

# Effect of Selenite and Selenate Uptake by Maize Plants on Specific Leaf Area

F. Garousi, Sz. Veres, É. Bódi, Sz. Várallyay, B. Kovács

**Abstract**—Specific leaf area (SLA;  $\text{cm}^2_{\text{leaf}} \text{g}^{-1}_{\text{leaf}}$ ) the ratio of leaf area to leaf dry mass is a key ecophysiological parameter influencing leaf physiology, photosynthesis, and whole plant carbon gain and also can be used as a rapid and diagnostic tool. In this study, two species of soluble inorganic selenium forms, selenite ( $\text{Se}^{\text{IV}}$ ) and selenate ( $\text{Se}^{\text{VI}}$ ) at different concentrations were investigated on maize plants that were growing in nutrient solutions during 2 weeks and at the end of the experiment, amounts of SLA for first and second leaves of maize were measured. In accordance with the results we observed that our regarded Se concentrations in both forms of  $\text{Se}^{\text{IV}}$  and  $\text{Se}^{\text{VI}}$  were not effective on maize plants' SLA significantly although high level of  $3 \text{ mg} \cdot \text{kg}^{-1} \text{ Se}^{\text{IV}}$  had negative affect on growth of the samples that had been treated by it but about  $\text{Se}^{\text{VI}}$  samples we did not observe this state and our different considered  $\text{Se}^{\text{VI}}$  concentrations were not toxic for maize plants.

**Keywords**—Maize, Sodium selenate, sodium selenite, specific leaf area.

## I. INTRODUCTION

THE trace element selenium (Se) has been well recognized as an essential micronutrient for human and animals [1] and agronomic biofortification is reported to be an effective method to increase Se concentration in the edible portion of crops and hence dietary intake of Se [2]. Despite substantial literature on Se uptake by plants and crops such as wheat, little consideration has been given to maize (*Zea mays*), a low “Se-indicator” plant but the world’s most widely grown cereal. To date there have been few publications on Se uptake and assimilation in this plant [3] and parallel to that, investigation of its effects on maize leaves’ specific leaf area (SLA).

Thick leaves allows a higher concentration of photosynthetic apparatus per unit leaf area, whereas thinner, but larger leaves, allows a higher light interception [4] for example fast-growing species develop more leaf area per unit leaf biomass, leading to a higher growth rate [5]. Since the determination of leaf thickness and density is not straightforward, a relative measure is used, namely, specific leaf area (SLA, leaf area to leaf biomass ratio,  $[\text{cm}^2 \cdot \text{g}^{-1}]$ ) [6].

F. Garousi is with the University of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management, Institute of Food Science, H-4032 Hungary (corresponding author to provide phone: 36-30-7597506; fax: 36-52-417572; e-mail: farzaneh@agr.unideb.hu).

Sz. Veres is with the University of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management, Institute of Crop Sciences, Department of Agricultural Botany, Crop Physiology and Biotechnology, H-4032 Hungary (e-mail: szveres@agr.unideb.hu).

É. Bódi, Sz. Várallyay and B. Kovács are with the University of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management, Institute of Food Science, H-4032 Hungary (e-mail: bodieva@agr.unideb.hu, varallyay.szilvia@agr.unideb.hu, kovacs@agr.unideb.hu).

In crop growth simulators, the daily increase of leaf area is often derived from the product of leaf mass increase and SLA, whereas the SLA of already formed and expanded leaves is assumed as conserved. The SLA is influenced by many factors and remarkable efforts have been made to isolate the most important ones, aiming to obtain a robust empirical prediction.

The objective of our study was to expose maize plants to Se in both forms of sodium selenite and sodium selenate as well as investigation of their uptake effects on maize leaves’ SLA.

## II. MATERIAL AND METHODS

### A. Materials

Sodium selenite and sodium selenate were obtained from Sigma-Aldrich Ltd. (Poole, UK)

### B. 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 in  $22^\circ\text{C}$ . Seedlings with 2.5-3.0 cm coleoptile were placed into aerated nutrient solution pots. Maize plants were grown up in a climate room under strictly regulated environmental conditions. Relative humidity was maintained between 65-75%, light/dark cycle was 16/8 hrs. with a respective  $25/20^\circ\text{C}$  temperature periodicity, and light intensity was kept in constant  $300 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  during daytime.

### C. Plant Growth in Nutrient Solution

The nutrient solution that was 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 [7].

Selenium was supplemented to the nutrient solution as two species of selenite in form of  $\text{Na}_2\text{SeO}_3$  and selenate in form of  $\text{Na}_2\text{SeO}_4$  in five different concentrations as follows: 0 (control), 0.1, 0.3, 0.9, and  $3 \text{ mg} \cdot \text{kg}^{-1} \text{ Se}^{\text{IV}}$  and  $\text{Se}^{\text{VI}}$ . Nutrient solution was changed every 3 days and evaporated water was replenished regularly. The experiment ended 2 weeks after planting when third leaf of control treatment grew completely and seedlings had approximately 40-30 cm long shoots and roots, respectively. Experiments were carried out in triplicates.

### D. SLA Measurements

From each plant, first and second mature, intact and erect leaves were sampled for determination of the specific leaf area (SLA). From each leaf, 5 leaf discs with a fixed surface area were punched out with a perforator. The samples were

collected at both sides of the main midrib, and subsequently dried at 60°C for at least 24 hrs till constant weight. The dry weight of all 5 leaf discs together was determined by an electronic balance with an accuracy of 0.001g (OHAUS, Swiss). Finally, SLA ( $\text{cm}^2 \cdot \text{g}^{-1}$ ) of each leaf was calculated by dividing leaf area by corresponding leaf dry weight.

*E. Weight Measurements*

At the end of the experiment shoots were separated from roots. Plant shoots were dried at 85°C until constant weight was achieved, then cooled to room temperature and weighed by an electronic balance with an accuracy of 0.001g (OHAUS, Swiss).

*F. Statistical Analysis*

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 Two-Way ANOVA and a significance level of  $p < 0.05$ .

Significant differences in the mean value of each treatment group are indicated by different lowercase letters based on the Duncan test ( $p < 0.05, n=3$ ).

III. RESULTS AND DISCUSSION

*A. Se<sup>IV</sup> Uptake Effects on First Leaves' SLA*

Fig. 1 displays SLA measurements in maize at different concentrations of Se<sup>IV</sup> for first leaves. According to our calculation, there was not any significant difference between the treatments although control and 3 mg.kg<sup>-1</sup> Se<sup>IV</sup> samples had the most and least amounts respectively.

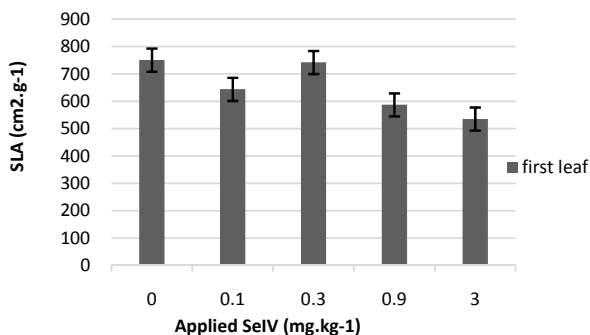


Fig. 1 Se<sup>IV</sup> uptake effects on first leaves' SLA

*B. Se<sup>IV</sup> Uptake Effects on Second Leaves' SLA*

Fig. 2 displays SLA measurements in maize at different concentrations of Se<sup>IV</sup> for second leaves. According to our calculation, there was not any significant difference between the samples.

Treatment by Se<sup>IV</sup> did not effect on both first and second maize plants leaves' SLA significantly and it shows applying our different regarded Se<sup>IV</sup> concentrations have not had positive effect on maize plants.

Table I shows changes of fresh weight of maize shoots by increasing the application of Se<sup>IV</sup> and as we see, Se<sup>IV</sup> has made significant differences between the treatments so that control

samples have the freshest weights. Meanwhile 3 mg.kg<sup>-1</sup> Se<sup>IV</sup> had a negative effect on maize growth and it was toxic for it.

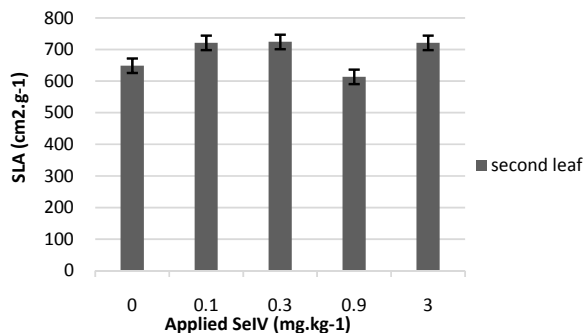


Fig. 2 Se<sup>IV</sup> uptake effects on second leaves' SLA

TABLE I  
DIFFERENT CONCENTRATIONS OF Se<sup>IV</sup> UPTAKE EFFECTS ON FRESH WEIGHT OF MAIZE SHOOT

Applied Se <sup>IV</sup> (mg.kg <sup>-1</sup> )	Fresh weight (g)
0	3.4760±0.2637 <sup>c</sup>
0.1	2.7697±0.2815 <sup>b</sup>
0.3	2.9544±0.6297 <sup>ab</sup>
0.9	2.6551±0.2834 <sup>b</sup>
3	0.5369±0.0264 <sup>a</sup>

Significant differences in the mean value of each treatment group are indicated by different lowercase letter based on the Duncan-test ( $p < 0.05, n = 3 \pm \text{s.e.}$ ).

Table II shows changes of dry weight of maize shoots by increasing the application of Se<sup>IV</sup> and as we see, Se<sup>IV</sup> has made significant differences between the treatments so that control samples have the driest weights. Meanwhile 3 mg.kg<sup>-1</sup> Se<sup>IV</sup> had a negative effect on maize growth and it was toxic for it.

TABLE II  
DIFFERENT CONCENTRATIONS OF Se<sup>IV</sup> UPTAKE EFFECTS ON DRY WEIGHT OF MAIZE SHOOT

Applied Se <sup>IV</sup> (mg.kg <sup>-1</sup> )	Dry weight (g)
0	0.2632±0.0255 <sup>c</sup>
0.1	0.2087±0.0234 <sup>b</sup>
0.3	0.2329±0.0319 <sup>ab</sup>
0.9	0.2315±0.0183 <sup>ab</sup>
3	0.0618±0.0036 <sup>a</sup>

Significant differences in the mean value of each treatment group are indicated by different lowercase letter based on the Duncan-test ( $p < 0.05, n = 3 \pm \text{s.e.}$ ).

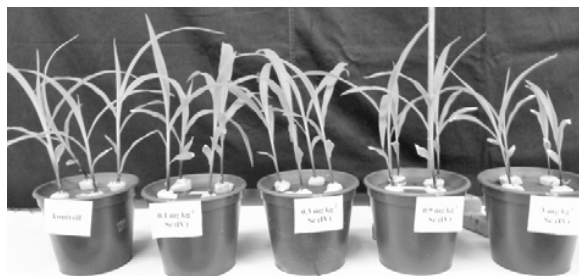


Fig. 3 Different concentrations of Se<sup>IV</sup> on maize, from left: 0, 0.1, 0.3, 0.9, and 3 mg.kg<sup>-1</sup> Se<sup>IV</sup>

Fig. 3 shows different concentrations of  $\text{Se}^{\text{IV}}$  effects on our maize samples and as we can see, sample that has been treated by  $3 \text{ mg.kg}^{-1} \text{Se}^{\text{IV}}$  has stayed small and this amount of  $\text{Se}^{\text{IV}}$  has been toxic for it.

#### C. $\text{Se}^{\text{VI}}$ Uptake Effects on First Leaves' SLA

Fig. 4 displays SLA measurements in maize at different concentrations of  $\text{Se}^{\text{VI}}$  for first leaves. According to our calculation, there was not any significant difference between the samples.

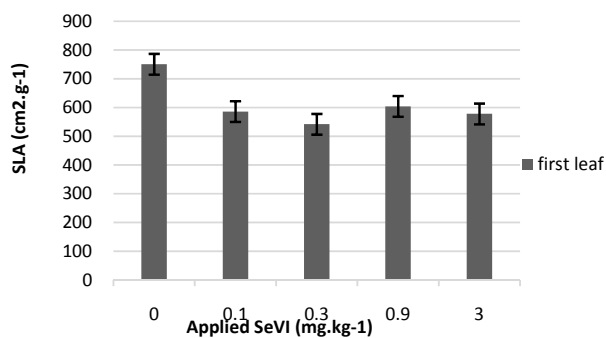


Fig. 4  $\text{Se}^{\text{VI}}$  uptake effects on first leaves' SLA

#### D. $\text{Se}^{\text{VI}}$ Uptake Effects on Second Leaves' SLA

Fig. 5 displays SLA measurements in maize at different concentrations of  $\text{Se}^{\text{VI}}$  for second leaves. According to our calculation, there was not any significant difference between the samples.

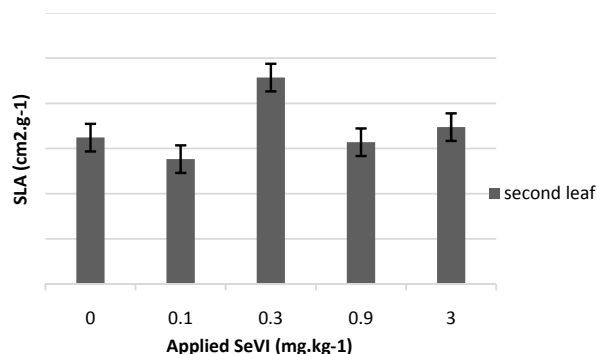


Fig. 5  $\text{Se}^{\text{VI}}$  uptake effects on second leaves' SLA

Treatment by  $\text{Se}^{\text{VI}}$  did not effect on both first and second maize plants leaves' SLA significantly.

Table III shows changes of fresh weight of maize shoots by increasing the application of  $\text{Se}^{\text{VI}}$  and as we see, samples that had been treated by  $0.1 \text{ mg.kg}^{-1}$  have the most fresh weights but on the whole there is not any significant difference between all of the treatments.

Table IV shows changes of dry weight of maize shoots by increasing the application of  $\text{Se}^{\text{VI}}$  and as we see, samples that had been treated by  $0.1 \text{ mg.kg}^{-1}$  have the most dry weights but on the whole there is not any significant difference between all of the treatments.

TABLE III  
DIFFERENT CONCENTRATIONS OF  $\text{Se}^{\text{VI}}$  UPTAKE EFFECTS ON FRESH WEIGHT OF MAIZE SHOOT

Applied $\text{Se}^{\text{VI}}$ (mg.kg <sup>-1</sup> )	Fresh weight (g)
0	3.4760±0.2637 <sup>a</sup>
0.1	4.1070±1.3455 <sup>a</sup>
0.3	3.2581±0.6369 <sup>a</sup>
0.9	2.9850±0.4136 <sup>a</sup>
3	3.2889±1.1539 <sup>a</sup>

The same lowercase letters after the mean values and standard deviations in both columns shows no significant difference between the treatments according to the Duncan-test ( $p < 0.05$   $n = 3 \pm \text{s.e.}$ ).

TABLE IV  
DIFFERENT CONCENTRATIONS OF  $\text{Se}^{\text{VI}}$  UPTAKE EFFECTS ON DRY WEIGHT OF MAIZE SHOOT

Applied $\text{Se}^{\text{VI}}$ (mg.kg <sup>-1</sup> )	Dry weight (g)
0	0.2632±0.0255 <sup>a</sup>
0.1	0.3011±0.0905 <sup>a</sup>
0.3	0.2471±0.0397 <sup>a</sup>
0.9	0.2336±0.0260 <sup>a</sup>
3	0.2683±0.0902 <sup>a</sup>

The same lowercase letters after the mean values and standard deviations in both columns shows no significant difference between the treatments according to the Duncan-test ( $p < 0.05$   $n = 3 \pm \text{s.e.}$ ).

Fig. 6 shows different concentrations of  $\text{Se}^{\text{VI}}$  effects on our maize samples and as we can see, none of these different amounts of  $\text{Se}^{\text{VI}}$  have had negative effects on them.

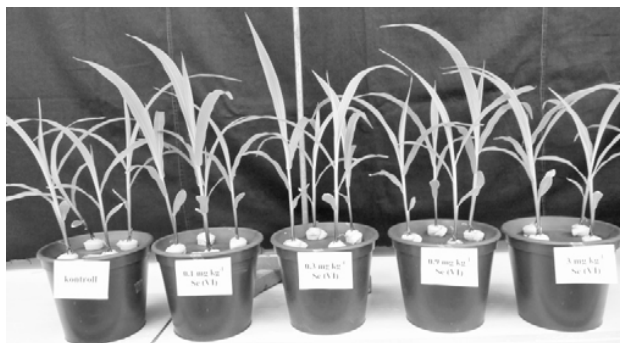


Fig. 6 Different concentrations of  $\text{Se}^{\text{VI}}$  on maize. From left: 0, 0.1, 0.3, 0.9, and  $3 \text{ mg.kg}^{-1} \text{Se}^{\text{VI}}$

#### ACKNOWLEDGMENT

The authors are very grateful the cooperation of Faculty of Agricultural and Food Sciences and Environmental Management, Institute of Crop Sciences, Department of Agricultural Botany, Crop Physiology and Biotechnology in Debrecen University.

#### REFERENCES

- [1] B. Yang and G. Y. Wei, "Preparation of selenium-enriched *Candida utilis* with fed-batch cultivation," 2<sup>th</sup> Conf. Selenium: Global perspectives of impacts on humans, animals and the environment China, 2011, p. 103.
- [2] A. D. C. Chilimba, S. D. Young, C. R. Black, M. C. Meacham, J. Lammel and M. R. Broadley, "The fate of applied selenium in a maize cropping system in Malawi," 2<sup>th</sup> Conf. Selenium: Global perspectives of impacts on humans, animals and the environment China, 2011, p. 81.
- [3] M. Longchamp, N. Angeli and M. Castrec-Rouelle, "Uptake of selenate and/or selenite in hydroponically grown maize plants and interaction with some essential elements (calcium, magnesium, zinc, iron,

- manganese, and copper),” 2<sup>th</sup> Conf. *Selenium: Global perspectives of impacts on humans, animals and the environment* China, 2011, p. 83.
- [4] J. W. White and R.C. Montes, “Variation in parameters related to leaf thickness in common bean (*Phaseolus vulgaris* L.),” *Field Crop Res.*, vol. 91, no. 1, pp. 7–21, Jan. 2005.
- [5] H. Poorter and A. Van der Werf, “Is inherent variation in RGR determined by LAR at low irradiance and by NAR at high irradiance. A review of herbaceous species,” *Inherent Variation in Plant Growth*, pp. 309–336, 1998.
- [6] W. Larcher, “Physiological Plant Ecology: Ecophysiology and Stress Physiology of Functional Groups,” *Springer*, Germany, 2001, p. 513.
- [7] I. Cakmak and H. Marschner, “Decrease in nitrate uptake and increase in proton release in zinc deficient cotton, sunflower and buckwheat plants,” *Plant and Soil*, vol. 129, pp. 261–268, 1990.