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RELATIVE CHLOROPHYLL CONTENT CHANGES DURING UPTAKING OF SELENITE AND SELENATE BY MAIZE PLANTS GROWN IN NUTRIENT SOLUTION

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ABSTRACT

Chlorophyll content (chl), one of the most important physiological parameters related to plant photosynthesis, is usually used to predict plant potential and portable, non-destructive chlorophyll meters could be a valuable and effective tool for estimating Relative Chlorophyll Content (RCC) in leaves. 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 changes of RCC within this time was monitored. It means chlorophyll content of three leaves of maize when everyone grew completely was measured according to Special Products Analysis Division (SPAD) value and the results revealed that high concentrations of Se^{IV} (10, 30 and 90 $mg.kg^{-1}$) were toxic for maize even lower amounts (1 and 3 $mg.kg^{-1}$) had effects of damage on it while this state wasn't adjusted for lower concentrations of Se^{VI} (1 and 3 $mg.kg^{-1}$) and treated samples didn't have significant differences with controls although in higher amounts (10 and 30 $mg.kg^{-1}$) toxic effects were seen in them, too.

Keywords: Relative chlorophyll content, sodium selenite, sodium selenite, maize

INTRODUCTION

Selenium (Se) is an essential micronutrient for the health of mammals and mainly enters food chains through plants. To provide recommended optimal Se values for target population, agronomic Se-biofortification (e.g., adding soluble selenite and selenate salts to mineral fertilizers) seems to be a good short-term strategy to accumulate higher levels of Se in plants. 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 **Castrec-Rouelle *et al.* (2011)** and parallel to that, investigation of its effects on maize leaves' chlorophyll content.

Chlorophyll is a frequent organic chemical component because it is naturally present in plants, giving their specific coloration **Withnallas *et al.* (2003)** as a photosynthetic pigment and an essential component of the plant photosystem. Leaf chlorophyll content affects photosynthetic ability and thus is one of the most important physiological traits affecting plants (**Czyczyło-Mysza *et al.*, 2013; Teng *et al.*, 2004; Wang *et al.*, 2008**) so that content of photosynthetic pigments is highly correlated with the nutrition condition **Gitelson *et al.* (2003)** and as an indicator for growth and survival of plants (**Foyer *et al.*, 1982; Peng and Gitelson, 2012**).

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' RCC.

MATERIAL AND METHODS

Materials

Sodium selenite and sodium selenate were obtained from SIGMA-ALDRICH LTD. (POOLE, UK) and Chlorophyll meter (MINOLTA SPAD-502) was supplied by KONICA-MINOLTA, JAPAN.

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.

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 (**Cakmak and Marschner, 1990**).

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 and four different concentrations respectively as follows: 0 (control), 1, 3, 10, 30 and 90 $mg.kg^{-1}$ Se^{IV} and 0 (control), 1, 3, 10 and 30 $mg.kg^{-1}$ 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

Chlorophyll measurements (SPAD)

According to SPAD value five different parts' average of three leaves were measured by portable, non-destructive chlorophyll meters when every leaf of maize plants grew completely.

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 $p < 0.05$.

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 Duncan test ($p < 0.05$, n=3).

RESULTS AND DISCUSSION

Se^{IV} uptake effects on RC

Fig. 1 displays chlorophyll contents (SPAD value) in maize leaves at different concentrations of Se^{IV} for three times of three leaves RCC measurements but samples that had been treated by more than 3 $mg.kg^{-1}$ didn't grow so that 90 $mg.kg^{-1}$ treatments got dried and RCC measurement was impossible for them. As is obvious in these figures, SPPAD value significantly increased for first leaf due to increasing application of Se^{IV} but about second time there wasn't significant increasing and again for third time just first leaf had significant changes.

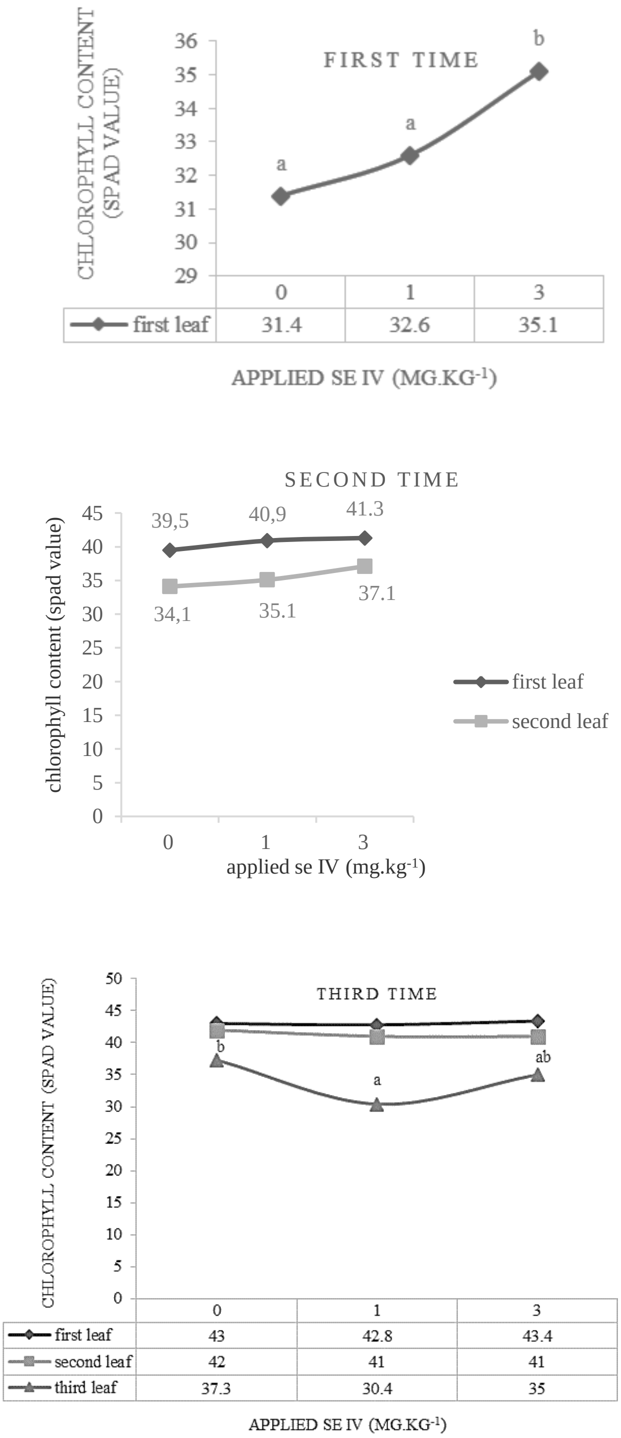

Figure 1 Se^{IV} uptake effects on RCC. Significant differences in the mean value of each treatment group are indicated by different lowercase letter based on the Duncan-test ($p < 0.05$, n = 3).

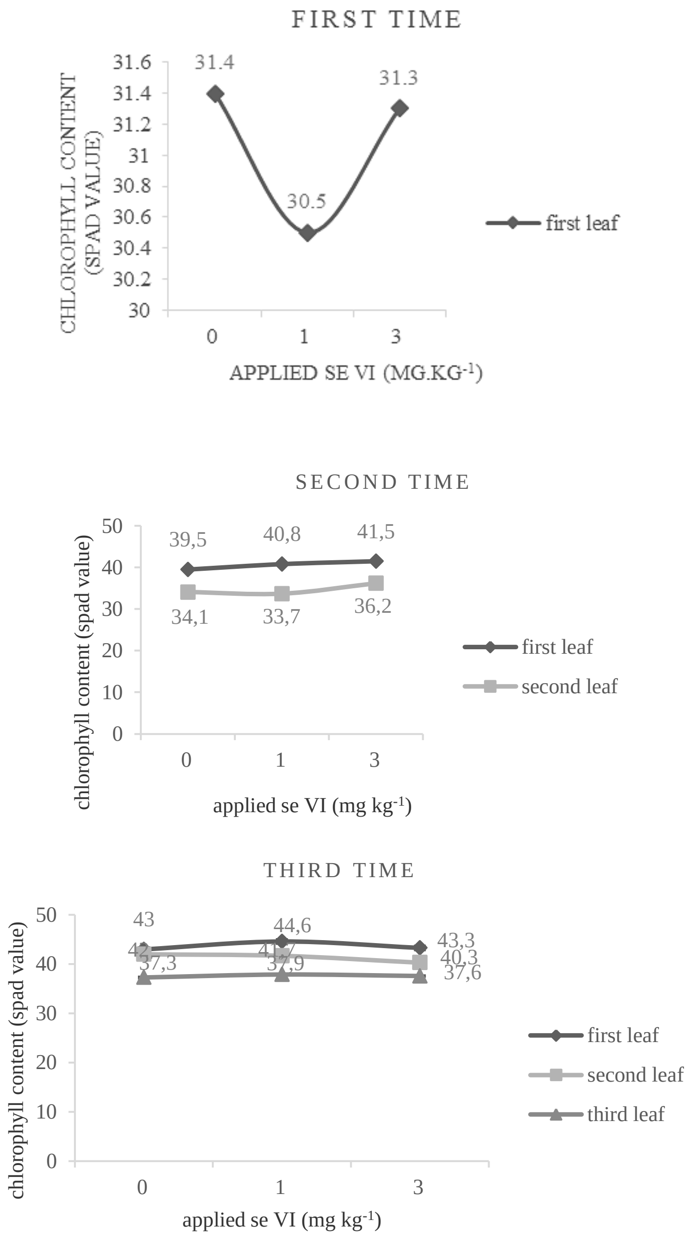

Figure 2 Se^{VI} uptake effects on RCC.

Changes of fresh and dry weight of maize shoots by increasing the application of Se^{IV} and process of weight decrease has been showed (Tab 1).

Table 1 Different concentrations of Se^{IV} uptake effects on fresh and dry weight of maize shoot.

Applied Se^{IV} (mg kg^{-1})	*Fresh weight (g)*	*Dry weight (g)*
0	8.6379 ± 0.8418^{c}	0.6083 ± 0.0742^{b}
1	5.5065 ± 0.9534^{b}	0.4543 ± 0.0796^{a}
3	3.6023 ± 0.6422^{a}	0.3510 ± 0.0451^{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.)

Se^{VI} uptake effects on RCC

Fig. 2 displays chlorophyll contents (SPAD value) in maize leaves at different concentrations of Se^{VI} for three times of three leaves RCC measurements but samples that had been treated by more than 3 $mg.kg^{-1}$ didn't grow so that 30 $mg.kg^{-1}$ treatments got dried and RCC measurement was impossible for them. SPPAD value didn't change significantly for all of three leaves due to increasing application of Se^{VI}.
Tab 2 shows changes of fresh and dry weight of maize shoots by increasing the application of Se^{VI}.

Table 2 Different concentrations of Se^{VI} uptake effects on fresh and dry weight of sunflower and maize shoot.

Applied Se^{VI} (mg kg^{-1})	*Fresh weight (g)*	*Dry weight (g)*
0	8.6379 ± 0.8418^{a}	0.6083 ± 0.0742^{a}
1	8.1375 ± 1.6916^{a}	0.6183 ± 0.1443^{a}
3	8.0187 ± 0.2765^{a}	0.6174 ± 0.0223^{a}

Legend: 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.).

CONCLUSION

The data in this study indicate that portable chlorophyll meter is an effective tool for rapid and nondestructive estimation of relative chlorophyll content in maize leaves during the growing and treatment by two Se forms of selenite and selenate. As has been investigated in this paper, high doses of Se in both forms of Se^{IV} and Se^{VI} are toxic for maize plant. Moreover, in lower doses according to SPAD value and weight amount results, samples which had been treated by Se^{IV} still had significant differences with control samples and effects of damage on maize growth whereas this state wasn't seen in Se^{VI} treatments and there wasn't significant difference between treated and control samples in three times of RCC measurement.

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