Journal of Plant Process and Function, Vol. 6, No. 20, Year 2017

Beneficial effects of low selenium levels in *Lallemantia iberica* plants subjected to salinity stress

Ghader Habibi *

Department of Biology, Payame Noor University (PNU), P.O.BOX 19395-3697 Tehran, Iran.
(Received: 25/03/2016-Accepted: 14/12/2016)

Abstract:

Selenium (Se) at high concentrations can cause toxic effects in plants, whereas at low concentrations, enhances resistance to certain abiotic stresses. The possible mechanisms of the Se-enhanced tolerance of plants to salinity stress remain elusive. In this work, effect of Se supplementation at 0, 0.1, 1 and 10 mg l^{-1} sodium selenate was studied in *Lallemantia iberica* plants subjected to salt stress. In pre-experiment design, the toxicity threshold for selenate was measured at the concentration of 10 mg l^{-1}, as the biomass of *L. iberica* plants decreased selenate concentrations in the growth medium reached 10 mg l^{-1}. Thereafter, we used 0.1 mg l-1 Se to mitigate salt stress in *L. iberica* plants. Se at low concentration (0.1 mg l^{-1}) mitigated salt-induced oxidative stress because the NaCl-induced lipid peroxidation was smallest in the plants treated with Se, and was highest in the plants grown without Se addition. This occurred as a result of glutathione (GSH) accumulation in Se-supplied plants. Additionally, Se-supplied plants exhibited higher glutathione peroxidase (GSH-Px) activity after 2 days salt stress. These data provided the first evidence that the low-level Se pretreatment alleviated salt stress in *L. iberica* by increasing GSH levels and by decreasing ROS production; and therefore less reduction of biomass production during salt stress.

Keywords: Antioxidant system, Glutathione, *Lallemantia iberica*, Salt stress, Selenium toxicity.

Introduction:

Salinity is one of the environmental stresses and negatively influences the crop growth and yield. Salt stress causes an initial water deficit and ion-specific toxicity that initiates chain reactions that produce more harm than oxygen radicals. Accumulation of reactive oxygen species (ROS) may cause cellular damage through oxidation of cellular macromolecules including DNA, proteins and carbohydrates (Miller *et al*., 2010; Ding *et al*., 2010). Plants have both enzymatic and nonenzymatic antioxidant defense systems for scavenging and prevention of ROS damage under salt stress conditions (Parida and Das, 2005).

Suitable plant nutrition is one of the strategies to avoid oxidative damage to cells (Kong *et al*., 2005). Numerous research results have demonstrated that the minor addition of Se to the growth medium can decrease the harmful effects of diverse environmental stressors, such as drought (Hasanuzzaman and Fujita, 2011; Habibi, 2013), salt (Hasanuzzaman *et al*., 2011; Diao *et al*., 2014), cold (Chu *et al*., 2010), ultraviolet-B (Yao *et al*., 2010), and high temperature (Djanaguiraman *et al*., 2010), whereas a high level of Se may lead to a ROS burst and hampering of plant growth. This increased production of ROS at high Se levels is mainly attributed to an imbalance in the levels of glutathione (GSH), thiols (SH), ferredoxins and/or NADPH, which can play vital roles in the assimilation of Se (Mroczek-Zdyrska and Wojcik, 2012).

Nevertheless, the possible mechanisms of the Se-enhanced tolerance of plants to environmental stresses have not been fully determined. Since exploring suitable ameliorants or stress alleviant is one of the tasks of plant biologists, there is no information about the physiological responses of the *L. iberica* to Se, which may be an increase salinity tolerance. In the study described here, *L. iberica* was examined with respect to the effects of Se on plant productivity and plant tolerance to salinity. The findings from this study give further insight into the evolution of the possible mechanisms of Se-enhanced tolerance of plants.

Materials and methods:

Plant material and harvest: Seeds of *Lallemantia iberica* L. were surface sterilized and germinated on filter paper moistened with distilled water. Ten-day-old seedlings were transferred to modified Hoagland nutrient solution (Johnson *et al*., 1957) for 30 days prior to the start of treatments. Composition of the modified Hoagland's solution is shown in Table 1. The pH of the nutrient medium was adjusted to 5.5–5.7. At 40 days after germination, the selenium (0.1, 1 or 10 mg l^{-1} Na_2SeO_4) and NaCl (0 or 80 mM) were applied together with the nutrient solution described above. Plants were grown under day/night temperature of 20-25/17-19 ºC, relative humidity of 60-65 % and daily photon flux density of about 800-950 µmol m^{-2} s^{-1} throughout the experimental period. Plants were harvested and analyzed

***Corresponding Author, Email: ebadi@uma.ac.ir**

[DOR: 20.1001.1.23222727.1396.6.20.4.4]

Table 1- Composition of a modified Hoagland nutrient solution was employed in the present study.

Macronutrients	Concentration	Micronutrients	Concentration
KNO_3	6 mM	H_3BO_3	50 µM
$Ca(NO_3)_2$	4 mM	$MnSO_4$	2 µM
$NH_4H_2PO_4$	2 mM	$ZnSO_4$	2 µM
$MgSO_4$	1 mM	$CuSO_4$	0.5 µM
		H_2MoO_4	0.5 µM
		$FeSO_4$-EDTA	0.02 mM

in a temporal (on different days after imposition of selenium and NaCl) manner. Full- grown and mature expanded leaves were used for measurement of enzymatic analysis. Shoots and roots were separated and washed with distilled water, blotted dry on filter paper and after determination of fresh weight (FW) they were dried for 48 h at 70 °C for determination of dry weight (DW).

Assay of antioxidative enzymes and related metabolites: The activities of superoxide dismutase (SOD, EC 1.15.1.1) and catalase (CAT, EC 1.11.1.6) were measured according to methods described elsewhere (Habibi and Hajiboland, 2012). The glutathione peroxidase (GSH-Px, EC 1.11.1.9) activity was evaluated by a moderation of the method of Flohé and Günzler (1984) using the H_2O_2 as substrate. Leaves were homogenized in ice bath with 50 mM phosphate buffer pH 7.0 and the supernatant was added to the reaction mixture contained 0.2 ml of the supernatant, 0.4 ml GSH (0.1 mM) and 0.2 mL $KNaHPO_4$ (0.067 M). After preheating the mixture on water bath at 28^{o}C for 5 min, 0.2 ml H_2O_2 (1.3 mM) was added to initiate the reaction. The reaction was stopped by adding 1 ml 1% trichloroacetic acid and the mixture was put into an ice bath for 30 min. Then, the mixture was centrifuged for 10 min at 1100 g, 0.48 ml the supernatant was placed into a cuvette and 2.2 ml of 0.32 M Na_2HPO_4 and 0.32 ml of 1.0 mM DNTB were added for colour development. The reaction was run for 5 min and measured at 412 nm. The enzyme activity was estimated as a decrease in GSH within the reaction time when compared with that in the non-enzyme reaction.

The hydrogen peroxide (H_2O_2) contents in the leaves were measured according to the method of Velikova *et al*. (2000). Leaves were homogenized in ice bath with 0.1% (w/v) TCA. The extract was centrifuged at 12,000 × g for 15 min, after which to 0.5 ml of the supernatant was added 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) and 1 ml of 1 M KI, the reaction was improved for 1 h in the dark and measured spectrophotometrically at 390 nm. The content of H_2O_2 was given on a standard curve.

Lipid peroxidation was assayed from the amount of malondialdehyde (MDA) formed in a reaction mixture containing thiobarbituric acid according to methods described elsewhere (Habibi and Hajiboland, 2012). The level of glutathione (GSH) was calculated according to Singh et al. (2006) with few modifications. Samples of 0.5 g were homogenized in 6% *m*-phosphoric acid (pH 2.8) containing 1 mM EDTA. Afterwards, two solutions were prepared. Solution A consisted of 110 mM $Na_2PO_4.7H_2O$, 80 mM $NaH_2PO_4.H_2O$, 15 mM EDTA, 0.3 mM 5, 5'–dithiobis (2-nitrobenzoic acid) and 0.4 ml l^{-1} BSA (final pH 7). Solution B was made up of 1 mM EDTA, 50 mM imidazole, 0.2 ml l^{-1} BSA and an equivalent of 1.5 units GR activity (Sigma). The absorbance at 412 nm was read after 2 min. The GSH concentration was determined from a standard curve by preparing solutions of 0.5–16 mM GSH.

Experiment was undertaken in complete randomized block design with 4 independent replications. Statistical analysis was carried out using Sigma Stat (3.5) with Tukey test. Results were given as mean ± standard deviation (SD). Differences between treatments were considered to be significant, when a P value was less than 0.05 ($P<0.05$).

Results and Discussion:

Selenium toxicity threshold in lemon balm during pre-experiment design: In pre-experiment design, we incorporated different concentrations of Se into the growth medium and thereafter measured the growth parameters in order to estimate the damage or favor effect of Se. Plants treated with a moderate concentration of Se (0.1 mg l^{-1} Se) exhibited an increase in plant dry weight with respect to plants in control medium (Table 2). In contrast, media supplemented by 10 mg l^{-1} Se induced an extreme stress for the growth parameters of *M. officinalis*, as demonstrated by the changes in plant dry weight was similar to that observed in 80 mM NaCl treatment (Table 2). Selenium plays two roles in plants: at low concentrations, it inhibits lipid peroxidation (Hawrylak-Nowak *et al*., 2015), whereas at high concentrations, it causes lipid peroxidation, membrane destruction and protein denaturation (Van Hoewyk, 2013). Our results coincide with those obtained in the field experiment of Zhang et al. (2014) and in the hydroponic experiment of Hawrylak-Nowak (2013); Se applied at high concentrations reduced the photosynthesis rate and plant's growth in rice and lettuce, respectively. Selenium toxicity in plants is imparted by formation of non-specific selenoproteins and selenium-induced oxidative stress (Van Hoewyk, 2013). Comparing the values of the toxicity threshold (Hawrylak-Nowak *et al*., 2015), it is obvious that lemon balm plant is a Se-sensitive plant, and the biomass of *Lallemantia iberica* plants decreased if selenate concentrations in the growth medium reached 10 mg l^{-1}. Thereafter, we used 0.1 mg l^{-1} Se to mitigate salt stress because growth at low-level Se was increased as growth at high-level Se (Table 2).

Selenium is an alleviant for the oxidative stress effects caused by NaCl: In this work, attention is focused on the effect of 80 mM NaCl since this

[DOR: 20.1001.1.23222727.1396.6.20.4.4]

Table 2- Effect of increasing levels of selenium (Se) and 80 mM NaCl on fresh and dry weight (g plant^{-1}), and Se content (mg kg^{-1} dry wt) in *L. iberica* plants.

Treatments	Fresh weight (g plant^{-1})	Dry weight (g plant^{-1})	Se content (mg kg^{-1} dry wt)
Shoots			
Control	2.33±0.30 ^{b}	0.30±0.03 ^{b}	-
0.1 mg l^{-1} Se	2.98±0.35 ^{a}	0.41±0.04 ^{a}	0.74±0.11 ^{b}
1 mg l^{-1} Se	2.18±0.34 ^{b}	0.27±0.02 ^{b}	0.99±0.12 ^{a}
10 mg l^{-1} Se	1.26±0.24 ^{c}	0.17±0.05 ^{c}	-
80 mM NaCl	1.06±0.15 ^{c}	0.14±0.03 ^{c}	-
NaCl+0.1 mg l^{-1} Se	2.08±0.13 ^{b}	0.25±0.04 ^{b}	0.80±0.10 ^{ab}
Roots			
Control	1.02±0.12 ^{a}	0.14±0.02 ^{a}	-
0.1 mg l^{-1} Se	1.07±0.10 ^{a}	0.16±0.03 ^{a}	0.62±0.12 ^{a}
1 mg l^{-1} Se	0.98±0.13 ^{a}	0.15±0.04 ^{a}	0.75±0.10 ^{a}
10 mg l^{-1} Se	0.66±0.11 ^{bc}	0.06±0.02 ^{b}	-
80 mM NaCl	0.58±0.07 ^{c}	0.05±0.02 ^{b}	-
NaCl+0.1 mg l^{-1} Se	0.89±0.09 ^{ab}	0.10±0.03 ^{a}	0.58±0.09 ^{a}

Data of each column within each defined plant part indicated by the same letter are not significantly different (*P*<0.05, Tukey test). Values are the mean ± SD (n=10).

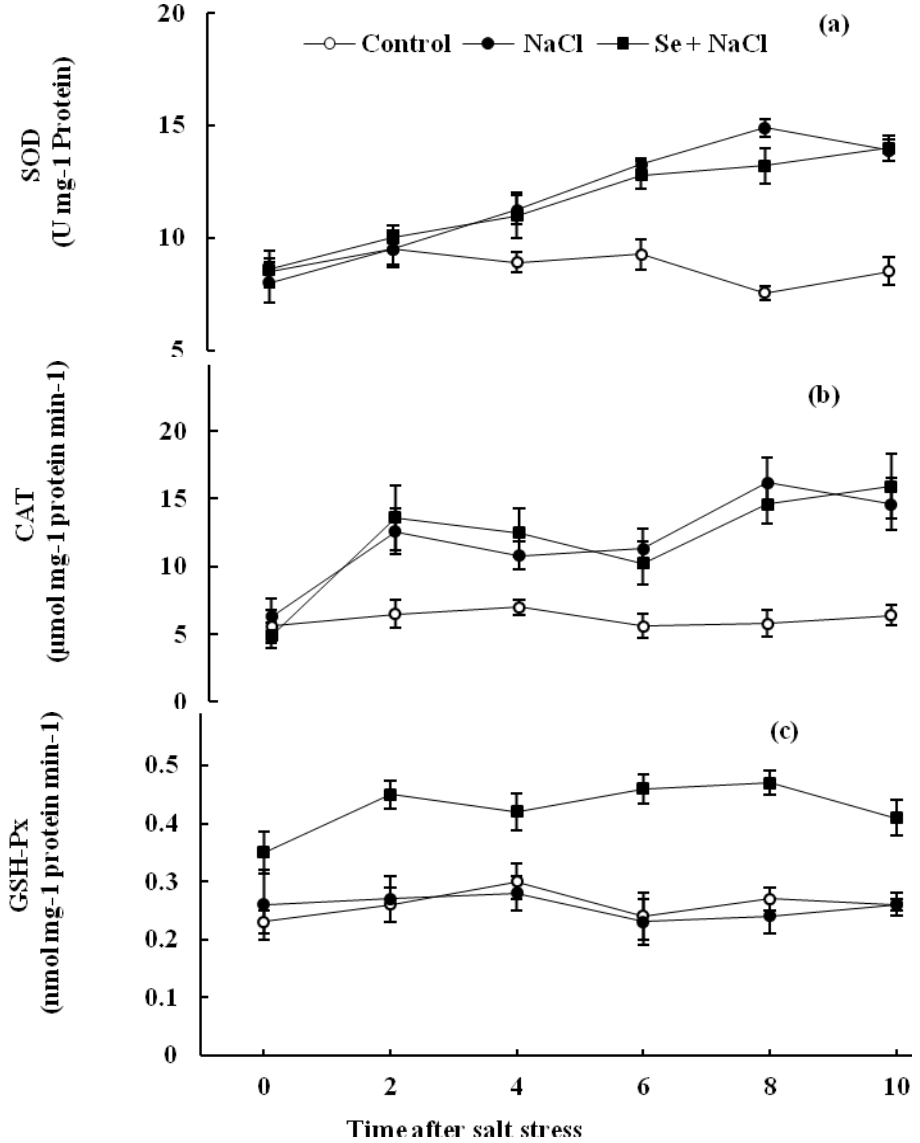

Fig. 1- Effect of Se supplementation on the activity of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) at different time intervals after NaCl treatment in *L. iberica* plants. Values are the mean ± SD (n=4).

[DOR: 20.1001.1.23222727.1396.6.20.4.4]

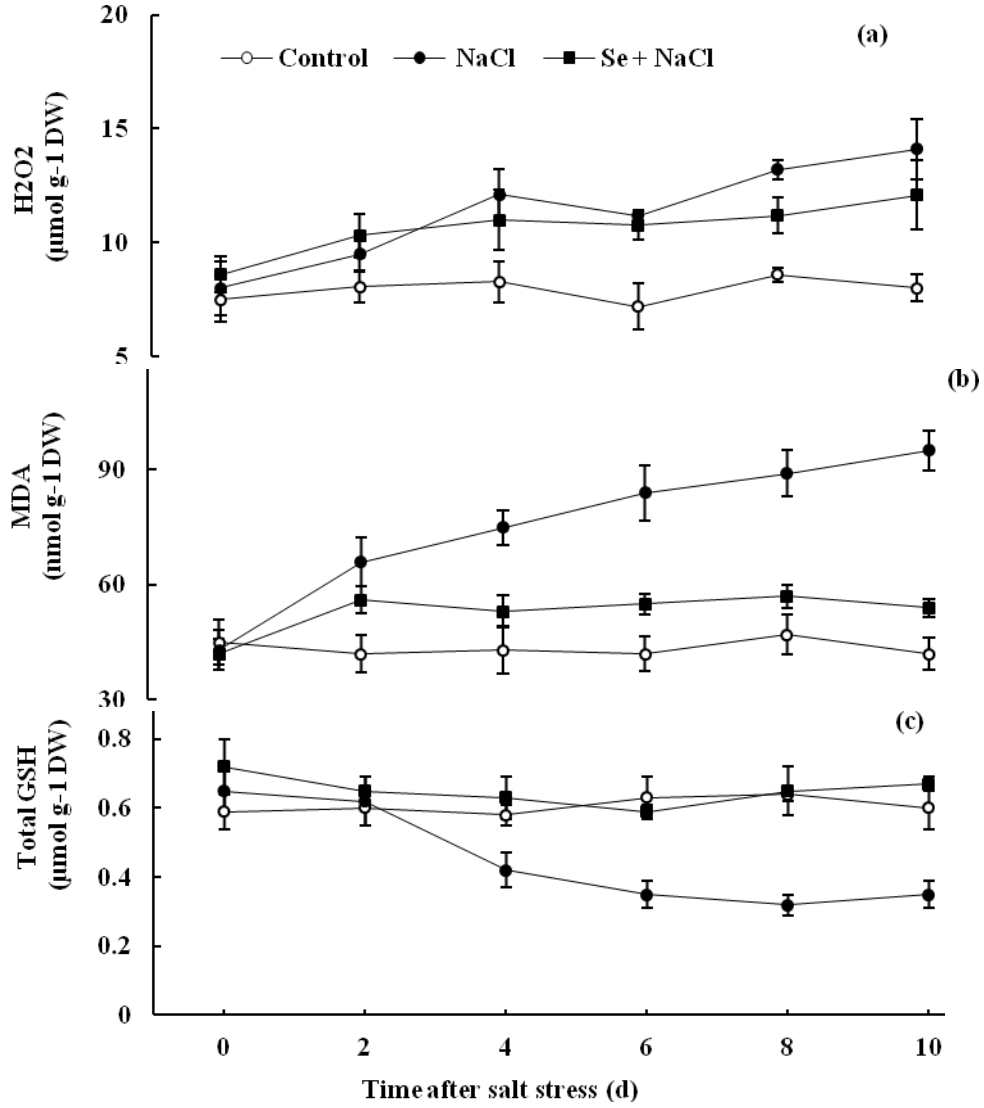

Fig. 2- Effect of Se supplementation on the concentration of hydrogen peroxide (H_2O_2), malondialdehyde (MDA) and glutathione (GSH) at different time intervals after NaCl treatment in *L. iberica* plants. Values are the mean ± SD (n=4).

treatment exhibits the toxic effect of Na ions. For plants treated with 80 mM NaCl, the biomass of roots and shoots decreased significantly, in contrast, the decline in growth parameters of *Lallemantia iberica* with respect to the control plants was not significant when media was supplemented by 0.1 mg l^{-1} Se (Table 2). Here, enrichment of NaCl-containing medium with 0.1 mg l^{-1} Se resulted in significant increase in roots and shoots biomass, when compared to NaCl-stressed plants grown without Se addition (Table 2). Similar responses were also observed in cucumber (Hawrylak-Nowak, 2009) and tomato (Diao *et al*., 2014) seedlings under salt stress. All of those results demonstrate that Se treatment results in the improvement of growth in plants exposed to salt stress.

Salt stress significantly increased the activity of SOD and CAT (Fig. 1). Interestingly, Se-supplied plants exhibited higher GSH-Px activity than –Se ones after 2 days salt stress. Therefore, a significantly rise in the activity of GSH-Px showed that Se exerts beneficial effects on salt stress tolerance by enhancing their antioxidative capacity (Hasanuzzaman *et al*., 2011). Like other abiotic stress conditions, salt stress increases accumulation of ROS which lead to oxidative stress of plants (Mittova *et al*., 2003). The occurrence of oxidative stress upon salt treatments was determined by the accumulation of MDA, a marker for the ROS-mediated cell membrane damage (Davey *et al*., 2005). In this study, a significant increase of MDA concentrations was observed under salt stress (Fig. 2), however, treatment of seedlings with Se was effective in reducing leaf MDA concentrations. In the current experiment, the greater induction of the synthesis and accumulation of antioxidants, such as GSH was observed by Se (Fig. 2*c*). These results are similar to those reported by Ríos et al. (2008) as these authors observed that the application of selenate in general more strongly induced the synthesis and accumulation of GSH antioxidant compounds in lettuce plants. The result of GSH is important in this work, as it indicates

[DOR: 20.1001.1.23222727.1396.6.20.4.4]

the degree of S assimilation and therefore the possibility of Se toxicity (Ríos *et al*., 2008). At higher Se levels, glutathione depletion is increased, and this leads to the accumulation of reactive oxygen species (ROS) and thereby induces oxidative stress. Therefore, our results are novel and could define the application of Se at low concentration under salinity stress resulted in greater formation of GSH and lower ROS production and therefore less reduction of biomass production with respect to plants treated with salinity alone.

Conclusions:

Our results indicated that Se at high concentrations may lead to toxicity and causes damage, as the biomass of *Lallemantia iberica* plants decreased when selenate concentrations in the growth medium reached 10 mg l^{-1}. However, low-level Se pretreatment during salt stress improved shoot growth of *Lallemantia iberica* and alleviated salt-induced oxidative stress.

References:

Chu, J., Yao, X., Zhang, Z. (2010) Responses of wheat seedlings to exogenous selenium supply under cold stress. Biological Trace Element Research 136: 355–363.

Davey, M.W., Stals, E., Panis, B., Keulemans, J. and Swennen, R. L. (2005) High-through put determination of malondialdehyde in plant tissues. Analytical Biochemistry 347: 201–207.

Diao, M., Ma, L., Wang, J. W., Cui, J. X., Fu, A. F., Liu, H. Y. (2014) Selenium promotes the growth and photosynthesis of tomato seedlings under salt stress by enhancing chloroplast antioxidant defense system. Journal Plant Growth Regululation 33: 671–682.

Djanaguiraman, M., Prasad, P. V. V., Seppanen, M. (2010) Selenium protects sorghum leaves from oxidative damage under high temperature stress by enhancing antioxidant defense system. Plant Physiol. Biochem. 48: 999–1007.

Flohe, L., Gunzler, W. A. (1984) Assays of Glutathione Peroxidase, in: Methods in Enzymology. Academic Press, New York, pp. 114–121.

Habibi, G., Hajiboland, R. (2012) Comparison of photosynthesis and antioxidative protection in *Sedum album* and *Sedum stoloniferum* (Crassulaceae) under water stress. Photosynthetica 50: 508–518.

Habibi, G. (2013) Effect of drought stress and selenium spraying on photosynthesis and antioxidant activity of spring barley. Acta Agriculturae Slovenica 101: 167-177.

Hasanuzzaman, M., Fujita, M. (2011) Selenium pretreatment upregulates the antioxidant defense and methylglyoxal detoxification system and confers enhanced tolerance to drought stress in rapeseed seedlings. Biological Trace Element Research 143: 1758–1776.

Hasanuzzaman, M., Hossain, M. A., Fujita, M. (2011) Selenium-induced up-regulation of the antioxidant defense and methylglyoxal detoxification system reduces salinity-induced damage in rapeseed seedlings. Biol. Trace Elem. Res. 143: 1704–1721.

Hawrylak-Nowak, B. (2009) Beneficial effects of exogenous selenium in cucumber seedlings subjected to salt stress. Biological Trace Element Research 132: 259–269.

Hawrylak-Nowak, B., Matraszek, R., Pogorzelec, M. (2015) The dual effects of two inorganic selenium forms on the growth, selected physiological parameters and macronutrients accumulation in cucumber plants. Acta Physiology Plant 37: 41–53.

Johnson, C. M., Stout, P. R., Broyer, T. C., Carlton, A. B. (1957) Comparative chlorine requirements of different plant species. Plant and Soil 8: 337–353.

Kong L., Wang M., Bi D. (2005) Selenium modulates the activities of antioxidant enzymes, osmotic homeostasis and promotes the growth of sorrel seedlings under salt stress. Plant Growth Regulation 45: 155–163.

Mittova, V., Theodoulou, F.L., Kiddle, G., Gomez, L., Volokita, M., Tal, M. (2003) Coordinate induction of glutathione biosynthesis and glutathione-metabolizing enzymes is correlated with salt tolerance in tomato. FEBS Letters 554: 417–421.

Mroczek-Zdyrska, M., Wojcik, M. (2012) The influence of selenium on root growth and oxidative stress induced by lead in *Vicia faba* L. minor plants. Biological Trace Element Research 147: 320–328.

Parida A. K., Das A. B. (2005) Salt tolerance and salinity effects on plants: a review. Ecotoxicology and Environmental Safety 60: 324–349.

Rios, J. J., Blasco, B., Cervilla, L. M., Rosales, M. A., Sanchez-Rodriguez, E., Romero, L., Ruiz, J. M. (2009) Production and detoxification of H_2O_2 in lettuce plants exposed to selenium.

Singh, N., Ma, L. Q., Srivastava, M., Rathinasabapathi, B. (2006) Metabolic adaptations to arsenic-induced oxidative stress in *Pteris vittata* L and *Pteris ensiformis* L. Plant Science 170: 274–282.

Van Hoewyk, D. (2013) A tale of two toxicities: malformed selenoproteins and oxidative stress both contribute to selenium stress in plants. Annals of Botany 112: 965–972.

Velikova, V., Yordanov, I., Edreva, A. (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants-protective role of exogenous polyamines. Plant Science 151: 59–66.

Yao, X., Chu, J., Ba, C. (2010) Responses of wheat roots to exogenous selenium supply under enhanced ultraviolet-B. Biological Trace Element Research 137: 244–252

[DOR: 20.1001.1.23222727.1396.6.20.4.4]

Zhang, Y., Gladyshev, V. N. (2010) General trends in trace element utilization revealed by comparative genomic analyses of Co, Cu, Mo, Ni and Se. Journal Biology Chemistry 285: 3393–3405

Zhang, M., Tang, S., Huang, X., Zhang, F., Pang, Y., Huang, Q., Yi, Q. (2014) Selenium uptake, dynamic changes in selenium content and its influence on photosynthesis and chlorophyll fluorescence in rice (*Oryza sativa* L.). Environ. Exprimental Botany 107: 39–45.

[DOR: 20.1001.1.23222727.1396.6.20.4.4]