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THE EFFECTS OF SELENIUM ON *LYCOPERSICON ESCULENTUM* MILL. SEEDLINGS

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Abstract

This study was planned to contribute the views examining the physiological mechanisms of selenium-induced growth reductions with regard to selenium and plant nutrient interactions. Growth inhibition in the seedlings and the changes in nutrient compositions of epidermal cells with administration of increasing concentrations of selenium were investigated at the initial growth stage, which is the most stress-sensitive stage, of plant. The effects of selenium on *Lycopersicon esculentum* Mill. seedlings were investigated by EDX (Energy Dispersive X-Ray Microanalysis) analysis of the regions, approximately 450 μm x 500 μm in size, with use of low-vacuum (~ 24 Pascal) Scanning Electron Microscope, and SEM images were obtained. Increasing concentrations of selenium in the nutrient solution led to decreased cell growth particularly in the hypocotyl and roots, and to increased selenium content in the cells. The growth inhibitions were found at the selenium concentrations of ≥ 100 ppm. Development of the glandular hairs in the hypocotyl epidermal system was significantly reduced with the administration of ≥ 200 ppm of selenium. Development of the absorbing hairs in the roots was decreased in parallel with the increasing selenium concentrations in the nutrient solution; and because the development of the root is often limited to only to the development of radicula at the selenium concentrations of 500 and 1000 ppm, no absorbing hairs was found in these roots. Some macro- and micronutrient contents of radicle and hypocotyl epidermal cells were changed in response to selenium toxicity. In conclusion, ≥ 200 ppm of selenium administered in the form of SeO_2 was certainly toxic for the initial growth period of *Lycopersicon esculentum* cv. H-2274.

Introduction

The chemical form of selenium in the soil has been reported to be mainly controlled by the redox potential and pH of the soil (Mayland *et al*., 1990). There are various factors also affecting the uptake and accumulation of selenium in the plants (Kacar, 2009). Alkaline soil pH is known to increase the selenium uptake by plants (Chaney, 1994). It has been reported that "the selenium concentration of the plants is associated with the sulfur content of plants and with the total and available selenium, $CaCO_3$, silt and clay contents of the soils and that the total and available selenium contents of the soils depend mainly on the salinity, $CaCO_3$, sand and silt contents of the soil" (Dhillon *et al*., 1992). In another study on *Lotus corniculatus*, *Trifolium subterraneum*, *Taraxacum densleonis*, *Helminthia echioides* and *Trifolium repens,* it has been reported that the selenium concentrations of the plants can be as high as 6 ppm in kaolinite textured soils, whereas it is 0.05 ppm in sandy and clay soils and varies between 1.18 and 0.01 ppm in calcareous soils (Arvy, 1992).

According to an opinion: ''growth tips and the roots of plants has the highest selenium content and the distribution and concentration of this element in various plant organs may vary with the plant species, growth stage and the amount of selenium added to the soil'' (Aller *et al*., 1990). It has been reported that the major difference between the high and low selenium-containing genotypes of the seedlings of *Oryza sativa* at the early growth stage results from the differences in the rate of selenium accumulation in the shoots, the differences in the selenium contents of shoots and the differences in the biomass of shoots (Zhang *et al*., 2006). After the sowing, the selenium has been administered during the various growth stages of cereals of *Triticum aestivum* and the highest concentrations of selenium in plant were achieved with administering the selenium during stem elongation or heading (Govasmark *et al*., 2008). On the other hand, selenium uptake and accumulation by *Festuca arundinacea* has been depended largely on plant dry weight production (Tennant & Wu, 1999).

Certain plant species grown in soils with high selenium content and called as ''indicator species'' have been found to accumulate 10 times more selenium than other plant species (Poole *et al*., 1989). For instance, the seeds of certain species of *Astragalus* and *Stanleya pinnata* have been reported to accumulate selenium by up to 10% of their dry weights (Peterson, 1993). The X-ray absorption spectroscopy analysis of *Populus tremula* x *Populus alba* hybrid has revealed that selenate is slowly but selenite is rapidly metabolized and converted into organic selenium in the plant (Pilon-Smits *et al*., 1998). Selenium accumulation in the shoots and roots of *Zea mays* has been found to exhibit dramatic increases with the increasing selenium concentrations in the solution (Huang *et al*., 2007), and it has been reported in another study that the foliar applied selenium increased the selenium content of *Oryza sativa* by 194.1% (Fang *et al*., 2008).

Selenium is known as a toxic element to the plants, particularly when applied in high concentrations (Semiz, 1984). According to an opinion: ''selenium is mostly antagonist of nitrogen, phosphorus, sulfur, manganese, zinc, copper, iron and amino acids in plants'' (Aller *et al*., 1990). According to the same opinion: ''increased concentrations of selenium in plants is likely to increase in the heavy metal uptake by the plant'' (Aller *et al*., 1990). In a study with *Phaseolus vulgaris*, it has been found that administration of selenium led to increased lipoxygenase activity and lipid peroxide levels and to inhibition of antioxidant enzyme systems including catalase and superoxide dismutase (Padmaja *et al*., 1995). On the other hand, the researchers have also found decreased chlorophyll (a + b) content and increased cytochrome P-450 levels with the administration of

selenium (Padmaja *et al*., 1995). In that study, the inhibitory effect of selenium on the synthesis of chlorophyll has been reported to result from the inhibition of the components of antioxidant defense system as well as from the effects of selenium on lipoxygenase mediated lipid peroxide levels and the constituent biosynthetic enzymes (Padmaja *et al*., 1995). A similarly designed study on *Hydrilla verticillata* has also indicated a significant increase in the peroxidase activity with the administration of selenium (Byl *et al*., 1994). Wu and colleagues (1994) have investigated the effects of increasing concentrations of selenium on the activity of symbiotic nitrogen fixation in *Melilotus indica* and have found that 500 µg/g of selenium can be accumulated without a reduction in the growth rates of the plants and that larger quantities of selenium can be accumulated in the plants forming no nodules, with being more sensitive to the selenium toxicity (Wu *et al*., 1994). In a study of *Daucus carota*, selenium administration to the soil has led to decreased root yield and it has been found that while selenium administration to the soil promoted an increase in the synthesis of alpha-carotene and lutein and a decrease in beta-carotene content, total carotenoid content of the roots was slightly increased (Biacs *et al*., 1995). Munshi & colleagues (1990) have found that exposure to selenium led to significantly higher protein content in the tubers of *Solanum tuberosum* than controls, however administration of selenium at a concentration of more than 11.2 kg/ha did not increase the protein content, but non-protein nitrogen content in the tubers was significantly decreased (Munshi *et al*., 1990). As seen, different aspects of the physiological basis of selenium toxicity have been examined in previous studies on certain plant species. On the other hand, we aimed to examine the effects of selenium on some macro- and micro nutritional element uptake from the nutrient solution by seedlings of 17-day old *Lycopersicon esculentum* Mill. and on mobilization of plant nutrients in the root and hypocotyls by using micro-analytical studies performed at the level of epidermal cells. In other words, we examined the growth inhibition observed by the SEM micrographs of the components of *L. esculentum* seedlings resulting from administration of increasing concentrations of selenium in terms of changes in the plant nutritional element composition in the epidermal cells. The earlier investigators using the same or similar study design on certain plant species have often conducted their study at the level of complete plant or organ. We anticipate that the findings of this study performed at the tissue level and during the initial growth stage -known as the most stress-sensitive growth stage- for *L. esculentum*, which is often preferred to use in the studies of plant physiology and plant biotechnology and is almost considered as a "model plant" (Tanrısever, 1993), may contribute to explain the interactions between selenium and nutritional elements and the physiological mechanisms of selenium-induced growth inhibition in plants.

Materials and Methods

The research material of this study was a culture variety of *Lycopersicon esculentum* Mill. belonging to the *Solanaceae* family. The seeds of *Lycopersicon esculentum* Mill. cv. H-2274 (tomato) constituting our research material were obtained from Eskisehir Anatolia Agricultural Research Institute (Turkey).

As is known, in the today's agriculture, cultivated plants are usually exposed to various biotic and abiotic stresses mostly as a result of agricultural applications (Anwar *et al*., 2011; Shakeel & Mansoor, 2012). The fertility of one of the world's most widely cultivated plants, *Lycopersicon esculentum*, is determined by a large number of genetic and environmental factors and has been reported that this plant, particularly the young seedlings, is also sensitive to various abiotic stresses (Ali *et al*., 2011; Zdravkovic *et al*., 2011).

At the beginning of the study, the plant seeds were subjected to serial superficial sterilization procedures with exposing to 96% ethyl alcohol for 1 minute and then to 5% sodium hypochlorite for 35 minutes. The surface of all the testa was ensured to be in contact with the sterilant solution. Then, they were cleared of sodium hypochlorite by passing through sterile distilled water baths. Following the sterilization procedures, 100 seeds were sown to each sterile Petri dishes containing sterile filter paper inside in sterile conditions. For sterilization and sowing, the techniques recommended for standard tissue cultures were used (Basaran, 1990; Babaoglu *et al*., 2001).

The macro- and micronutrients of Murashige and Skoog medium (Murashige & Skoog, 1962) were used as the nutrient solution. Selenium was concurrently administered to the plant seeds incubated in petri dishes containing Murashige and Skoog medium (Murashige & Skoog, 1962) in the form of SeO_2: Selenium (IV) oxide, and at 6 different concentrations (1, 50, 100, 200, 500 and 1000 ppm).

After completing the sterilization and sowing processes, the plant seeds were incubated in a culture room under 16/8 h light/dark photoperiod and at 25 +/- 2^{o}C for 17 days. At the end of the incubation period, the root, hypocotyl and cotyledon upper and lower epidermal cells of the seedlings isolated from the nutrient solutions were analyzed for their carbon, nitrogen, oxygen, sulfur, phosphorus, potassium, calcium, magnesium, iron, copper, zinc, manganese, chlorine, sodium, cobalt and selenium content by the EDX analysis (Energy Dispersive X-Ray Microanalysis) performed on a local region of approximately 450 µm x 500 µm in size with using low vacuum (~ 24 Pascal) Scanning Electron Microscope (Jeol_JSM5600LV.Pioneer).

Results

In our study, there was no significant anatomical change in hypocotyl epidermal cells of the seedlings of *L. esculentum* cv. H-2274 incubated in the nutrient solutions containing 1-50 ppm selenium. However, growth inhibition was recorded in the cells from the concentration of 100 ppm of selenium and it was increased markedly from the concentration of 200 ppm. Development of glandular hairs in the hypocotyl epidermal system was decreased particularly from the concentration of 200 ppm. The general morphological appearance of cells was also changed from the concentration of 200 ppm of selenium. There was a significant decrease in longitudinal growth and a slight increase in transverse growth of the cells, which were more remarkable with the administration of 500 and 1000 ppm of selenium (Figs. 1-6). It should be noted that toxic concentrations of selenium resulted in collapse of the hypocotyl epidermal cells and deformation of the root tips in some seedlings. The development of cotyledon (positivity of cotyledon opening frequency) in the seedlings was also reduced significantly from the concentration of 200 ppm of selenium.

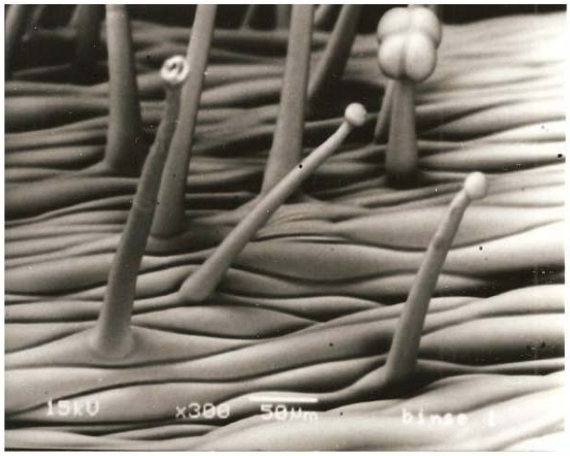

Fig. 1. SEM image of the hypocotyl epidermal cells of *L. esculentum* cv. H-2274 seedlings incubated in the nutrient solution containing 1 ppm of selenium.

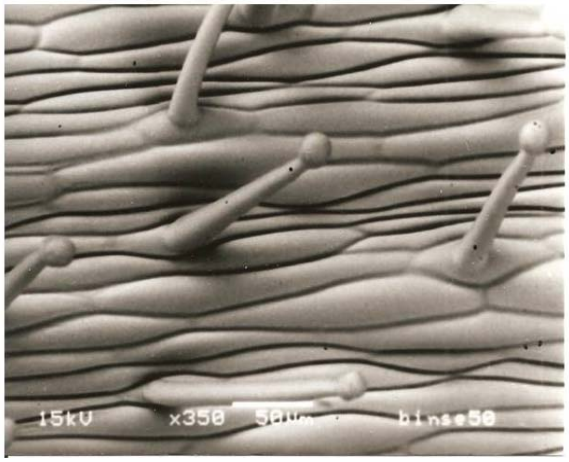

Fig. 2. SEM image of the hypocotyl epidermal cells of *L. esculentum* cv. H-2274 seedlings incubated in the nutrient solution containing 50 ppm of selenium.

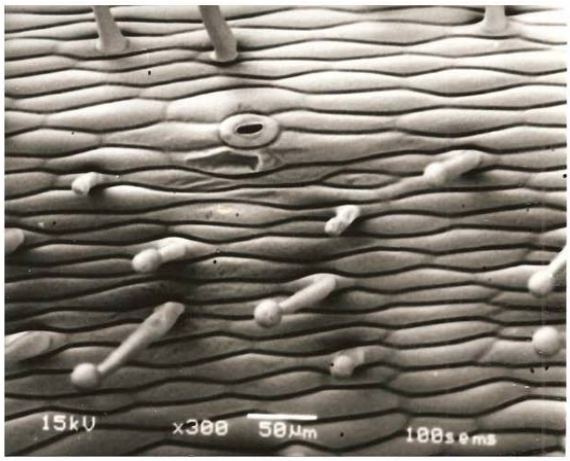

Fig. 3. SEM image of the hypocotyl epidermal cells of *L. esculentum* cv. H-2274 seedlings incubated in the nutrient solution containing 100 ppm of selenium.

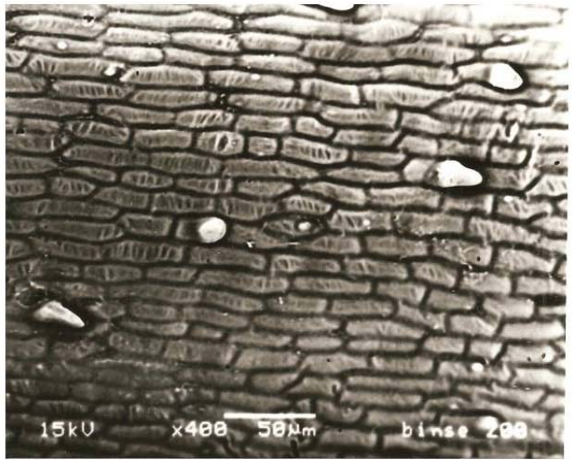

Fig. 4. SEM image of the hypocotyl epidermal cells of *L. esculentum* cv. H-2274 seedlings incubated in the nutrient solution containing 200 ppm of selenium.

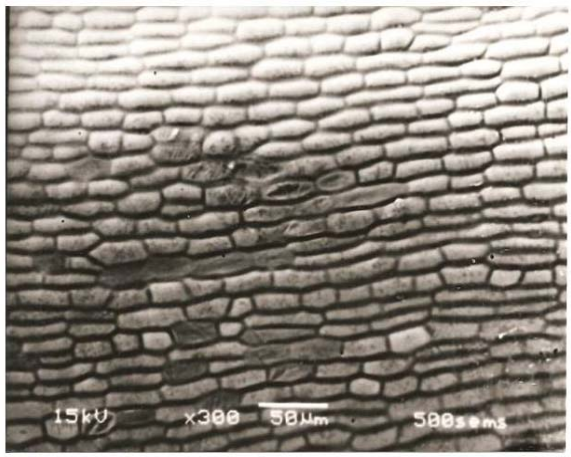

Fig. 5. SEM image of the hypocotyl epidermal cells of *L. esculentum* cv. H-2274 seedlings incubated in the nutrient solution containing 500 ppm of selenium.

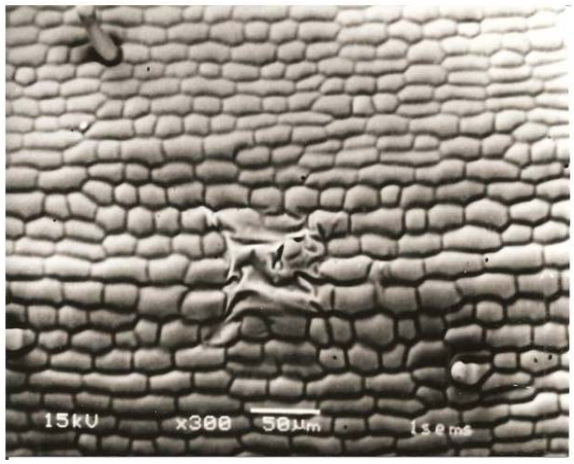

Fig. 6. SEM image of the hypocotyl epidermal cells of *L. esculentum* cv. H-2274 seedlings incubated in the nutrient solution containing 1000 ppm of selenium.

During the incubation period, the density of absorbent hairs was excessive in the roots of seedlings incubated in Murashige-Skoog nutrient solutions without administration of selenium (Fig. 7), and it -although not significantly- began to decline from the concentration of 1 ppm of selenium (Figs. 8 and 9). On the other hand, nodule-like abnormal morphology was found in the root epidermal system of the seedlings incubated in only 1 ppm of selenium for 17 days without Murashige-Skoog nutrient solution. Because the root development was often only limited to the radicle development from the concentration of 500 ppm and particularly of 1000 ppm of selenium, no absorbent hair was found in these roots (Figs. 10 and 11).

Selenium was undetectable in the root epidermal cells of *L. esculentum* cv. H-2274 seedlings after an incubation period of 17 days in the nutrient solution containing 1 ppm of selenium. On the other hand, selenium was detectable in the cells with the administration of 100 and 200 ppm of selenium (% elemental weights: 0.05 and 0.10, respectively); administration of 500 ppm of selenium to the nutrient solution led to a remarkable increase in the selenium content of root epidermal cells (% elemental weight: 0.64). However, 1000 ppm of selenium resulted in a lower selenium content (% elemental weight: 0.46) in the cells than 500 ppm of selenium. While selenium was undetectable in hypocotyl epidermal cells after administration of 1, 50 and 100 ppm of selenium to the nutrient solution, the cells began to accumulate selenium from administration of 200 ppm of selenium (% elemental weight after administration of 200, 500 and 1000 ppm of selenium: 0.276, 0.36 and 0.09, respectively). Selenium was undetectable in the cotyledon upper and lower epidermal cells after an incubation period of 17 days in the nutrient solution containing 1 ppm of selenium (Figs. 12 and 13). In the case of administration of 50 ppm of selenium, % elemental weight was 0.04 for the cotyledon upper epidermal cells and 0.06 for the cotyledon lower epidermal cells.

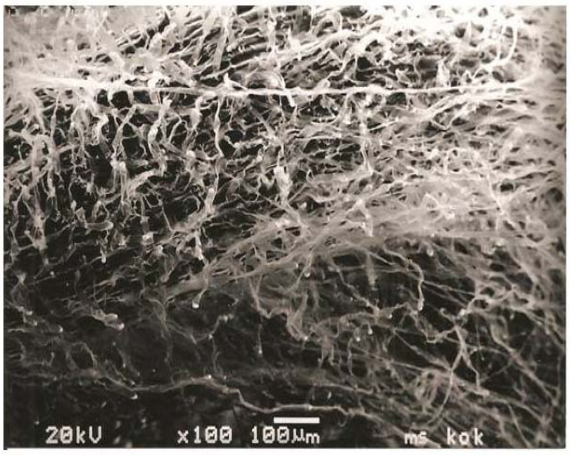

Fig. 7. SEM image of the absorbent hairs in *L. esculentum* cv. H-2274 seedlings incubated in Murashige-Skoog nutrient solution.

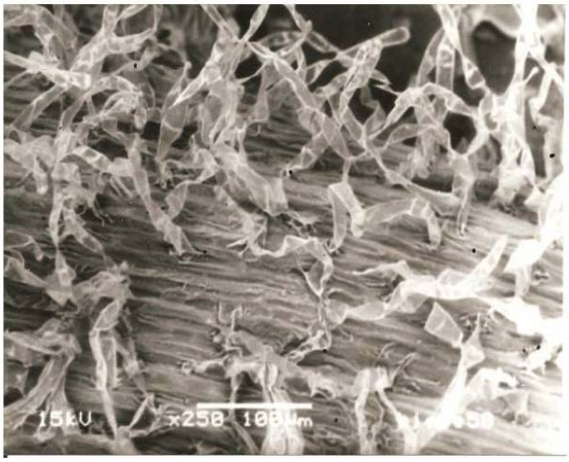

Fig. 8. SEM image of the roots of *L. esculentum* cv. H-2274 seedlings incubated in nutrient solution containing 50 ppm of selenium.

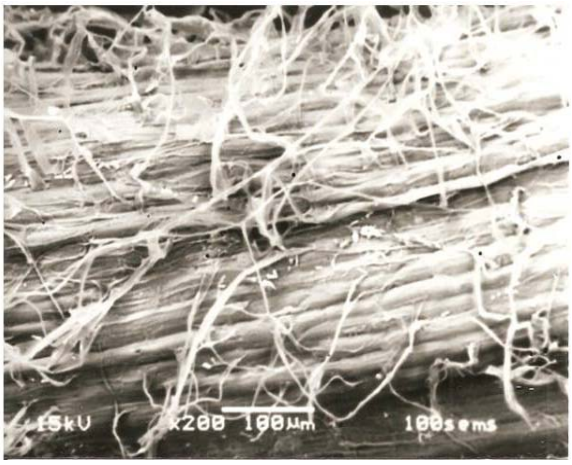

Fig. 9. SEM image of the roots of *L. esculentum* cv. H-2274 seedlings incubated in nutrient solution containing 100 ppm of selenium.

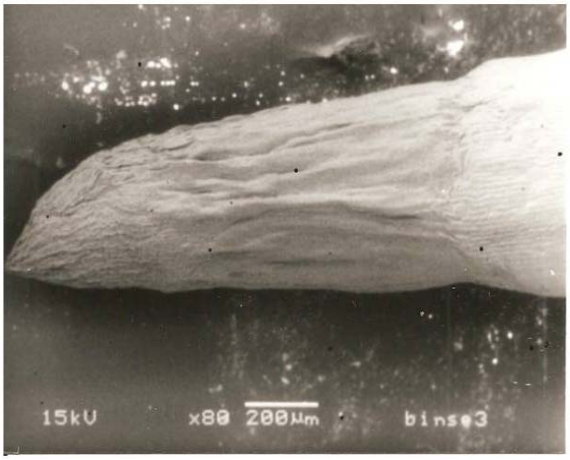

Fig. 10. SEM image of the roots of *L. esculentum* cv. H-2274 seedlings incubated in nutrient solution containing 1000 ppm of selenium.

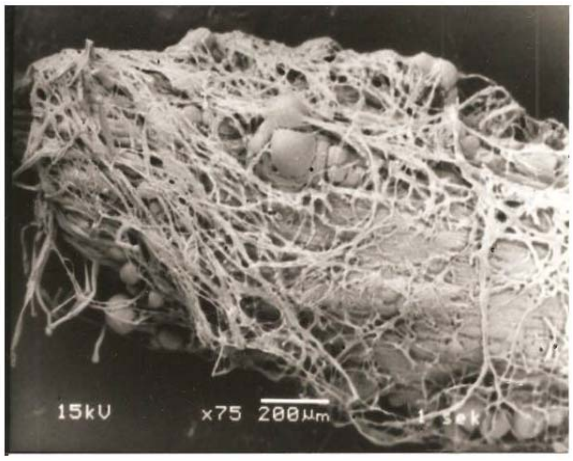

Fig. 11. SEM image of the roots of *L. esculentum* cv. H-2274 seedlings grown in incubation medium containing 1 ppm of selenium.

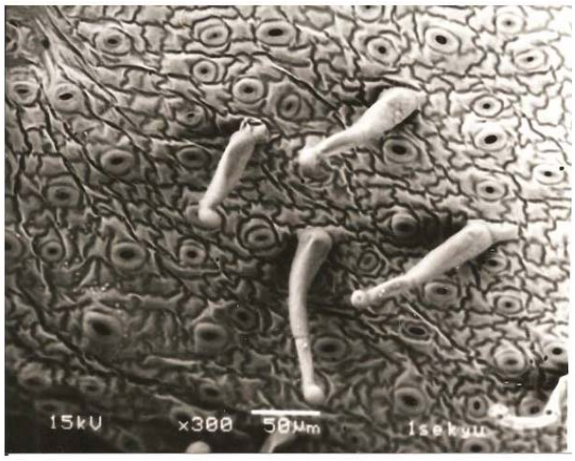

Fig. 12. SEM image of cotyledon upper epidermal cells of *L. esculentum* cv. H-2274 seedlings incubated in nutrient solution containing 1 ppm of selenium.

Fig. 13. SEM image of cotyledon lower epidermal cells of *L. esculentum* cv. H-2274 seedlings incubated in nutrient solution containing 1 ppm of selenium.

In present study, some macro- and micronutrient contents of the root and hypocotyl epidermal cells were determined after administration of the highest (1000 ppm) and lowest (1 ppm) concentrations of selenium, so that resultant decline in cell growth and changes in morphology of the cells were sought an answer in terms of the changes in nutrient uptake and accumulation. Although they are not considered as a nutrient for *L. esculentum,* but because they are present in Murashige and Skoog medium, sodium and cobalt were also included to the analysis.

In the root epidermal cells of the seedlings incubated in nutrient solutions containing 1000 ppm of selenium, carbon content was found to be significantly increased. The same epidermal cells also had increased sulfur, phosphorus, magnesium, manganese, chlorine and cobalt content. However, administration of toxic concentration of selenium led to decreased nitrogen, oxygen, potassium, calcium, sodium, iron, copper and zinc content in the root epidermal cells (Table 1).

The carbon content of the hypocotyl epidermal cells of the seedlings tended to increase with the administration of 1000 ppm of selenium. Oxygen, magnesium, sulfur, phosphorus, copper and zinc content of these cells, which were significantly affected from the selenium toxicity, were also increased. However, the same cells had lower nitrogen, potassium, calcium, manganese, iron, chlorine and sodium content than the control group (Table 1).

In this study, magnesium content of lower epidermal cells of cotyledons did not change. The most significant change in the lower epidermal cells of cotyledons with the increased concentration of selenium in nutritional solutions was the decrease in nitrogen content. The oxygen and potassium contents of the cells tended to increase, while there was no significant change for other macro-nutrients. No significant change was also observed for the micro-nutrients, zinc and chlorine. Although 1ppm of selenium resulted in manganese and iron elements at a level of 0.06% and 0.05% respectively, they were not detected in lower epidermal cells of the cotyledons of the seedlings with the administration of 50 ppm of selenium (Table 2).

Discussion

In a study conducted to identify the differential accumulation of selenium in the different parts of *Hordeum vulgare,* the highest selenium content has been found in the root tissues, and moderate levels has been reported in grains and lowest levels in the stem tissue (Ilbas *et al*., 2012). Another study examining the selenium uptake by 8-16 day old young seedlings of *Hordeum vulgare* found approximately 10-fold higher uptake and catabolic rates in the roots than grains (Huang & Clausen, 1994).

In a study of various genotypes of *Lycopersicon lycopersicum*, *Lycopersicon pennellii* and *Lycopersicon peruvianum*, although selenium has been easily transferred from shoots to the fruits, the edible parts of plants included much less total selenium than the inedible plant parts (Pezzarossa *et al*., 1999). In a study conducted with *Phaseolus vulgaris,* the highest selenium content has been found in the roots, followed by stems and leaves (Wallace *et al*., 1980). The researchers examining organic selenium

uptake by *Astragalus bisulcatus* and *Pascopyrum smithii* has reported that the concentration of selenium in the shoots is proportional to that in the nutrient solution when both plants were exposed to sodium selenite and selenocystine and when *Pascopyrum smithii* was exposed to selenomethionine (Williams & Mayland, 1992). The researchers also determined the shoot/root selenium rates for sodium selenite, selenomethionine and selenocystine to be 1.2, 0.7 and 0.4 in *Astragalus bisulcatus* and 0.1, 0.5 and 0.1 in *Pascopyrum smithii* (Williams & Mayland, 1992). Selenium accumulation in a fast-paced life-cycled *Brassica oleracea* population has been found to vary between 551 and 1.916 μgg^{-1} in the leaf tissues, 267 and 1.165 μgg^{-1} in the stem tissues and 338 and 1.636 μgg^{-1} in the root tissues (Kopsell & Randle, 1999a). Accordingly, the highest concentrations of selenium have been found in the floret and leaf tissues of *Brassica oleracea* var. *capitata*, *Brassica oleracea* var. *botrytis*, *Brassica oleracea* var. *acephda* and *Beta vulgaris* var. *cicla* (Banuelos & Meek, 1989). While the highest concentrations of selenium have been found also in the leaves of *Medicago sativa*, *Trifolium pratense*, *Brassica oleracea* var. *italica*, *Brassica oleracea* var. *gemmifera* and *Brassica napobrassica,* the stems had the lowest concentrations (Gupta, 1991). In another study examining accumulation of selenium in the floret, leaves and stems of *Broccoli oleracea* varieties, the highest and lowest concentrations of selenium have been found in the floret and stem, respectively (Banuelos *et al*., 2003).

Table 1. The results of EDX analysis of the root and hypocotyl epidermal cells of the *Lycopersicon esculentum* cv. H-2274 seedlings grown in nutrient solutions containing increased concentrations of selenium.

Element	1 ppm Selenium				1000 ppm Selenium			
	Root		Hypocotyl		Root		Hypocotyl	
	% Atom	% Elemental weight	% Atom	% Elemental weight	% Atom	% Elemental weight	% Atom	% Elemental weight
C	55.08	48.76	29.30	24.64	67.24	60.85	30.21	25.16
N	18.31	18.90	29.36	28.79	11.77	12.42	22.21	21.57
O	25.92	30.57	41.12	46.05	20.07	24.19	47.28	52.45
Se	0.00	0.00	0.00	0.00	0.08	0.46	0.02	0.09
S	0.10	0.23	0.02	0.04	0.18	0.42	0.05	0.12
P	0.03	0.06	0.03	0.07	0.12	0.27	0.05	0.11
Na	0.11	0.18	0.05	0.07	0.08	0.14	0.04	0.06
Mg	0.04	0.08	0.01	0.01	0.09	0.17	0.02	0.04
K	0.15	0.44	0.03	0.07	0.10	0.29	0.02	0.06
Ca	0.11	0.34	0.03	0.08	0.05	0.15	0.01	0.02
Mn	0.00	0.00	0.01	0.03	0.00	0.01	0.00	0.00
Fe	0.01	0.02	0.01	0.03	0.00	0.00	0.00	0.00
Co	0.00	0.00	0.00	0.00	0.01	0.03	0.00	0.00
Cu	0.01	0.06	0.01	0.03	0.01	0.04	0.03	0.12
Zn	0.03	0.12	0.00	0.00	0.02	0.11	0.03	0.12
Cl	0.05	0.14	0.02	0.04	0.07	0.19	0.01	0.02

Table 2. The results of EDX analysis of the cotyledon lower epidermal cells of the *Lycopersicon esculentum* cv. H-2274 seedlings grown in nutrient solutions containing increased concentrations of selenium.

Element	1 ppm Selenium		50 ppm Selenium	
	% Atom	% Elemental weight	% Atom	% Elemental weight
C	22.06	17.83	22.71	18.17
N	18.99	17.90	13.44	12.55
O	58.44	62.91	63.05	67.21
Se	0.00	0.00	0.01	0.06
S	0.02	0.05	0.02	0.04
P	0.10	0.21	0.10	0.20
Na	0.05	0.08	0.14	0.21
Mg	0.05	0.08	0.05	0.08
K	0.04	0.10	0.06	0.17
Ca	0.04	0.12	0.04	0.11
Mn	0.02	0.06	0.00	0.00
Fe	0.01	0.05	0.00	0.00
Co	0.00	0.00	0.01	0.04
Zn	0.04	0.19	0.04	0.18
Cl	0.03	0.07	0.04	0.09

Although more selenium accumulation has been reported in the shoots than roots in a phytoremediation study of *Eichhornia crassipes* (Zhu *et al*., 1998), selenium content of the roots was much higher than shoots in a similar study conducted with *Cyperus alternifolius*, *Wedelia trilobata*, *Polygonum hydropiperoides*, *Pistia stratiotes*, *Hippuris vulgaris*, *Baumia rubiginosa*, *Myriophyllum brasiliense* and *Mimulus guttatus* (Qian *et al*., 1998). For *Astragalus bisulcatus* and *Stanleya pinnata,* a specific flow of selenium has been mentioned from roots to young leaves in the spring, from aging leaves to reproductive tissues in the summer and then again to the roots in the autumn (Galeas *et al*., 2007).

In present study, increasing concentrations of selenium in nutrient solutions led to reduced cell growth particularly in the hypocotyl and root and to increased content of selenium in the cells. Selenium element was undetectable in the root and cotyledon epidermal cells of the seedlings incubated in the nutrient solution containing 1 ppm of selenium as well as in the hypocotyl epidermal cells of the seedlings incubated in the nutrient solutions containing 1, 50 and 100 ppm of selenium. Selenium accumulation was detected in the root epidermal cells from the concentration of 100 ppm and in hypocotyl epidermal cells from the concentration of 200 ppm of selenium. Moreover, selenium accumulation was found to be much higher in the root epidermal cells than the hypocotyl epidermal cells.

In the study of Ilbas *et al*., (2012) the higher selenium accumulation in the root has been associated with the selenium form used. The authors suggested that "selenate (SeO_4) may be easily transferred from the roots to the shoot tissues, while selenite (SeO_3) often accumulates in the root tissues" (Ilbas *et al*., 2012). Accordingly, Smith and Watkinson (1984) have found that compared to the plants exposed to selenite, a greater proportion of the absorbed selenium could be transported to the shoots of plants exposed to selenate (Smith & Watkinson, 1984). Mikkeisen *et al*., (1987) showed that selenium was accumulated in higher concentrations in the shoots when supplied as Se (VI) and in the roots when supplied as Se (IV) (Mikkeisen *et al*., 1987). On the other hand, in the study of Zayed *et al*., (1998) the highest selenium concentrations in the shoots were recorded by applying of SeO_4 followed by L-selenomethionin and SeO_3, and in the roots by L-selenomethionin followed by SeO_3 and SeO_4 (Zayed *et al*., 1998). Selenium was supplied as the form of SeO_2 in our study and the main site of accumulation was found to be the roots of seedlings. When this finding was considered together with the results of the studies presented above, the effect of "selenium oxidation state" previously brought up by Mikkeisen and colleagues (1987) for accumulation and transport of the element in their study with *Medicago sativa* (Mikkeisen *et al*., 1987) can be a critical factor that should be considered to explain the accumulation at the level of epidermal cells in our study. Because the selenium at 4+ oxidation step in SeO_2: Selenium (IV) oxide compound is at the same oxidation step in SeO_3 anion but at 6+ oxidation step in SeO_4 anion.

The studies examining the effects of toxic concentrations of selenium on nutrient uptake by plants mainly point out the relation of selenium and sulfur. Some researchers have attributed this relationship to chemical and physical similarities between these two elements (Mikkelsen & Wan, 1990; Banuelos *et al*., 2003; Ilbas *et al*., 2012). In this context, the effects of various concentrations of sulfate in the incubation medium on selenium concentrations of plant tissues have been studied (Asher *et al*., 1977; Severson & Gough, 1992; Zayed & Terry, 1992; Wu *et al*., 1994; Bailey *et al*., 1995; Zayed *et al*., 1998; Pezzarossa *et al*., 1999). The results of a study of different genotypes of *Lycopersicon lycopersicum*, *Lycopersicon pennellii* and *Lycopersicon peruvianum* has revealed that selenium translocation from the roots to shoots was more affected than selenium uptake by the roots in the case of administration of high concentrations of sulfate (Pezzarossa *et al*., 1999). According to an opinion: "the concentration of sulfate in plant tissues increases with the increasing concentration of sulfate in the nutrient solutions, leading to the competition with selenate for the enzymes of the sulfur assimilation pathway and this internal competition often leads to a decrease in production of seleno-aminoacids, particularly of selenomethionin" (Zayed & Terry, 1992). In our study, sulfur content of *L. esculentum* seedlings was evaluated at the level of epidermal cells after applying of increasing concentrations of selenium.

In a study of 4 culture varieties of *Allium cepa,* selenium has been found to reduce total sulfur content in the bulbs of all the genotypes included in the study but to increase the percentage of total sulfur deposited as sulfate in 3 genotypes (Kopsell & Randle, 1999b). On the other hand, when the leaf samples of *Brassica oleracea* var. *botrytis* grown hydroponically with the increasing concentrations of selenium were analyzed for selenium and sulfate concentrations, selenium concentrations have been found to correlate negatively with the sulfate concentrations in the leaf tissue (Banuelos & Meek, 1989).

In our study, the applying of toxic concentrations of selenium led to increases in sulfur content of the root and hypocotyl epidermal cells. It was found that the percent elemental weight determined as 0.23 in the control root epidermal cells was increased to 0.42% in the root epidermal cells exposed to 1000 ppm of selenium; it was also increased from 0.04% in the control hypocotyl epidermal cells to 0.12% in the hypocotyl epidermal cells exposed to 1000 ppm of selenium. Accordingly, in the study conducted with *Brassica oleracea*, addition of selenium to the nutrient solutions has led to linear increments in sulfur accumulation in the leaves and stems of the plant (Kopsell & Randle, 1999a). In another study with *Lolium perenne* and *Trifolium repens,* the researchers have mentioned synergistic effects of applying of increasing concentrations of selenate on concentration of sulfur in the shoots (Smith & Watkinson, 1984). The synergistic interaction between sulfate and Se^{+6} in *Hordeum vulgare* and *Oryza sativa* incubated in increasing concentrations of Se^{+6} with low concentrations of sulfate has led to increased concentrations of sulfur in the shoots, while no synergism has been found with high concentrations of sulfate; Se^{+6} has been found to be less effective on sulfur concentrations of the plant roots (Mikkelsen & Wan, 1990). Increasing concentrations of selenium in the nutrient solution has found to lead

increased concentration and accumulation of sulfur in the shoots but not in the roots of *Zea mays* (Huang *et al*., 2007). Selenium and sulfur levels have showed a negative correlation in *Astragalus bisulcatus* and *Stanleya pinnata* and a positive correlation in *Astragalus sericoleucus, Oxytropis sericea* and *Thlaspi montanum* (Galeas *et al*., 2007). However, the effects of selenium on the sulfur content of *Hordeum vulgare* exposed to increasing concentrations of selenium under field conditions were not significant (Ilbas *et al*., 2012).

As it is seen, the increase we found in our analysis at the level of root and hypocotyl epidermal cells of the seedlings of *L. esculentum* has been also previously demonstrated in the studies conducted at the level of various organs by Smith & Watkinson (1984) on *Lolium perenne* and *Trifolium repens*, by Kopsell & Randle (1999a) on *Brassica oleracea* and by Galeas *et al*., (2007) on *Astragalus sericoleucus, Oxytropis sericea* and *Thlaspi montanum*. In the study of Galeas and colleagues (2007), the positive correlation between the levels of selenium and sulfur has been also explained as a feature of non-hyperaccumulator plant species.

Selenium has been reported to be mostly antagonistic to nitrogen, phosphorus, sulfur, manganese, zinc, copper, iron and cadmium in plants (Aller *et al*., 1990). In a study aimed to determine the physiological mechanism of selenium accumulation in *Festuca arundinacea,* the researchers have found genetic variations in selenium and salt tolerance (Wu & Huang, 1991), and it has been reported that selenium tolerance and salt tolerance are independent of each other and that show negative correlations with tissue selenium and salt concentrations (Wu & Huang, 1991). In a similar study of the same group of researchers, selenium assimilation and nutrient element uptake has been investigated in *Festuca arundinacea* and *Trifolium repens* and it has been found that administration of selenium led to increased calcium concentration and decreased phosphorus concentration in the plant tissues (Wu & Huang, 1992), and that increased tissue selenium concentrations were associated with increased amount of iron in *Trifolium repens* and also copper, manganese and zinc concentrations were increased in *Trifolium repens* under the conditions of severe growth inhibition (Wu & Huang, 1992). In a study with *Phaseolus vulgaris*, high levels of selenium has been reported to lead to decreased phosphorus, calcium, magnesium, manganese and potassium concentrations in the leaves and phosphorus and calcium concentrations in the roots; silicon and titanium levels have been found to be higher with the highest selenium level (Wallace *et al*., 1980). Tissue selenium concentration has been reported to have positive correlations with phosphorus, manganese, zinc, nickel, cobalt and cadmium levels in *Helminthia echioides* and with potassium, cobalt and molybdenum levels in *Trifolium repens* (Arvy, 1992), selenium absorption has been also found to be inhibited by iron, aluminum, nickel and arsenic (Arvy, 1992). The increased selenium content in the grains of all genotypes of *Hordeum vulgare* exposed to increased concentrations of selenium has resulted in decreased nitrogen content in the grains (Ilbas *et al*., 2012). When selenite and selenate supplied alone or in combination with cadmium in a study examining the effects of selenium on cadmium uptake and toxicity in *Triticum aestivum* and *Pisum sativum,* although selenite has increased cadmium concentration in the pea roots by up to 300%, selenate has led to increased cadmium content up to 50% in the shoots of wheat (Landberg & Greger, 1994). However, in another study on the effects of selenite and selenate on cadmium uptake by *Zea mays,* the researchers have reported that cadmium content in the roots and shoots was reduced by selenite and selenate and usually much more remarkable reductions have been found in the roots than shoots (Shanker *et al*., 1996). As seen in previous studies, interaction of selenium with other elements in the uptake and accumulation processes has been variable at the level of species and genotypes.

Germination of some of the *L. esculentum* seeds incubated in the nutrient solutions containing 500 and particularly 1000 ppm of selenium was found to remain only at the level of development of the radicle in our study. However, extremely reduced root and hypocotyl developments were recorded in some of the seeds incubated in nutrient solutions containing 1000 ppm of selenium. When percent element content of the epidermal cells of such extremely reduced roots was compared with the control values, it was found that administration of toxic concentrations of selenium led to decreased nitrogen, oxygen, potassium, calcium, sodium, iron, copper and zinc content in the epidermal cells. However, carbon, sulfur, phosphorus, magnesium, manganese and chlorine contents were increased in these cells. Toxic concentrations of selenium also led to cobalt accumulation in the root epidermal cells. Extremely reduced hypocotyl development was also recorded in some of the embryos with the addition of 1000 ppm of selenium to the culture nutrient solution. With regard to the elemental composition of these epidermal cells, the carbon, oxygen, sulfur, phosphorus, magnesium, copper and zinc contents were found to be increased. However, there was a remarkable decrease in nitrogen, potassium, calcium, manganese, iron, chlorine and sodium content of the cells, with being more pronounced particularly for the elements nitrogen and calcium. As a result, in our opinion, nutrition disorders induced by selenium-stress might lead to anomalous metabolic deviations in the vital processes mediated by these elements that are integral parts of many macromolecules and play an important role in enzyme and redox systems, which was resulted in the growth inhibition demonstrated by the SEM images of the cells.

Some researchers have suggested that ''the naturally occurring forms of the available selenium, SeO_3 and SeO_4'' (Zhang *et al*., 2006), ''are the most soluble and most mobile forms of selenium in the soil'' (Ilbas *et al*., 2012), therefore, ''plants mainly uptake the selenium as selenate or selenite ions but selenite is much more toxic to higher plants than selenate when it was provided in solution culture'' (Ilbas *et al*., 2012), and it has been also reported that ''the differences in the mode of absorption of selenite and selenate and in the chemical form and distribution of selenium found in the plant after the absorption may explain the disparity in their toxicity'' (Smith & Watkinson, 1984). Nielsen (1987) has also reported ''that selenium metabolism in plants depends on the oxidation state of the applied selenium''. Accordingly,

we can also suggest that the compound of SeO_2: Selenium (IV) oxide, which is at the same oxidation step with SeO_3 anion, is definitely phytotoxic to the seedlings of *Lycopersicon esculentum* cv. H-2274 at the selenium concentrations of 200 ppm or more. The effects of the selenium, in the form of SeO_2, on the seedlings were remarkable for both the roots and hypocotyls. Development of the cotyledons in the seedlings was also significantly reduced with the applying of selenium at the concentrations of 200 ppm or more. Although the underlying mechanisms were not investigated in this study, the changes in cell morphology recorded in the SEM images with the applying of selenium at the concentrations of 200 ppm or more also suggest that the selenium in the form of SeO_2 is certainly metabolized by these cells. In addition, selenate and selenite are not the sole selenium sources used in the studies of selenium phytotoxicity. In fact, certain organoselena compounds including 4-(1-bromoundecanoyl) selenamorpholine, 4-benzoyl selenamorpholine and selenamorpholine hydrochloride have been found to be more effective than sodium selenite for selenium uptake by *Raphanus sativus* (Wu *et al*., 2000). Therefore, a much less studied inorganic selenium source SeO_2: (Selenium (IV) oxide) compound was preferred to use in our study instead of selenate and selenite anions which were much more studied previously on different plant species.

Conclusion

In conclusion, ≥ 200 ppm of selenium administered in the form of SeO_2 was certainly toxic for the initial growth period of *Lycopersicon esculentum* cv. H-2274. It was concluded that the growth inhibition in the cells is directly related to the increasing concentrations of selenium in the cells and that the changes in nutrient compositions of the cells can be considered as an effective parameter to explain the physiological mechanisms of selenium-induced growth reductions.

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