

## Assessment and comparison of selenium-enriched maize with sodium selenite and sodium selenate

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### SUMMARY

*Selenium is an element of environmental interest owing to the narrow range between its nutritionally required and toxic concentrations in many organisms. Its mobility and bioavailability differ greatly depending on individual Se species. In this regard, in present study, the uptake and distribution of Se, the changes in Se content, and the effects of different concentration of Se in two forms of sodium selenite and sodium selenate on maize plants were measured in nutrient solution experiments to clarify their response to the two forms of Se. The results revealed that the Se content in shoots and roots of maize plants significantly increased as the Se level increased. Two Se forms behaved differently and the effects of toxic damage in samples which had been treated with selenite were much more than in the selenate treatments.*

**Keywords:** maize, sodium selenite, sodium selenate

### ÖSSZEFOGLALÁS

*A szelénrel kapcsolatos kutatásokat elsősorban az indokolja, hogy számos élőlény esetén a táplálkozási szempontból szükséges és a toxikus koncentráció nagyon közel esik egymáshoz. A szelén mobilitása és biológiai hozzáférhetősége elsősorban az adott szelén speciestől (módsulattól) függ. A kutatásunk célja ebből adódóan a szelén felvételének és növényen belüli megoszlásának vizsgálata, illetve annak meghatározása, hogy a különböző szelénformák (nátrium-szelenit, nátrium-szelenát) hogyan hatnak a kukorica csiranövények szeléntartalmára tápoldatos kísérletben. Kísérletünk során azt tapasztaltunk, hogy a szelén kezelések hatására a kukorica hajtásának és gyökerének szelén koncentrációja szignifikánsan emelkedett. A két szelénforma hatásának tekintetében azonban eltérést figyeltünk meg, a toxikus hatás sokkal erőteljesebben jelentkezett szelénit kezelés esetén.*

**Kulcsszavak:** kukorica, nátrium-szelenit, nátrium-szelenát

### INTRODUCTION

Selenium (Se) is a naturally occurring trace element which is toxic at high concentrations, but it is also an essential element for many organisms (Fan et al. 2002). An optimal supply of Se is important because it can support protection against several diseases, like different type of cancers, prostate, lung, gastrointestinal (Clark et al. 1998, Della Rovere et al. 2006, Grau et al. 2006). Agronomic bio fortification can increase Se concentration in crops and hence dietary intake of Se (Euroola et al. 2004, Euroola 2005, Lyons et al. 2005, White and Broadley 2009, Broadley et al. 2010).

Se can occur in four oxidation states (elemental (Se<sup>0</sup>), selenite (Se<sup>4+</sup>), selenate (Se<sup>6+</sup>) and selenide (Se<sup>2-</sup>) (Canton and Van Derveer 1997). Due to the high solubility of selenite (Se<sup>IV</sup>) and selenate (Se<sup>VI</sup>), these forms are more available to plants (Carvalho and Martin 2001) and are important in bio geological and biochemical cycle of Se but since they exhibit different biochemical properties, their toxicity and energy consumption during uptake and metabolism are different (Shen et al. 1997, Weiller et al. 2004).

Despite substantial literature on Se uptake by plants and crops such as wheat, little consideration has been given to maize, low “Se-indicator” plant (Ježek et al. 2012). To date there have been few publications on Se uptake and assimilation in this plant. Therefore, in this study we selected maize (*Zea mays* L.) because it is widely used plant cultured throughout the world and is

important source of Se for human diet. Maize is non-Se-accumulator plant and the threshold Se toxicity concentration is dependent on the form of Se accumulated (Terry et al. 2000).

Then, we decided to enrich maize plants with Se in both forms of sodium selenite and sodium selenate as well as investigation and comparison of their uptake and distribution to two main parts of shoot and root.

### MATERIAL AND METHODS

#### Materials

Sodium selenite and sodium selenate were obtained from Sigma-Aldrich Ltd. (Poole, UK). Nitric acid (69% ACS, VWR, Lutter-worth, UK) hydrogen-peroxide (30%, Suprapure grade), and rhodium (1000 mg L<sup>-1</sup>) for internal standard were obtained from Fluka (Poole, UK). Selenium (1000 mg L<sup>-1</sup>) reference solution for ICP-MS calibration was supplied by Scharlau Chemie (Germany).

#### General plant propagation

Maize (*Zea mays* L. cv. Norma SC) as a monocotyledon plant was chosen for our research. Disinfected maize seeds were geotropically germinated between moist filter papers at 22 °C. Maize seedlings with 2.5–3.0 cm coleoptile were placed into aerated nutrient solution pots. Maize plants were grown in a climate

room under strictly regulated environmental conditions. Relative humidity was maintained between 65–75%, the light/dark cycle was 16/8 hrs. with a respective 25/20 °C temperature periodicity, and light intensity was kept at the constant  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$  during daytime.

#### Plant growth in nutrient solution

The nutrient solution used for plant growth had the following composition: 2.0 mM  $\text{Ca}(\text{NO}_3)_2$ , 0.7 mM  $\text{K}_2\text{SO}_4$ , 0.5 mM  $\text{MgSO}_4$ , 0.1 mM  $\text{KH}_2\text{PO}_4$ , 0.1 mM  $\text{KCl}$ , 0.1  $\mu\text{M}$   $\text{H}_3\text{BO}_3$ , 0.5  $\mu\text{M}$   $\text{MnSO}_4$ , 0.5  $\mu\text{M}$   $\text{ZnSO}_4$  and 0.2  $\mu\text{M}$   $\text{CuSO}_4$ . Iron was supplied in the form of  $10^{-4}$  M Fe-EDTA, too (Cakmak and Marschner 1990).

Selenium was supplemented to the nutrient solution as two species of selenite in the form of  $\text{Na}_2\text{SeO}_3$  and selenate in the form of  $\text{Na}_2\text{SeO}_4$  in five different concentrations, as follows: 0 (control), 0.1, 0.3, 0.9 and 3  $\text{mg L}^{-1}$ .

Nutrient solution was changed every 3 days and evaporated water was replenished regularly. The experiment ended 2 weeks after planting, when the third leaf of the control treatment had completely grown and seedlings had approximately 40–30 cm long shoots and roots respectively. Experiments were carried out in triplicates (three pots) that every pot had four seedlings.

#### Sample preparation

At the end of the experiment shoots were separated from roots. Plant parts were dried at 70 °C until constant weight was achieved, then cooled to room temperature and weighed using an analytical scale (OHAUS, Swiss). Dried samples (0.01, 0.5 or 1 g, depended on our samples' amount) were homogenized and digested by  $\text{HNO}_3$ - $\text{H}_2\text{O}_2$  treatment (Kovács et al. 1996). Briefly, samples were kept in 1, 5 or 10 ml concentrated  $\text{HNO}_3$  overnight, then heated to 60 °C for 45 min in a LABOR MIM OE 718/A block digestion apparatus. Following the first digestion step, 0.3, 1.5 or 3 ml 30%  $\text{H}_2\text{O}_2$  were added to the samples and digestion was 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.

#### Quantification of selenium

Total selenium content was measured on a Thermo Fisher Scientific model X-Series II inductively coupled plasma mass spectrometer (ICP-QMS) equipped with Hexapole Collision Cell Technology (CCT). For quantification of selenium content 1 ml of digested sample was diluted to 5 ml by the addition of 3.9 ml water and 0.1 ml 5 mg rhodium  $\text{l}^{-1}$  solution as an internal standard.

As collision/reaction gas 7% hydrogen 93% helium gas mixture was applied at a flow rate  $6 \text{ ml min}^{-1}$ . The sample introduction system consisted of a Meinhard type concentric nebulizer interfaced with a quartz

conical spray chamber with impact bead cooled down to 2 °C by Peltier chiller. Nickel sampler and skimmer cones were used with 1.0 mm and 0.7 mm orifice ID respectively. The sample solutions were pumped at a rate of  $0.5 \text{ ml min}^{-1}$  by a peristaltic pump from tubes arranged on a CETAC ASX 520 Model auto sampler (CETAC, Omaha, Nebraska, USA). Instrument was controlled by Plasma Lab (ver. 2.5.10.319, Thermo Fisher Scientific, Bremen, Germany) software.

The system operation e.g. ion lens voltage, torch position etc. were daily optimized with a multielement standard solution (Thermo Fisher Scientific, Bremen, Germany) ( $10 \mu\text{g L}^{-1}$ ) according to the standard daily optimization procedure recommended by the manufacturer. Optimization was performed with respect to the maximum ion intensity reaching  $>400\,000$  integrated counts per second for cobalt and uranium, and  $>800\,000$  for indium, also the oxides and doubly charged ion formation was minimized by monitoring on the  $^{156}\text{CeO}^+ / ^{140}\text{Ce}^+$  and  $^{138}\text{Ba}^{2+} / ^{138}\text{Ba}^+$ ; ratios which were kept below 1%. The ions were detected with a secondary electron multiplier operating in dual mode (pulse counting or analogue mode). Typical instrument settings were, RF power 1.4 kW, plasma gas flow rate  $14.0 \text{ L min}^{-1}$ , auxiliary gas flow rate  $1.00 \text{ L min}^{-1}$  and nebulizer gas flow rate of  $0.90 \text{ L min}^{-1}$ . Signals were measured using 100 ms dwell time and 9 sweeps for all isotopes as a main run with 3 replications.

#### Statistical analyses

All data were statistically analyzed using SPSS 17.0 software, and the mean values of each treatment group were subjected to multiple comparisons analysis using the One-Way ANOVA and a significance level of  $p < 0.05$ .

The bars indicate the standard error of the mean. Significant differences in the mean value of each treatment group are indicated by different lowercase letters based on the LSD test ( $p < 0.05$ ,  $n=3$ ) when the distribution of data were homogenous and Games-Howell test ( $p < 0.05$ ,  $n=3$ ) when the distribution of data were not homogenous.

## RESULTS AND DISCUSSION

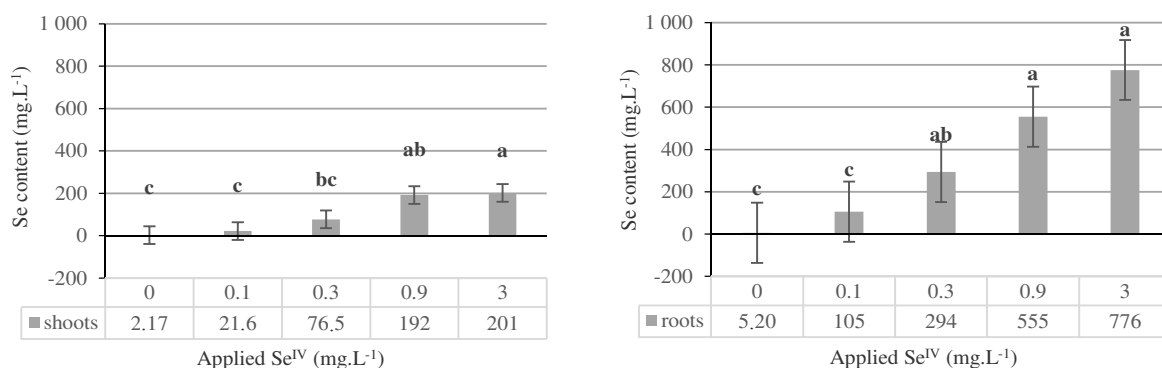
#### Comparison of $\text{Se}^{\text{IV}}$ uptake effects on maize shoot and root

Figure 1 displays Se contents of shoot and root at different concentrations of  $\text{Se}^{\text{IV}}$ . Se content significantly increased due to increasing the application of  $\text{Se}^{\text{IV}}$  and its amount in root is more than shoot in all of treatments.

Table 1 shows changes of fresh and dry weight of shoots and roots by increasing the application of  $\text{Se}^{\text{IV}}$ . Control and 3  $\text{mg L}^{-1}$   $\text{Se}^{\text{IV}}$  samples have the most and the least fresh and dry weights respectively in both shoots and roots.

Figure 2 illustrates  $\text{Se}^{\text{IV}}$  uptake effect on maize at different concentrations and high dose  $\text{Se}^{\text{IV}}$  toxicity in samples which had been treated with 3  $\text{mg L}^{-1}$   $\text{Se}^{\text{IV}}$ , is obvious.

Figure 1: Comparison of Se<sup>IV</sup> uptake effect on maize shoot and root



Note: significant differences in the mean value of each treatment group are indicated by different lowercase letter based on the Games-Howell test ( $p < 0.05$ ,  $n = 3 \pm s.e.$ )

Table 1.

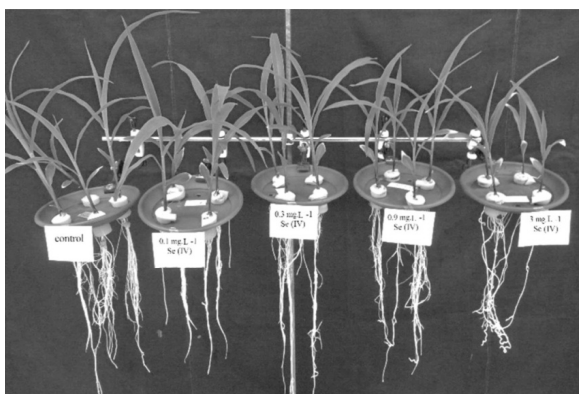
Comparison of different concentrations of Se<sup>IV</sup> uptake effects on fresh and dry weight of shoots and roots

| Applied Se (mg l <sup>-1</sup> ) | Shoots                      |                             | Roots                      |                             |
|----------------------------------|-----------------------------|-----------------------------|----------------------------|-----------------------------|
|                                  | Fresh weight (g)            | Dry weight (g)              | Fresh weight (g)           | Dry weight (g)              |
| 0                                | 3.4760±0.2637 <sup>a</sup>  | 0.2632±0.0255 <sup>a</sup>  | 1.6431±0.1630 <sup>a</sup> | 0.0989±0.0100 <sup>ab</sup> |
| 0.1                              | 2.7697±0.2815 <sup>b</sup>  | 0.2087±0.0234 <sup>b</sup>  | 1.3972±0.1319 <sup>a</sup> | 0.0816±0.0073 <sup>b</sup>  |
| 0.3                              | 2.9544±0.6297 <sup>ab</sup> | 0.2329±0.0319 <sup>ab</sup> | 1.7253±0.3283 <sup>a</sup> | 0.1007±0.0169 <sup>ab</sup> |
| 0.9                              | 2.6551±0.2834 <sup>b</sup>  | 0.2315±0.0183 <sup>ab</sup> | 1.7792±0.3092 <sup>a</sup> | 0.1096±0.0136 <sup>a</sup>  |
| 3                                | 0.5369±0.0264 <sup>c</sup>  | 0.0618±0.0036 <sup>c</sup>  | 0.4850±0.0399 <sup>b</sup> | 0.0377±0.0039 <sup>c</sup>  |

Note: significant differences in the mean value of each treatment group are indicated by different lowercase letter based on the LSD test ( $p < 0.05$ ,  $n = 3 \pm s.e.$ )

Figure 2: Se<sup>IV</sup> uptake effect on maize at different concentrations

(from left: control, 0.1, 0.3, 0.9, 3 mg L<sup>-1</sup> Se<sup>IV</sup> treatments)



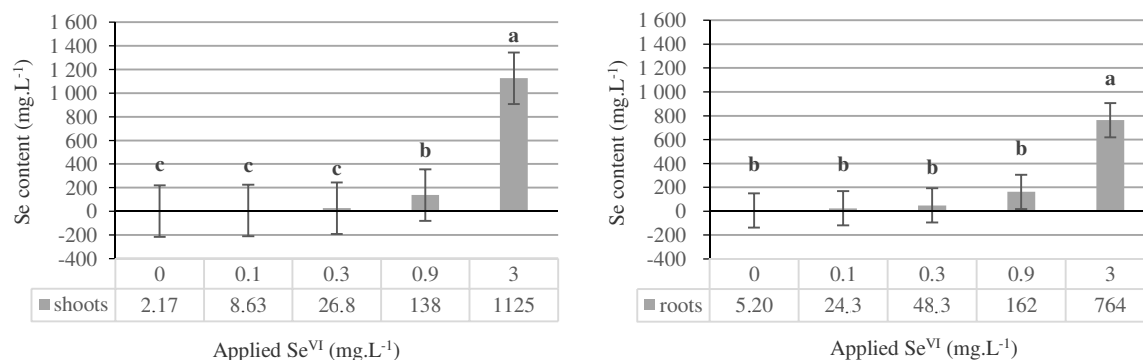
### Comparison of Se<sup>VI</sup> uptake effects on maize shoot and root

Se content of shoots and roots significantly increased due to increasing the application of Se<sup>VI</sup> as it has been shown in Figure 3 and its amount in root is more than shoot in all of the treatments but in the case of 3 mg L<sup>-1</sup> treatment, there is a contrast that shoot samples have more Se content.

Table 2 shows changes of fresh and dry weight of maize shoots and roots by increasing the application of Se<sup>VI</sup> and as we see, samples that had been treated with 0.1 mg L<sup>-1</sup> Se<sup>VI</sup> have the most fresh and dry weights although on the whole there is not any significant difference between all of the treatments.

Figure 4 illustrates Se<sup>VI</sup> uptake effect on maize at different concentrations.

Figure 3: Comparison of Se<sup>VI</sup> uptake effect on maize shoot and root



Note: significant differences in the mean value of each treatment group are indicated by different lowercase letter based on the Games-Howell test ( $p < 0.05$ ,  $n = 3 \pm s.e.$ )

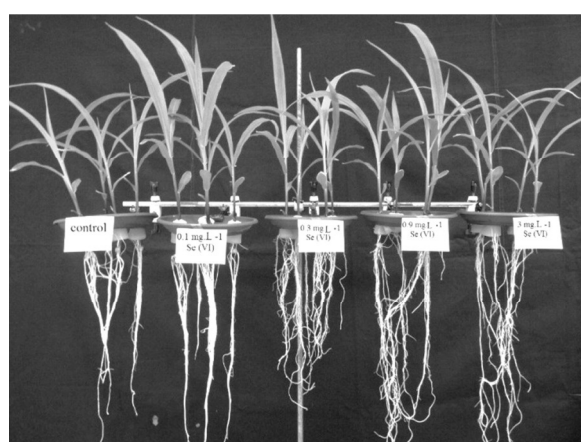
Table 2.

Comparison of different concentrations of Se<sup>VI</sup> uptake effects on fresh and dry weight of shoots and roots

| Applied Se (mg l <sup>-1</sup> ) | Shoots                     |                            | Roots                      |                            |
|----------------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
|                                  | Fresh weight (g)           | Dry weight (g)             | Fresh weight (g)           | Dry weight (g)             |
| 0                                | 3.4760±0.2637 <sup>a</sup> | 0.2632±0.0255 <sup>a</sup> | 1.6431±0.1630 <sup>a</sup> | 0.0989±0.0100 <sup>a</sup> |
| 0.1                              | 4.1070±1.3455 <sup>a</sup> | 0.3011±0.0905 <sup>a</sup> | 1.8678±0.6821 <sup>a</sup> | 0.1067±0.0368 <sup>a</sup> |
| 0.3                              | 3.2581±0.6369 <sup>a</sup> | 0.2471±0.0397 <sup>a</sup> | 1.6172±0.1869 <sup>a</sup> | 0.0919±0.0109 <sup>a</sup> |
| 0.9                              | 2.9850±0.4136 <sup>a</sup> | 0.2336±0.0260 <sup>a</sup> | 1.5506±0.3685 <sup>a</sup> | 0.0871±0.0192 <sup>a</sup> |
| 3                                | 3.2889±1.1539 <sup>a</sup> | 0.2683±0.0902 <sup>a</sup> | 1.4476±0.4550 <sup>a</sup> | 0.0977±0.0330 <sup>a</sup> |

Note: significant differences in the mean value of each treatment group are indicated by different lowercase letter based on the LSD test ( $p < 0.05$ ,  $n = 3 \pm s.e.$ )

Figure 4: Se<sup>VI</sup> uptake effect on maize at different concentrations (from left: control, 0.1, 0.3, 0.9, 3 mg L<sup>-1</sup> Se<sup>VI</sup> treatments)



## CONCLUSIONS

Due to the high demand of food across the world, its enrichment with essential micronutrients, such as Se, is crucial and therefore, plants play an important role in Se supplementation (Finley 2005). However, Se can also be toxic when ingested in high concentrations (Gasó et al. 2000, Hartikainen 2005).

Our experiment showed the Se content in shoots and roots of maize increased as the Se concentration applied increased.

Furthermore, although the mobility amount of selenite and selenate in hydroponic systems are the same, due to the lower energy consumption required for uptake, selenite (+4) exhibited higher toxicity than selenate (+6), as the results for fresh and dry weight of shoots and roots confirm.

Moreover, the presented results allow us to conclude Se content in the maize roots are more than the shoots.

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