### SUNFLOWER SEEDLINGS HYPERACCUMULATE SELENIUM

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Selenium (Se) is an essential element for animals and humans, but not plants. However, the capacity of some plants to accumulate and transform Se into bioactive compounds has important implications for human nutrition and health. In this study, sunflower (*Helianthus annuus*) and maize (*Zea mays*) seedlings were cultivated in soil to investigate the effect of different rates of sodium selenite (1–90 mg kg<sup>-1</sup> soil) and sodium selenate (1–30 mg kg<sup>-1</sup> soil) on absorption and translocation of Se and sulphur (S). Sodium selenate decreased growth of sunflower roots at all applied rates and of maize roots at the highest rate applied. In contrast, sodium selenite up to 30 mg kg<sup>-1</sup> for sunflower and 3 mg kg<sup>-1</sup> for maize resulted in increase in Se concentration in soil resulted in an increase in Se and a decrease in S accumulation in roots and shoots of both maize and sunflower. Selenium translocation from roots to shoot was higher in sunflower than maize. Root-to-shoot translocation of Se was 5 to 30 times greater in sunflower, as a Se-hyperaccumulator with up to 1.8 g kg<sup>-1</sup> in shoots (with no significant decrease in shoot biomass) can be a valuable plant in biofortification to improve animal/human nutrition, as well as in phytoremediation of contaminated sites to restore ecosystem services.

*Keywords:* Sodium selenite – sodium selenite – sunflower – maize – rhizobox

### INTRODUCTION

Selenium (Se) is a naturally occurring chemical element found almost everywhere on earth. Deficiency or excess of Se can cause adverse effects on organisms [1, 12]. In the last decades, its physiological importance as a micronutrient fundamental to animal and human health has been established [11]. Selenium deficiency increases the risk of oxidative damage and important associated pathologies, i.e. cancer and HIV [7, 27].

Although essentiality of Se has been confirmed for bacteria, animals and humans, experimental attempts to identify its role in plants have not led thus so to recognizing Se as plant nutrient. However, the available reports showed that Se improved plant

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growth [31], increased tolerance to biotic and abiotic stresses [18, 29, 44] and was benefitial for other physiological parameters [5, 42, 44].

Plant-based foods are the major dietary sources of Se in most countries in the world [35], and a mineral imbalance in the soil-plant system can lead to production of Se-deficient food with negative consequences for human and animal nutrition [15]. The awareness that the intake of Se by plants is the first step of Se entry into the human food chain could explain why biofortification with this element has received great attention. For instance, field treatments with Se or Se-rich compounds can be considered a safe, low-cost approach to achieving biofortification in Se-deficient areas [2, 4, 13, 20, 25, 32, 33].

Although Se occurs in soil at different valence states [particularly Se(IV), Se(VI), Se(II) and Se(0)], Se can be absorbed by plants in the form of selenite and selenate [19]. Se shows some chemical similarities with sulphur; therefore, they can be taken up by sulphate transporters and metabolized in the sulphur metabolic pathways [39].

Despite a substantial literature on Se uptake by crops, such as wheat [2, 8, 13, 25, 26], little attention was given to maize and sunflower, with only a few publications on Se uptake and assimilation in these plants [22]. Therefore, the aim of this study was to characterize the effects of two Se forms [sodium selenite (Na<sub>2</sub>SeO<sub>3</sub>; active form: Se<sup>IV</sup>) and sodium selenite (Na<sub>2</sub>SeO<sub>4</sub>; active form: Se<sup>VI</sup>)] on the shoot and root growth and accumulation of Se in sunflower and maize.

#### MATERIALS AND METHODS

Sodium selenite and sodium selenate were obtained from Sigma-Aldrich Ltd. (Poole, UK). Nitric acid (69% v/v ACS, VWR, Lutterworth, UK) hydrogen-peroxide (30% v/v, Suprapure grade), and rhodium (1,000 mg  $L^{-1}$ ) for internal standard were obtained from *Fluka* (Poole, UK). The selenium (1,000 mg  $L^{-1}$ ) reference solution for *ICP-MS* and *ICP-OES* calibration was supplied by Scharlau Chemie (Germany).

#### Soil

Calcareous Chernozem soil – a very dark mollic horizon (thick, brownish to blackish surface horizon with a significant accumulation of organic matter and high base saturation) [10] at the depth of 0–0.3 m was obtained from the Látókép Experimental Station (N: 47°33', E: 21°27', 113–118 m above the sea level), University of Debrecen, Hungary. The soil properties (Table 1) were essentially the same as previously described by Nagy et al. [28]. No additional fertilization was applied. Selenium was supplemented to the soil as aqueous solution (prepared with distilled water) of sodium selenite (Na<sub>2</sub>SeO<sub>3</sub>) or sodium selenate (Na<sub>2</sub>SeO<sub>4</sub>) in concentrations of 0 (control), 1, 3, 10 and 30 mg kg<sup>-1</sup> soil. In addition, sodium selenite concentration of 90 mg kg<sup>-1</sup> soil was also tested.

nemozem son used
5.71
6.58
loamy clay
2.0 g kg <sup>-1</sup>
35.4 g kg <sup>-1</sup>
8.04 mg kg <sup>-1</sup>
199 mg kg <sup>-1</sup>
451 mg kg <sup>-1</sup>
332 mg kg <sup>-1</sup>

*Table 1* Properties of calcareous Chernozem soil used

<sup>1</sup>AL: 0.1 mol dm<sup>-3</sup> ammonium-lactate and 0.1 mol dm<sup>-3</sup> acetic-acid.

#### Plant growth

Sunflower (*Helianthus annuus* L. cv. Arena PR) as a dicotyledonous and maize (*Zea mays* L. cv. Norma SC) as a monocotyledonous species were chosen for experiments. Sunflower and maize seeds were surface-sterilised using 6% (v/v)  $H_2O_2$  for 2 min, rinsed extensively with deionised water and were germinated geotropically between moist filter papers at 22 °C. Sunflower seedlings with 1.5- to 2.0-cm long hypocotyl and maize seedlings with 2.5- to 3.0-cm long coleoptile were placed into soil in rhizoboxes (length 24.5 cm, width 10.5 cm; depth 2 cm) to easily monitor root development. Sunflower and maize plants were grown in a controlled-climate room. Relative humidity was maintained between 65–75%, the light-dark cycle was 16–18 h at respective 25–20 °C temperatures, and light intensity was 300 mmol m<sup>-2</sup> s<sup>-1</sup> during daytime.

In order to ensure uniform water supply to plants, wet fluted filter papers were placed at the bottom of the rhizoboxes before the soil was added. After planting seedlings in the soil, the transparent front wall of the rhizoboxes was covered with black foil. The rhizoboxes were placed at the 45 degree angle to force root growth along the transparent front wall of the box, thus allowing convenient monitoring of roots. The rhizoboxes were watered to weight daily.

#### Sampling and measurements

Root length was measured daily using millimeter paper. The experiment was harvested 6 days (maize) or 7 days (sunflower) after planting, when the longest roots reached the bottom of the rhizobox. Experiments were carried out in triplicates (three rhizoboxes), and every rhizobox had three seedlings.

At the end of the experiment, shoots were separated from roots. These plant parts were dried at 70 °C until constant mass, then cooled to room temperature and weighed using an analytical scale (OHAUS, Switzerland). Dried samples (0.01, 0.5 or 1 g, depending on the sample amounts) were homogenized and digested in the HNO<sub>3</sub>-H<sub>2</sub>O<sub>2</sub> mixture [21]. Briefly, samples were kept in 1.5 or 10 mL concentrated HNO<sub>3</sub> (according to the sample weight) overnight, and then heated to 60 °C for 45 min in a LABOR MIM OE 718/A block digestion apparatus. Following this first digestion step, 0.3, 1.5 or 3 mL of H<sub>2</sub>O<sub>2</sub> (30% v/v) was added to the samples, and digestion continued at 120 °C for another 90 min. After cooling the samples to room temperature, volume was adjusted to 5, 25 or 50 mL with deionized water. Samples were then mixed by shaking and filtered through FILTRAK 388 filters.

#### Transport factor (TF) calculation

The capacity of sunflower and maize plants to transport and accumulate Se in roots and shoots was calculated using the transport factor (TF) as follows:  $TF = (Se \text{ concentration} \times dry \text{ matter})$  in shoot/[(Se concentration  $\times dry$  matter) in shoot+(Se concentration  $\times dry$  matter) in roots], expressed as percentage.

### Quantification of selenium and sulphur

Elemental analysis was carried out by inductively coupled plasma optical emission spectroscopy (ICP-OES) (Perkin Elmer OPTIMA 3300 DV, Bremen, Germany) for measurement of sulphur and inductively coupled plasma mass spectrometry (ICP-MS) (Thermo Elemental X7, Bremen, Germany) for measurement of selenium. The concentration of sulphur was determined because selenium has chemical properties similar to sulphur. The instrument settings and parameters were the same as described previously [34].

#### Statistical analysis

All data were statistically analyzed using *SPSS 19.0* software (2010). Standard deviation was calculated and analysis of variance (ANOVA) was performed on the data to determine the least significance difference (Tukey) between treatment means with the level of significance at  $P \le 0.05$ .

These rhizobox experiments were carried out in three independent and different times under identical conditions.

#### RESULTS

## Root growth responses of sunflower and maize to sodium selenite and sodium selenate

Sodium selenite enrichment caused an increase in the total root length of sunflower from average of 10.2 cm in the control to the maximum average of 22.1 in the 30 mg kg<sup>-1</sup> sodium selenite treatment after 7 days (data not shown). In maize, the average longest root (22.5 cm) was measured in the 3 mg kg<sup>-1</sup> sodium selenite treatment in comparison with the control (17 cm) after 6 days. In both sunflower and maize, only the rate of 90 mg kg<sup>-1</sup> sodium selenite inhibited significantly root elongation at the end of the experiment.

Sodium selenate enrichment caused severe and quick root growth inhibition. In sunflower, at 30 mg sodium selenite kg<sup>-1</sup>, inhibition (compared with control) was significant even after 1 day, and no increase in total root length occurred after day 3; hence, after 7 days, total root length was 5-fold greater in control than at the highest sodium selenate rate. In contrast, total root length of maize seedlings was slightly (non-significantly) longer at 1 mg kg<sup>-1</sup> sodium selenate than in the control after 6 days (data not shown). Moreover, maize total root length at the highest sodium selenate dose was 1/2 that in the control after 6 days (compared with only 1/5 of control in sunflower), suggesting greater resistance to sodium selenate in maize than sunflower.

Toxic doses of either sodium selenite at 90 mg kg<sup>-1</sup> or sodium selenate at 30 mg kg<sup>-1</sup> caused decreases in root radius and root hair formation in sunflower and maize (data not shown).

## Effect of different Se forms on root and shoot dry weights in sunflower and maize

There was a non-significant trend of increases in root and shoot mass with an increase in sodium selenite rates up to 30 mg kg<sup>-1</sup> in sunflower (Table 2) and up to 3 mg kg<sup>-1</sup> in maize roots (Table 3). In both sunflower and maize, there was a slight (non-significant) reduction in root and shoot mass in the treatment with the highest sodium selenite rate of 90 mg kg<sup>-1</sup> compared with the control (Tables 2, 3).

Increasing sodium selenate rates did not significantly alter sunflower shoot mass, but there was a significant decline (compared with control) in root mass even at the low sodium selenate rate of 3 mg kg<sup>-1</sup> soil (Table 2). In maize, there was no significant difference in root and shoot mass over the range of sodium selenate rates (0–30 mg kg<sup>-1</sup> soil) compared with the control (Table 3). In both sunflower and maize, rough comparisons suggested that sodium selenate was more toxic to root and shoot growth than sodium selenite.

Applied Se compounds	Dry weight of s	hoots (mg/plant)	Dry weight of roots (mg/plant)				
(mg kg <sup>-1</sup> soil)	sodium selenite	sodium selenate	sodium selenite sodium selen				
0	$44\pm 6^{a}$	$44\pm 6^a$	$24\pm8^{a}$	$24\pm8^a$			
1	47±3ª	$43\pm15^a$	24±4 <sup>a</sup>	19±3ª			
3	$50\pm15^a$	$42\pm16^a$	25±3ª	12±2 <sup>b</sup>			
10	$51\pm6^{a}$	$41\pm9^{a}$	$29\pm8^a$	$8\pm 2^b$			
30	$54\pm6^{a}$	$39\pm 6^a$	$29\pm5^a$	$7\pm2^{b}$			
90	35±2ª		20±4ª				

Table 2 Dry weight of sunflower shoots and roots (means  $\pm$  SD, n = 9) after 7 days of Se treatments

Significant differences among means in a column are indicated by different lower case letters based on the Tukey test ( $p \le 0.05$ ).

*Table 3* Dry weight of maize shoot and roots (means  $\pm$  SD, n = 9) after 6 days of Se treatment

Applied Se compounds	Dry weight of s	hoots (mg/plant)	Dry weight of roots (mg/plant)				
(mg kg <sup>-1</sup> soil)	sodium selenite	sodium selenate	sodium selenite sodium seler				
0.0	26±3ª	$26\pm 3^a$	46±2ª	$46\pm 2^a$			
1.0	$28\pm12^a$	$27\pm3^{a}$	$49\pm10^{a}$	$62\pm12^{a}$			
3.0	28±3ª	$24\pm5^{a}$	$61\pm10^a$	54±11ª			
10.0	24±1ª	$23\pm5^{a}$	46±13ª	$42\!\pm\!22^a$			
30.0	$21\pm 6^a$	14±2ª	43±3ª	$31 \pm 2^{a}$			
90.0	14±2ª		40±1ª				

Significant differences among means in a column are indicated by different lower case letters based on the Tukey test ( $p \le 0.05$ ).

# *Effect of different Se forms on Se and S accumulation in shoots and roots of sunflower and maize*

Selenium concentration and content in both roots and shoots significantly increased with increasing the rates of applied Se in either form in sunflower and maize (Tables 4, 5). This increase was greater in sunflower than maize at different concentrations of the two Se forms. Supply of sodium selenate resulted in higher tissue Se concentrations than the supply of selenite. Compared with maize, shoot Se concentration in sunflower was 2.2-fold higher in the sodium selenate treatment, and 6.3-fold higher in the sodium selenate treatment, accumulated greater total Se amounts than maize, and accumulated more Se in the sodium selenate than sodium selenate treatments.

Effects of	different Se forms	on Se and S concen to	trations in sunflow tal Se amounts per	er shoots and roots, shoot and root	, as well as the S	e transfer factor (Tl	() and
Applied Se compounds (mg kg <sup>-1</sup> soil)	Se concentration in shoots (mg kg <sup>-1</sup> )	Total Se amount per shoot (μg)	Se concentration in roots (mg kg <sup>-1</sup> )	Total Se amount per root (μg)	TF (%)	Total S concentration in shoots (g kg <sup>-1</sup> )	Total S concentration in roots (g kg <sup>-1</sup> )
Sodium selenite							
0	$7.04 \pm 0.28^{e}$	$0.31\pm0.03^{\rm e}$	$3.26 \pm 0.47^{d}$	$0.08\pm0.01^{ m e}$	$79 \pm 1.91^{a}$	$18\pm0.3^{e}$	$5.4 \pm 0.7^{b}$
1	8.11±0.71e	$0.39 \pm 0.01^{e}$	$4.52 \pm 0.93^{d}$	$0.11 \pm 0.01^{de}$	$78\pm1.34^{a}$	$28\pm3.9^{a}$	$7.0\pm0.5^a$
3	$13.3 \pm 1.0^{d}$	$0.66 \pm 0.01^{d}$	$11 \pm 1.0^d$	$0.28 \pm 0.13^{d}$	$70 \pm 1.19^{b}$	$26\pm0.1^{b}$	$5.5 \pm 0.3^{b}$
10	28.8±1.8c	$1.47 \pm 0.12^{\circ}$	$28.4 \pm 1.3^{\circ}$	$0.82 \pm 0.33^{\circ}$	$64\pm1.20^{\circ}$	25±2.8°	$4.2 \pm 0.1^{\circ}$
30	44.0±2.2 <sup>b</sup>	$2.37 \pm 0.21^{a}$	$116\pm15^{b}$	$3.36 \pm 0.23^{b}$	41±1.01 <sup>d</sup>	$21\pm 3^d$	$4.0\pm0.6^{\circ}$
06	$60.3 \pm 2.6^{a}$	$2.09 \pm 0.25^{b}$	$262\pm29^{a}$	$5.24 \pm 0.62^{a}$	$29\pm0.50^{e}$	$11 \pm 0.1^{f}$	$3.9 \pm 1.2^{\circ}$
Sodium selenate							
0	$7.04 \pm 0.28^{d}$	$0.31 \pm 0.01^{d}$	$3.26\pm0.47^{e}$	$0.08\pm0.00^{\rm e}$	$79 \pm 1.32^{d}$	$18 \pm 0.3^{b}$	$5.4\pm0.7^{a}$
1	$1203\pm138^{\circ}$	$51.2 \pm 0.6^{d}$	$141 \pm 32^d$	$2.69 \pm 0.28^{b}$	95±2.43°	$20\pm0.5^{a}$	$1.4 \pm 0.2^{b}$
3	$1210 \pm 182^{\circ}$	$50.8 \pm 0.1^{\circ}$	$151 \pm 39^{\circ}$	$1.86\pm0.85^{\rm c}$	$96 \pm 2.01^{b}$	$19\pm7^{b}$	$1.0 \pm 0.1^{b}$
10	$1580 \pm 11^{b}$	$64.6 \pm 0.3^{b}$	$215\pm60^{\mathrm{b}}$	$1.66 \pm 0.20^{d}$	$97\pm2.27^{a}$	$19\pm 3^{b}$	$0.1\pm0.1^d$
30	$1803\pm72^a$	$70.3 \pm 0.5^{a}$	$448\!\pm\!82^a$	$3.23\pm0.16^a$	$96 \pm 2.19^{b}$	$11 \pm 1.7^{c}$	$0.5\pm0.1^{\circ}$
Significant differences a	mong means in a col	lumn are indicated by	y different lower cas	e letters based on the	e Tukey test (p≤0	.05). Means±SD (n	= 9).

### SE hyperaccumulation in sunflower

Table 4

Acta Biologica Hungarica 69, 2018

and	Total S concentration in shoots (g kg <sup>-1</sup> )		$6.3\pm0.1^{b}$	$7.5 \pm 0.2^{a}$	$4.7 \pm 0.6^{\circ}$	$4.7\pm0.4^{\circ}$	$4.5 \pm 0.4^{\circ}$	$4.0\pm0.2^d$		$6.3\pm0.1^d$	$8.0\!\pm\!1.4^a$	$6.8\pm2.0^{b}$	$6.7 \pm 0.3^{\circ}$	$5.6\pm0.1^{e}$	(= 9).
ransfer factor (TF)	Total S concentration in shoots (g kg <sup>-1</sup> )		$9.4\pm0.8^{b}$	$10\pm0.5^a$	$9.1 \pm 1.1^{b}$	$6.6 \pm 0.5^{\circ}$	$5.3\pm0.1^d$	$4.2 \pm 0.1^{e}$		$9.5 \pm 0.8^{b}$	$10.7\pm2.0^a$	7.4±0.1°	$5.5 \pm 0.1^{d}$	$3.2 \pm 0.2^{e}$	.05). Means ± SD (n
s well as the Se t	TF (%)		$0 \pm 0.01^{f}$	$9\pm 1.12^{a}$	$5\pm 1.65^{\circ}$	$4\pm0.25^d$	$7 \pm 1.20^{b}$	$3\pm0.51^{e}$		$0\pm0.02^{e}$	$4\pm0.02^{d}$	$11 \pm 1.30^{\circ}$	$17 \pm 1.52^{b}$	$20\pm1.21^a$	e Tukey test (p≤0.
shoots and roots, a shoot and root	Total Se amount per root (μg)		$0.04 \pm 0.01^{f}$	$0.10 \pm 0.01^{e}$	$0.40 \pm 0.01^{d}$	$1.08 \pm 0.06^{\circ}$	$2.50 \pm 0.73^{b}$	$4.39 \pm 0.89^{a}$		$0.04 \pm 0.01^{e}$	$44.3 \pm 5.3^{d}$	$58.4 \pm 5.4^{b}$	$63.1\pm6.0^{a}$	$47.0 \pm 5.9^{\circ}$	e letters based on th
of different Se forms on Se and S concentrations in maize total Se amounts per	Se concentration in roots (mg kg <sup>-1</sup> )		$0.90 \pm 0.48^{e}$	$2.01 \pm 0.83^{e}$	$6.53 \pm 0.23^{d}$	$23.35 \pm 1.77^{\circ}$	58.00±9.19 <sup>b</sup>	$110 \pm 20^{a}$		$0.90 \pm 0.48^{e}$	$711 \pm 145^d$	$1081 \pm 32^{\circ}$	$1491 \pm 139^{b}$	$1521\pm75^a$	different lower case
	Total Se amount per shoot (µg)		$0.00 \pm 0.01^{d}$	$0.01\pm0.06^{\rm d}$	$0.02 \pm 0.01^{d}$	$0.05 \pm 0.01^{\circ}$	$0.19 \pm 0.07^{a}$	$0.13 \pm 0.01 a^{b}$		$0.00 \pm 0.01^{e}$	$1.79 \pm 0.02^{d}$	7.16±0.1°	$12.7\pm1.8^a$	$11.4 \pm 2.3^{b}$	umn are indicated by
	Se concentration in shoots (mg kg <sup>-1</sup> )		$0.11\pm0.00^{f}$	$0.47 \pm 0.12^{e}$	$0.86 \pm 0.08^{d}$	$2.11\pm0.84^{\circ}$	$8.96 \pm 2.79^{b}$	$9.47 \pm 2.47^{a}$		$0.11\pm0.00^{e}$	$67\pm12^d$	$302\pm 38^{\circ}$	$562\pm79^{b}$	$807\pm33^{a}$	nong means in a col
Effects o	Applied Se compounds (mg kg <sup>-1</sup> soil)	Sodium selenite	0.0	1.0	3.0	10.0	30.0	0.06	Sodium selenate	0.0	1.0	3.0	10.0	30.0	Significant differences ar

Table 5

Acta Biologica Hungarica 69, 2018

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The calculated value of the Se transfer factor (a proportion of total plant Se found in shoots) decreased significantly with increasing rate of sodium selenite applied, in both sunflower and maize (Tables 4, 5) but it remained relatively unaltered (sunflower, Table 4) or increased significantly (maize, Table 5) in the sodium selenate treatments. The Se transfer factor was greater in sunflower than maize regardless of the treatment, indicating a strong capacity of sunflower to transport Se from roots to shoots (Table 4). That strong Se-transporting capacity in sunflower was expressed to a greater extent in the sodium selenate than sodium selenite treatments.

The total S content in both plants' shoots and roots tended to decrease in response to both sodium selenite and sodium selenate increase; shoots had a higher amount of S than roots and this increase was significant in sunflower. However, the 1 mg kg<sup>-1</sup> sodium selenite and sodium selenate treatments resulted in an increase in S content compared to the control treatments in both plants. Furthermore, sunflower shoots had much higher S content than maize shoots, while maize roots had more S content than sunflower roots in both the sodium selenite and sodium selenite for the control treatments is both plants. Furthermore, sunflower shoots had much higher S content than maize shoots, while maize roots had more S content than sunflower roots in both the sodium selenite and sodium selenate treatments (Tables 4, 5).

#### DISCUSSION

Our results agree with those obtained in previous works, which reported that Se application at low concentration may positively influence plant growth. In a pot experiment, Se-treated potato plants produced higher tuber yields than the control plants, a result which was related to the antioxidative effect of Se in delaying senescence [41]. Similarly, in a hydroponic experiment, the Se treatment was associated with a 43% increase in *Brassica rapa* seed production, which was attributed to increased total respiratory activity in leaves and flowers [24]. Foliarly-applied Se also increased the respiratory potential in young chicory plants [14]. Addition of Se significantly enhanced the antioxidant activity, antioxidant level and maize grain yield when drought stress level was increased [36].

Singh et al. [38] were the first authors to report about the positive effect of Se on plant growth. They showed the application of 0.5 mg kg<sup>-1</sup> Se, as selenite stimulated the growth and dry matter yield of Indian mustard (*Brassica juncea* L.). They also found that Se applied in low concentrations can increase the growth and antioxidative volume of both mono- and dicotyledonous plants. Hartikainen et al. [17] demonstrated a positive response of lettuce (*Lactucasativa* L.) growth to Se, while Djanaguiraman et al. [7] obtained the same results in case of to soybean (*Glycine max* L.). At a higher supplementation level than 29 mg kg<sup>-1</sup> soil, Se inhibited the growth and germination of tomato, lettuce, and radish (*Raphanus sativus* L.) seeds [4]. Hence, Se has an effect on germination. But according to Hasanuzzaman et al. [16] the positive effect on germination was linked to antioxidative activity, and selenite improved germination of bitter gourd (*Momordica charantia* L.) seeds at sub-optimal temperatures [5]. Depending on the plant species, growth stage, and plant organs, Se uptake and metabolism will be different. Broccoli (*Brassica oleracea* var. *italica*) is

known for its ability to accumulate high levels of Se, with a greater number of the selenoamino acids in the form of Se-Met (SeMeSeCys) [23]. What we can conclude from all the results is as follows. The physiological importance of Se for higher plants could be evaluated within the following topics: anti-oxidative and pro-oxidative effects of Se and the role of Se under abiotic stresses.

Increasing concentrations of Se in a growth media can evoke an increase in Se content in crop plants [3]. In our experiments, the total Se concentrations in sunflower and maize increased in a dose-dependent manner after Se addition. It is well known that selenate is more easily transferred from the root to aboveground organs than selenite or organic Se, since much of selenite is retained in the root tissues, where it is rapidly transformed into organic Se compounds [45].

In nonhyperaccumulator species Se is thought to be accumulated nonspecifically by the sulfate transport system [30, 43]. There is overwhelming evidence that sulfate transporters also transport selenate: mutants with nonfunctional sulfate transporter genes show selenate resistance [37, 43] and overexpression of a sulfate transporter led to an increase in selenate uptake [40]. Also, Se competes with S for uptake into plant roots [46]. In nonhyperaccumulators the sulfate transporters have not been reported to have a preference for sulfate over selenate. Thus, their activity affects the uptake of Se and S alike, explaining the observed correlation between tissue Se and S levels. In hyperaccumulators one or more sulfate transporters may have evolved into Se-specific transporters, as suggested by the 100-fold higher Se–S ratio in these plants. The preferential uptake of Se appears to be at the cost of S, as suggested by the observed negative correlation between tissue S and Se levels. In this experiment, the high internal Se levels may be sensed by the plants as S, leading to the downregulation of the nonspecific S transporters, causing a decrease in S accumulation as plant Se accumulation via the Se specific transporter continues.

Finally in our study, the poor translocation of applied Se as sodium selenite from root to shoot was also found, since under sodium selenite exposure, plants accumulated great amounts of Se in their roots. The Se content in the shoots of sunflower was higher than that in the roots in all of the sodium selenate treatments, while this finding was not consistent for sodium selenate maize treatments.

#### CONCLUSIONS

The maximum Se concentration in sunflower shoots reached 1.8 g Se kg<sup>-1</sup> in the sodium selenate-treated soil; therefore, sunflower could be classified as a Se hyperaccumulator. Translocation of sodium selenate from roots to shoot was significantly greater in sunflower than maize. There was a direct relation between Se and S concentrations in roots and shoots of both maize and sunflower. Our future work should concentrate on growing sunflower in Se-enriched soils to test biofortification of sunflower grain and oil, which are important from the points of view of the economy and human health.

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