreased in the peel of fruits from plants cultivated with Se with the exception of chlorogenic acid, which was unaffected. Whereas the concentrations of two dicaffeoylquinic acid isomers (5-*O*-caffeoylquinic acid and tricaffeoylquinic acid) were strongly

Table 4. Content of Secondary Metabolites in *Solanum lycopersicon* Fruit Peels of Plants Cultivated in Soil and Treated at the Foliar Level with Se Doses Ranging from 0 (Control) to 20 mg of Se per Plant[a]

	mg kg^{-1} fw		
	control	5 mg of Se per plant	20 mg of Se per plant
phenolic acids			
chlorogenic acid	73.5 ± 18.6a	59.7 ± 19.9a	80.3 ± 11.7a
dicaffeoylquinic acid 1	30.5 ± 14.1a	0.0 ± 0.0b	0.0 ± 0.0b
dicaffeoylquinic acid 2	71.2 ± 25.2a	0.0 ± 0.0b	0.0 ± 0.0b
dicaffeoylquinic acid 3	62.2 ± 18.9a	36.3 ± 9.1a	35.3 ± 11.5a
5-*O*-caffeoylquinic acid	28.2 ± 11.9a	0.0 ± 0.0b	0.0 ± 0.0b
tricaffeoylquinic acid	17.9 ± 1.2a	0.0 ± 0.0b	0.0 ± 0.0b
flavonoids			
rutin	510.5 ± 211.8a	411.5 ± 117.1a	461.0 ± 47.8a
kaempferol	0.0 ± 0.0c	139.8 ± 60.5a	45.6 ± 4.6b
kaempferol glucose rhamnose	57.4 ± 4.8a	45.2 ± 5.0a	49.2 ± 5.6a
quercetin-dihexose-deoxyhexose pentose	156.3 ± 35.7a	124.5 ± 35.6a	207.3 ± 74.1a
quercetin-hexose-deoxyhexose pentose	0.0 ± 0.0b	66.0 ± 9.7a	88.3 ± 17.3a
quercetin-hexose-deoxyhexose-pentose-*p*-coumaric	77.8 ± 23.9a	47.5 ± 5.0a	49.3 ± 9.0a
quercetin-hexose-deoxyhexose pentose glucose	107.6 ± 39.4a	98.1 ± 38.6a	91.7 ± 26.2a
quercetin	0.0 ± 0.0b	0.0 ± 0.0b	462.2 ± 92.2a
naringenin chalcone	2926.9 ± 1898.1b	7443.9 ± 4351.9ab	12558.2 ± 6005.4a
carotenoids			
lycopene	72.9 ± 48.3a	61.4 ± 18.6a	78.1 ± 37.0a
β-carotene	10.3 ± 5.2a	12.3 ± 5.1a	10.5 ± 2.9a

[a]Different letters across rows indicate statistical differences between treatments ($P < 0.05$).

reduced in fruit peel of Se-treated plants, the Se-associated decrease in concentration of dicaffeoylquinic acid 3 was not significant.

The content of several flavonoid compounds (rutin, kaempferol glucose rhamnose, quercetin-dihexose-deoxyhexose pentose, quercetin-hexose-deoxyhexose-pentose-*p*-coumaric, quercetin-hexose-deoxyhexose pentose glucose) did not change after application of Se to plants. Conversely, the amounts of quercetin-hexose-deoxyhexose pentose, kaempferol, and quercetin markedly increased in fruit peels when plants were exposed to Se. Naringenin chalcone was the most abundant flavonoid recovered in the peels of tomato fruits, and its level rose significantly in response to Se application to plants, especially at the dose of 20 mg of Se per plant.

No variation of both carotenoid compounds (lycopene and *β*-carotene) was observed in fruit peels of plants after Se exposure.

As far as fruit flesh was concerned, seven phenolic acids and eight flavonoids were identified (Table 5). The amounts of most of the phenolic acids (chlorogenic, dicaffeoylquinic, feruloylquinic, coumaroyl quinic acid, 5-*O*-caffeoylquinic) were comparable between fruit flesh of control and Se-treated plants. Tricaffeoylquinic acid was detectable only in fruit flesh of plants sprayed with Se, whereas caffeoyloside hesoside acid decreased in response to Se treatment to the point that it was not measurable anymore.

Selenium application to plants apparently did not alter the concentration of most flavonoids. However, Se did reduce the level of phloretin dihexose and increased naringenin chalcone in fruit flesh.

Se and S Quantification in Soil. The soil mixture used to grow tomato plants contained a very low (<2 ppm) Se concentration, like most soils worldwide (Figure 5). However, at the end of the experimental period the level of Se increased in soils in which plants treated with selenate had been cultivated. When the dose of Se sprayed onto plants was 20 mg

Table 5. Content of Secondary Metabolites in *Solanum lycopersicon* Fruit Flesh of Plants Cultivated in Soil and Treated at the Foliar Level with Se Doses Ranging from 0 (Control) to 20 mg of Se per Plant[a]

	mg kg^{-1} fw		
	control	5 mg of Se per plant	20 mg of Se per plant
phenolic acids			
chlorogenic acid	17.0 ± 7.9a	11.2 ± 3.6a	30.7 ± 19.0a
dicaffeoylquinic acid	4.5 ± 2.0a	1.5 ± 0.5a	5.4 ± 2.7a
tricaffeoylquinic acid	0.0 ± 0.0b	0.3 ± 0.1a	0.5 ± 0.4a
feruloylquinic acid	3.5 ± 1.8a	1.6 ± 0.6a	4.2 ± 2.7a
coumaroyl quinic acid isomer	1.4 ± 0.1a	1.2 ± 0.3a	1.2 ± 0.2a
5-*O*-caffeoylquinic acid	3.8 ± 1.1a	3.1 ± 0.8a	4.7 ± 2.3a
caffeoyloside hesoside	1.9 ± 0.5a	0.0 ± 0.0b	0.0 ± 0.0b
flavonoids			
rutin	9.1 ± 5.4a	7.4 ± 2.7a	9.5 ± 5.7a
kaempferol rutinoside pentoside	0.8 ± 0.2a	0.4 ± 0.1a	1.1 ± 0.5a
quercetin-dihexose-deoxyhexose pentose	3.9 ± 1.5a	2.9 ± 0.4a	4.5 ± 2.7a
phloretin dihexose	0.9 ± 0.4a	0.0 ± 0.0b	0.0 ± 0.0b
quercetin-hexose-deoxyhexose-pentose-*p*-coumaric	1.0 ± 0.7a	0.6 ± 0.2a	0.9 ± 0.4a
quercetin-hexose-deoxyhexose pentose glucose	1.4 ± 0.8a	0.6 ± 0.1a	1.9 ± 1.2a
naringenin chalcone	0.0 ± 0.0b	0.7 ± 0.3a	0.7 ± 0.3a
naringenin-dihexose	3.1 ± 1.1a	2.8 ± 0.8a	4.3 ± 1.4a

[a]Different letters across rows indicate statistical differences between treatments ($P < 0.05$).

per plant, the resulting level of Se in the soil was close to the minimum Se level that would classify soil as seleniferous (4–100 ppm).

The soil S concentration did not show significant variation between the soil mixtures used for the growth of control plants and plants exposed to Se (Figure 5).

DISCUSSION

The relationship between diet and health is an active area of research, and substantial evidence indicates that food components can influence physiological processes in humans. Thus, functional foods are of increasing interest in the prevention and/or treatment of diseases.

The current study was aimed at estimating the potential of Se enrichment of tomato plants cultivated under different experimental conditions (hydroponics or soil) and in the presence of various Se doses. Two different ways of increasing Se in plants were tested: (i) supplementation of Se in the nutrient solution of plants grown in hydroponics and (ii) foliar spray of Se in soil trials. In the first case, the effects of Se were evaluated on the nonedible plant tissues (leaves and roots) in short-term experiments, mainly to get information about the health status of plants upon Se treatment. In the second case, the effects of Se application were primarily assayed in fruits, being the part of plants channelled into the food chain.

When plants were cultivated hydroponically, low Se supply improved plant growth. Because the maximum values of plant fresh weight were measured at 10 μM Se, this dose of Se appeared to be optimal to obtain the highest biomass production of tomato. Stimulation of plant growth by Se at low doses has been also reported for a number of plant species, including Se hyperaccumulators and some non-hyper-accumulators (ryegrass, lettuce, potato, duckweed).[43,44] Tomato plants supplied with 25 and 50 μM Se displayed symptoms of toxicity at the end of the experimental period, in the form of darkness and edge dryness of older leaves. No apparent Se toxicity was observed in the tomato roots. This observation was in contrast to previous studies, which established roots as the main target of Se toxicity in *Arabidopsis thaliana* and *Brassica juncea*.[39,45]

Toxicity induced by Se at high doses could be associated with the high levels of Se measured in tomato plants and with the significant rate of Se translocation from the root to the shoot. In support of this, similar values of Se contents in leaves of the Se accumulator *B. juncea* were previously reported to be responsible for a reduction of plant growth.[39] However, several authors indicated that the [S]/[Se] ratio in plants is more important than the Se content alone for determining Se toxicity.[45−47] In particular, plants that manifest toxicity symptoms in response to Se application usually have a low [S]/[Se] ratio.[46] In our study, tomato plants treated with 5 and 10 μM Se had a higher [S]/[Se] ratio than plants supplied with 25 and 50 μM Se, because of both low Se accumulation and high S content. These differences in [S]/[Se] ratio may in part explain the observed variation in plant tolerance to Se. The increase of total S and sulfate content observed in tomato plants supplied with 5 and 10 μM Se is in agreement with a study by White et al.,[46] who reported the stimulation of plant sulfate uptake by low external Se concentrations in *A. thaliana*.

Selenium was furnished to plants as selenate, and in this form it was found to decrease in plant tissues between 24 h and 5 days of Se treatment. The reduction of selenate content in plants can be explained with the consumption of this ion to produce Se−organic compounds, as inorganic selenate can be converted to Se amino acids through the sulfate assimilatory pathway.[3−5,48] Our results indicate that the rate of Se assimilation was more pronounced when plants were supplied with high doses of Se (25 and 50 μM), especially at the root level and beyond the first 24 h of selenate supply.

Because selenate competes with sulfate for access to the S assimilation pathway, it is reasonable to assume that the content of the amino acid Cys could decrease in plants as a consequence of the synthesis of its analogue amino acid selenocysteine (SeCys), as previously reported in S-sufficient *B. juncea* plants.[39] At high doses of Se, a weak reduction of root Cys level was indeed observed, as well as of GSH, for which the Cys is a precursor. However, a concomitant strong enhancement of both Cys and GSH contents in foliar tissues of tomato plants was detected and could be explained by the need of plants to maintain high levels of these S compounds to counteract Se toxicity.[36,39] GSH is, in fact, one of the principal antioxidants of cells that plays a key role in cell defense and protection processes.[49,50] Given that total S levels in Se-treated plants were comparable to values measured in the control plants, increased levels of Cys for the synthesis of GSH at high doses of Se may have derived from enhanced degradation of unneeded or damaged proteins.

Other nonenzymatic substances with known antioxidant properties are phenolic compounds, which plants need for pigmentation, growth, and reproduction.[51,52] Phenols also act as signaling agents and metal chelators and are involved in the resistance to pathogens and abiotic stress.[52]

The alteration of these phytochemicals in response to selenate application has been reported in some plant species such as broccoli, lentils, and lettuce.[34−36] In our study, a linear correlation between the content of phenolic compounds and the dose of Se supplied to plants was not observed. Rather, a differential accumulation trend of individual phenolic acids and flavonoids depending on the dose of Se given to plants and on the time of plant exposure to this element was evident.

Our findings indicate that Se at low doses can efficiently stimulate the phenylpropanoid metabolic pathway. Indeed, in the short time (24 h) many phenolic acids were synthesized at elevated levels in leaves of plants supplied with 10 μM Se, whereas at 5 days enhanced accumulation of these compounds was detected in leaves of plants cultivated with 5 μM Se. Furthermore, Se at such doses stimulated the production of several flavonoids.

It is noteworthy that the levels of total phenolic compounds produced upon low Se treatment were apparently not deleterious to plants, as confirmed by the lack of evident toxicity symptoms. Higher phenol accumulation may result also in enhanced capacity of plants to overcome stress conditions, as reported by other authors.[53,54] Rutin, in particular, is known to protect plants against biotic stress and was significantly accumulated in tomato plants, especially upon short-term (24 h) Se treatment.

On the other hand, Se at low doses reduced the concentration of some micronutrients in tomato plants, especially at the root level. The mechanisms that cause this reduction are still not elucidated and need further investigation. In the case of Mo, it is noteworthy that the total content of this element in plants did not change in response to Se treatment, but Se stimulated Mo reallocation from the root to the shoot. Due to the chemical similarity of selenate and molybdate, the main form of Mo taken up by plants, translocation of selenate

from the root to the shoot may have favored the transfer of Mo, concomitantly.

In soil experiments, the application of Se to plants through foliar spray allowed the transfer of this element to the fruits at amounts low enough to be considered safe and nutritious for consumers. As the concentrations of Se in fruits reported on a fresh weight basis were 19 and 256 mg kg^{-1} at the 2 and 20 mg Se doses, respectively, the ingestion of two to six fruits obtained from plants treated with 20 mg of Se could be recommended to satisfy the RDA of 55−200 μg of Se per day.

The increase of Se in the soil after plant treatment with the dose of 20 mg of Se was indicative of the capacity of tomato plants to transport this element from the leaves to roots, and from the roots to the soil, in small amounts. It is known from the literature that Se can be released from the roots to the rizosphere in the form of dimethylselenide (DMSe).[29] Furthermore, due to the high chemical similarity shared by Se and S, it cannot be excluded that part of Se in the soil was due to the efflux of selenate from the root cells into the rizosphere through sulfate transporters. Selenium treatment caused a reduction in plant yield in terms of number of fruits produced. However, the size and weight of fruits were not substantially affected, and neither was the level of the main carotenoids. The most interesting effects of Se on secondary metabolites were observed in the fruit peel, as the phenylpropanoid metabolism was mainly shifted toward the synthesis of flavonoids to the detriment of phenolic acids.

Naringenin chalcone was the flavonoid that showed the maximum increase in content in fruit peels in response to both Se doses (2 and 20 mg Se), whereas quercetin aglycone was abundantly present only in plants treated with 20 mg of Se. In plants these flavonoids are strongly synthesized under stress conditions including UV light and bacterial or fungal infection.[55,56] Previous studies have highlighted a role for naringenin chalcone in the suppression of allergic responses in humans,[57,58] as well as the involvement of quercetin aglycone in the prevention of the onset of allergic chronic diseases.[59] Naringenin chalcone is also recognized as a potent compound that improves adipocyte metabolic functions and exerts insulin-sensitizing effects by activating an adiponectin-related pathway.[60]

We conclude that supplementation of Se at low doses to tomato plants cultivated hydroponically exerted beneficial effects on the plants with respect to growth and biosynthesis of antioxidant compounds implied in plant development and responses to stresses. When Se was furnished to tomato plants through a foliar spray, the fruits were enriched in Se, as well as in some compounds that display important nutraceutical properties. The enrichment of tomato fruits with naringenin chalcone and quercetin, in particular, may represent an important result due to the known therapeutic potential of these compounds in overweight/obese and allergic patients. However, it must be recognized that Se affected the level of phenolic acids in peels, especially cinnamic acid derivatives, which exert other important antioxidant effects on health.

Therefore, our findings on the whole provide insights for the utilization of *S. lycopersicon* as a potential functional food candidate in Se fertilization programs, but also suggest that the impact of Se on phenolic acid production in the fruits should be carefully considered.

■ ASSOCIATED CONTENT

Supporting Information

Additional tables and figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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