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Role of Selenite and Selenate Uptake by Maize Plants in Chlorophyll A and B Content

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

***Abstract*—**Extracting and determining chlorophyll pigments (chlorophyll a and b) in green leaves are the procedures based on the solvent extraction of pigments in samples using N,N-dimethylformamide as the extractant. In this study, two species of soluble inorganic selenium forms, selenite (Se^{IV}) and selenate (Se^{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 chlorophyll a and b 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 Se^{IV} and Se^{VI} were not effective on maize plants' chlorophyll a and b significantly although high level of 3 $mg.kg^{-1}$ Se^{IV} had negative affect on growth of the samples that had been treated by it but about Se^{VI} samples we did not observe this state and our different considered Se^{VI} concentrations were not toxic for maize plants.

***Keywords*—**Maize, sodium selenate, sodium selenite, chlorophyll a and b.

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' chlorophyll a and b.

Chlorophylls (Chl) are photosynthetic pigments that are widely distributed in nature. These pigments possess a basic skeleton structure of porphyrine with a magnesium ion in the centre and a long phytol group in the tail [4]. The major chlorophylls in plants include Chl-a and Chl-b. They differ only slightly, in the composition of a side chain (in Chl-a it is $–CH_3$, in Chl-b it is CHO). Both chlorophylls are genuine components of the photosynthetic membranes, and they are usually present at a ratio of 3:1 [5]. Growth conditions and environmental factors can modify this a/b ratio [6]. Both of these two chlorophylls are very effective photoreceptors because they contain a network of alternating single and double bonds, and the orbitals can delocalise stabilising the structure. Such delocalised polyenes have very strong absorption bands in the visible regions of the spectrum, allowing the plant to absorb the energy from sunlight [7].

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' chlorophyll a and b.

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).

S. 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, S. 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, kovacsb@agr.unideb.hu).

II. Material and Methods

A. Materials

Sodium selenite, sodium selenate and N,N-Dimethylformamide (N,N-DMF) 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°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°C temperature periodicity, and light intensity was kept in constant 300 $\mu mol.m^{-2}s^{-1}$ during daytime.

C. Plant Growth in Nutrient Solution

The nutrient solution that was used for plant growth had the following composition: 2.0 mM $Ca(NO_3)_2$, 0.7 mM K_2SO_4, 0.5 mM $MgSO_4$, 0.1 mM KH_2PO_4, 0.1 mM KCl, 0.1 µM H_3BO_3, 0.5 µM $MnSO_4$, 0.5 µM $ZnSO_4$ and 0.2 µM $CuSO_4$. Iron was supplied in the form of 10^{-4} M Fe-EDTA, too [8].

Selenium was supplemented to the nutrient solution as two species of selenite in form of Na_2SeO_3 and selenate in form of Na_2SeO_4 in five different concentrations as follows: 0 (control), 0.1, 0.3, 0.9, and 3 $mg.kg^{-1}$ Se^{IV} and Se^{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. Chlorophyll a and b Measurements

From each plant, first and second mature, intact and erect leaves were sampled for extraction and determination of the chlorophyll a and b. 50 mg of each leaf were collected and with 5ml N,N-Dimethylformamide (N,N-DMF) blended. This solution cooled at 4°C for 72 hours and finally, the extraction content of the pigment was determined using UV–vis spectrophotometry (Metertech SP-830 PLUS, Taiwan) at two characteristic wavelengths, 647 and 664 nm, which are the maximum absorption wavelengths for chlorophylls b and a, respectively. Calibration graph was obtained by using the wavelength of 480 nm and each concentration level was analysed in triplicate.

According to the formula that was proposed by Morgan and Porath (1981) [9], the following was processed mathematically for quantifying chlorophyll a and b:

Chlorophyll a ($mg.g^{-1}$) = (11.65 a664-2.69 a647)

Chlorophyll b ($mg.g^{-1}$) = (20.81 a647-4.53 a664).

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^{IV} Uptake Effects on First Leaves' Chlorophyll a and b

Fig. 1 displays chlorophyll a and b measurements in maize at different concentrations of Se^{IV} for first leaves. According to our calculation, there was not any significant difference between the treatments.

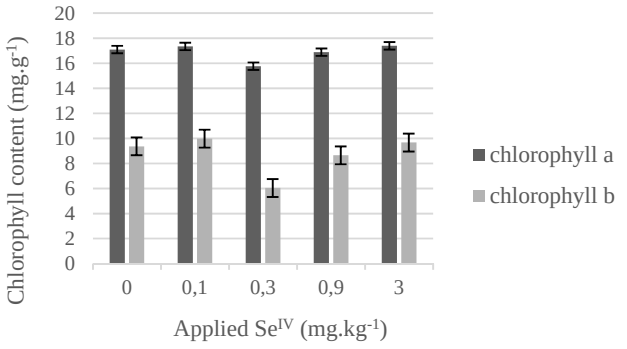

Fig. 1 Se^{IV} uptake effects on first leaves' chlorophyll a and b

B. Se^{IV} Uptake Effects on Second Leaves' Chlorophyll a and b

Fig. 2 displays chlorophyll a and b measurements in maize at different concentrations of Se^{IV} for second leaves. According to our calculation, there was not any significant difference between the samples.

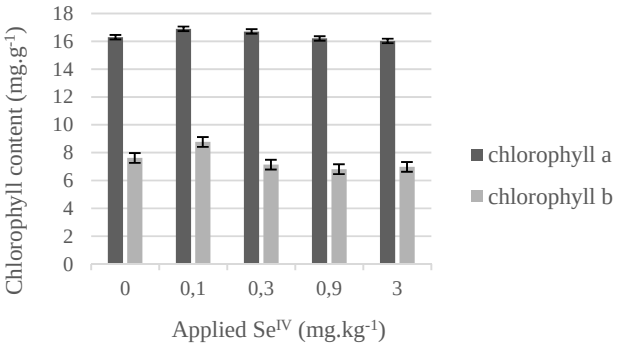

Fig. 2 Se^{VI} uptake effects on second leaves' chlorophyll a and b

Treatment by Se^{IV} did not effect on both first and second maize plants leaves' chlorophyll a and b significantly and it shows applying our different regarded Se^{IV} concentrations has not had positive effect on maize plants.

Table I shows changes of fresh weight of maize shoots by increasing the application of Se^{IV} and as we see, Se^{IV} has made significant differences between the treatments so that control samples have the freshest weights. Meanwhile 3 $mg.kg^{-1}$ Se^{IV} had a negative effect on maize growth and it was toxic for it.

TABLE I
DIFFERENT CONCENTRATIONS OF SE^{IV} UPTAKE EFFECTS ON FRESH WEIGHT OF MAIZE SHOOT

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

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±s.e.).

TABLE II
DIFFERENT CONCENTRATIONS OF SE^{IV} UPTAKE EFFECTS ON DRY WEIGHT OF MAIZE SHOOT

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

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±s.e.).

Table II shows changes of dry weight of maize shoots by

increasing the application of Se^{IV} and as we see, Se^{IV} has made significant differences between the treatments so that control samples have the driest weights. Meanwhile 3 $mg.kg^{-1}$ Se^{IV} had a negative effect on maize growth and it was toxic for it.

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

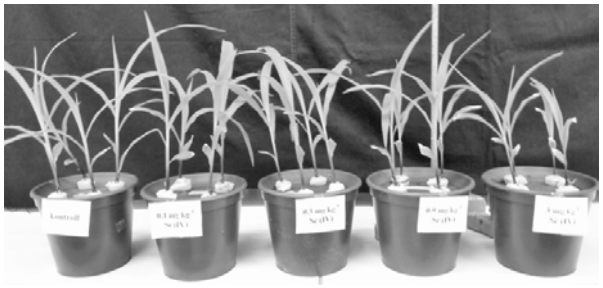

Fig.3 Different concentrations of Se^{IV} on maize. From left: 0, 0.1, 0.3, 0.9, and 3 $mg.kg^{-1}$ Se^{IV}

C.Se^{VI} Uptake Effects on First Leaves' Chlorophyll a and b

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

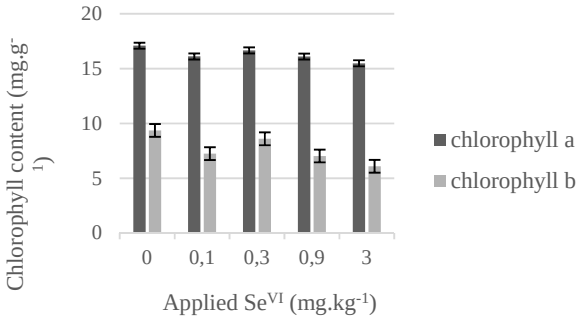

Fig. 4 Se^{VI} uptake effects on first leaves' chlorophyll a and b

D.Se^{VI} Uptake Effects on Second Leaves' Chlorophyll a and b

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

Treatment by Se^{VI} did not effect on both first and second maize plants leaves' chlorophyll a and b significantly.

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

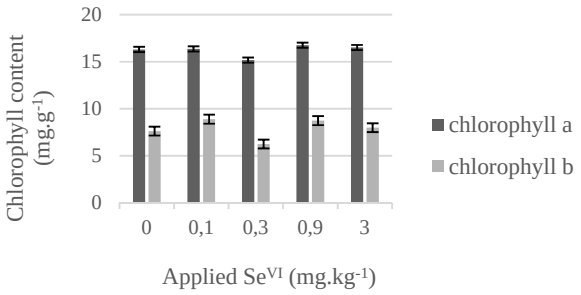

Fig. 5 Se^{VI} uptake effects on second leaves' chlorophyll a and b

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

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

The same lowercase letters after the mean values and standard deviations in both culomns shows no significant defference between the treatments according to the Duncan-test ($p < 0.05$ n = 3±s.e.).

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

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

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

The same lowercase letters after the mean values and standard deviations in both culomns shows no significant defference between the treatments according to the Duncan-test ($p < 0.05$ n = 3±s.e.).

Fig. 6 shows different concentrations of Se^{VI} effects on our maize samples and as we can see that none of these different amounts of Se^{VI} have not had negative effects on them.

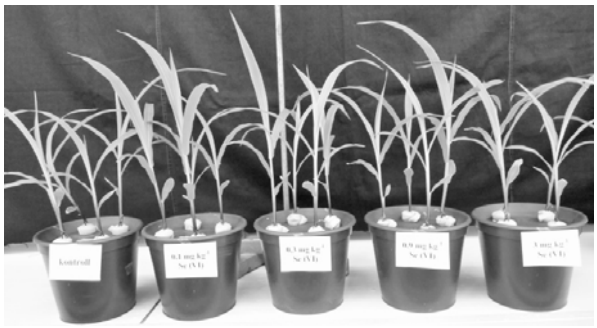

Fig.6 Different concentrations of Se^{VI} on maize. From left: 0, 0.1, 0.3, 0.9, and 3 $mg.kg^{-1}$ Se^{VI}

ACKNOWLEDGMENT

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