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SELENITE IS MORE EFFICIENT THAN SELENATE IN ALLEVIATION OF SALT STRESS IN LETTUCE PLANTS

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There is increasing evidence showing that low selenium (Se) concentrations may increase tolerance of crop plants to several environmental stresses. The aim of this study was to compare the influence of two chemical forms of Se (selenite or selenate) at different concentrations (2 or 6 μM) on the resistance of butterhead lettuce (*Lactuca sativa* L. var. *capitata*) cv. Justyna to NaCl-induced stress (40 mM NaCl). Plant growth was negatively affected by salinity, but the level of photosynthetic pigments was not reduced. Se application at a concentration of 2 μM significantly improved the growth of salt-stressed plants, but selenite was much more effective than selenate in enhancing salt-tolerance of lettuce. The growth-promoting effect of Se was also noted at 6 μM of selenite, but did not appear at 6 μM of selenate. The beneficial effect of Se in salt-stressed lettuce could be due to antioxidative activity of Se, root system growth stimulation, and/or increase in photosynthetic pigment concentration after Se supplementation; however, it was not related to either increase in proline accumulation or reduction in foliar Na^+ or Cl^- concentration. These results imply that Se application, especially in the form of selenite, can enhance antioxidant defense of lettuce under salt stress, and Se supplementation may be recommended for areas of lettuce cultivation with excessive salt accumulation.

Key words: *Lactuca sativa* L., oxidative stress, proline, salinity, selenium

INTRODUCTION

Soil salinity is one of the major factors limiting production of crop plants, particularly in arid and semi-arid regions; however, the problem of excessive salt accumulation occurs worldwide. Salt stress induces a wide range of physiological and biochemical changes in salt sensitive plants (Kumar et al., 2003). The adverse effects of high salinity on plants are related to the following factors: (1) low water potential of soil solution (water stress), (2) nutritional imbalance and disturbing ionic homeostasis (ionic stress), (3) specific ion effect (salt stress), (4) overproduction of reactive oxygen species – ROS (oxidative stress) (Parvaiz and Satyawati, 2008; Hasanuzzaman et al., 2013). In recent years, exogenous protectants such as osmoprotectants, phytohormones, polyamines, antioxidants and various trace elements have been found useful to alleviate the salt-induced damages (Hasanuzzaman et al., 2013). One of these protectants, which showed the ability to improve growth and stress tolerance of plants grown under excessive salinity, is selenium (Se). Selenium is an essential trace element for humans and animals; however, the question of whether or not Se is a micronutrient for higher plants is still considered unexplained. Although Se has not been classified as an essential plant nutrient, its role as a beneficial trace element in plant organisms (especially in Se hyperaccumulators) has been confirmed in a lot of research (Kopsell and Kopsell, 2007 and references therein). Moreover, Se-enriched crops have shown enhanced tolerance to certain abiotic stresses, e.g. drought (Yao et al., 2012), salinity (Kong et al., 2005, Diao et al., 2014), and excess of some heavy metals (Filek et al., 2008; Mozafariyan et al., 2014). The protective role of low Se concentrations in plants exposed to stressful conditions in most cases has been attributed to activation of antioxidative defense systems in plant cells (Kaur et al., 2014; Sieprawska et al., 2015).

Lettuce is a very popular leafy vegetable and ranks high in both production and economic value

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among all vegetables. It is an important source of dietary antioxidants, particularly those with high peroxyl radical scavenging activity (Oh et al., 2009). Lettuce was demonstrated to be a moderately salt sensitive vegetable crop (Shannon and Grieve, 1999). Although some reports have shown that Se presents a promising potential for use in the conditions of salt stress (Hawrylak-Nowak, 2009; Walaa et al., 2010; Hasanuzzaman et al., 2011; KeLing et al., 2013), the influence of the applied chemical form of Se on its activity in salt-stressed plants had not been studied earlier. Therefore, the aim of the present study was to compare the effectiveness of two inorganic chemical forms of Se (selenate and selenite) in mitigation of NaCl-induced salt stress in lettuce plants.

MATERIALS AND METHODS

PLANT MATERIAL, GROWTH CONDITIONS AND TREATMENTS

The experiments were carried out on butterhead lettuce (*Lactuca sativa* L. var. *capitata*) cv. Justyna grown in a controlled-climate vegetation chamber (Sanyo MRL 350 HT). Lettuce seeds germinated in wet quartz sand at 25°C for 14–18 days. The best-developed seedlings were transferred to 1 L glass jars (two plants each) filled with 1.5-times strength Hoagland's No. 2 nutrient solution (Hoagland and Arnon, 1950), with about 3 cm air space above the level of the solution in order to improve breathing of the root system. The pH of the growth media was adjusted to 6.0 using diluted NaOH. Then, the medium was differentiated in regard to the concentration of NaCl (0 or 40 mM) and Se (0, 2 or 6 μM) applied as selenite (Se IV; Na_2SeO_3) or selenate (Se VI; Na_2SeO_4). The experimental treatments were as follows: control (0 NaCl/0 Se), 40 mM NaCl, 40 mM NaCl + 2 μM Se IV, 40 mM NaCl + 6 μM Se IV, 40 mM NaCl + 2 μM Se VI, 40 mM NaCl + 6 μM Se VI.

The plants were cultured at PPFD of 250–270 μmol m^{-2} s^{-1}, 12-h day length, temperature 22/18°C (day/night) and relative humidity of 50–60%. The nutrient solution was aerated for 15 min every three days and replenished with a full-strength Hoagland's solution, when the medium level was depleted to about 70% of the initial level. After 21 days of plant growth under the differentiated conditions, the biometric and the physiological parameters were determined. Then, the plant samples were dried at 70°C and ground before analysis of the Na^+, K^+, Cl^-, and total Se concentrations.

PLANT ANALYSIS

The effect of NaCl on lettuce growth was evaluated by determining the root and shoot fresh weight (FW) as well as the leaf area (LA). Fresh second true leaves were scanned using a CI-202 laser area meter (CID Bio-Science, USA) and the LA was expressed in square centimetres (cm^2). The photosynthetic pigment concentrations were determined in the second true leaves by extraction of the fresh leaf samples in 80% acetone. The concentrations of chlorophyll *a* and *b* and total carotenoids were calculated from the equations given by Lichtenthaler and Wellburn (1983).

The oxidative stress intensity was determined by measuring the biomembrane lipid peroxidation and hydrogen peroxide (H_2O_2) accumulation in the second true leaves and roots. The level of lipid peroxidation was measured by estimating the concentration of thiobarbituric acid reactive substances (TBARS) following the method of Heath and Packer (1968), with slight modifications (Hawrylak-Nowak, 2013). The concentration of H_2O_2 was measured colorimetrically by the method of Jana and Choudhuri (1982), with slight modifications described previously (Hawrylak-Nowak, 2013).

Proline colorimetric determination proceeded according to Bates et al. (Bates et al., 1973). Briefly, fresh leaf samples were homogenized in aqueous solution of 3% (w:v) sulphosalicylic acid. For proline determinations, the plant extract, acid ninhydrin and glacial acetic acid (1:1:1) were incubated at 100°C for 1 h. The reaction was terminated in an ice bath. The reaction mixture was extracted with 4 mL of toluene and the chromophore-containing toluene phase was sucked. Proline content was measured spectrophotometrically at 520 nm using toluene as a blank and calculated as μg g^{-1} FW against standard proline.

For the measurements of K^+, Na^+, and Cl^- content in the aboveground organs, the plant samples were dry-ashed in a muffle furnace at 500°C for 6 h. The content of Cl^- was determined by a nephelometric method using acetic acid and silver nitrate (Nowosielski, 1974). The atomic absorption spectrometry (AAS) was used for the analysis of K^+ and Na^+ concentrations (Nowosielski, 1974). Hydride generation atomic absorption spectroscopy (HG-AAS) was used to determine the total Se concentrations, according to the method described in the previous paper (Hawrylak-Nowak, 2013).

STATISTICAL ANALYSIS

The experiments involved six treatments and four replications per each treatment. The values in the figures and tables represent the means (± SD) from three independent experiments. All the data were subjected to one-way analysis of variance (ANOVA) with the Tukey's post hoc test at $p < 0.05$.

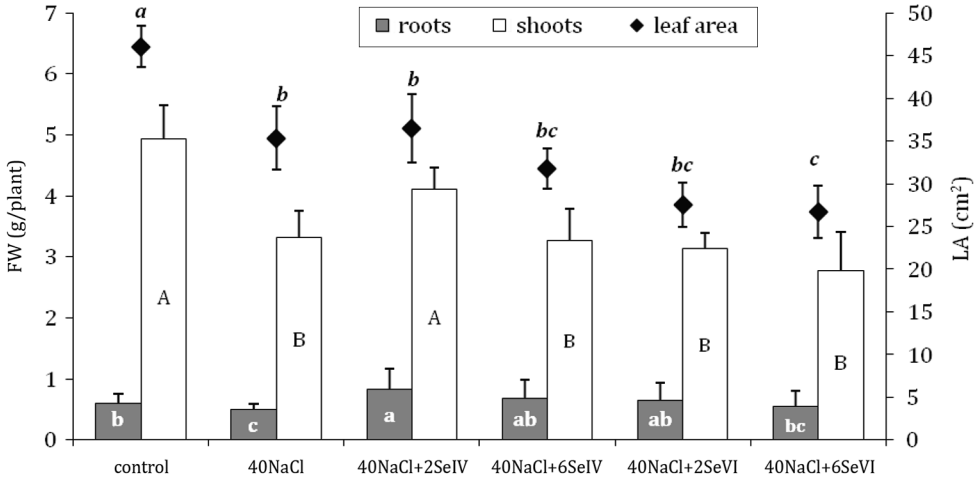

Fig. 1. The effect of selenium supplementation on fresh weight (FW) and leaf area (LA) of NaCl-stressed lettuce plants. The mean values (±SD, n=24) followed by different letters indicate significant differences between treatments at $p < 0.05$.

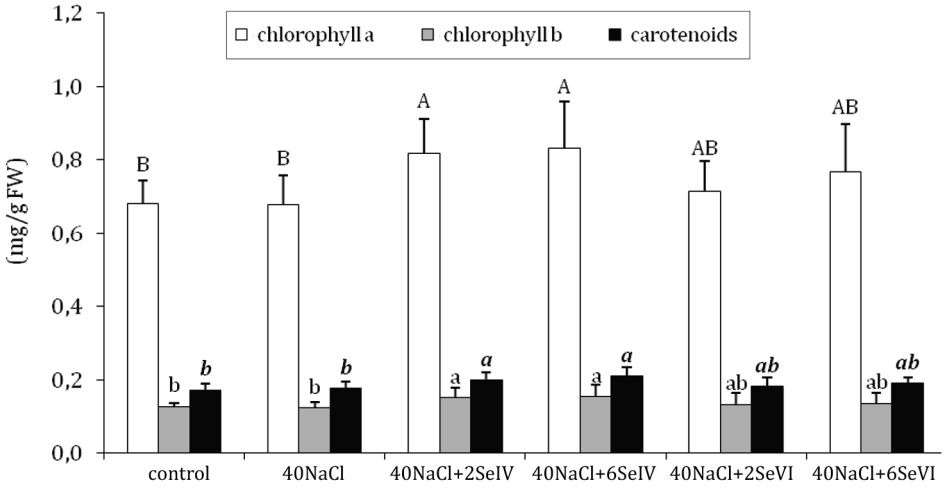

Fig. 2. The effect of selenium supplementation on photosynthetic pigments concentration in leaves of NaCl-stressed lettuce plants. The mean values (±SD, n=9) for each pigment class followed by different letters indicate significant differences between treatments at $p < 0.05$.

RESULTS

Under 40 mM NaCl treatment the growth of lettuce was negatively affected, as root and shoot FW were reduced by 19 and 33%, respectively, compared to the control. Also, the LA was decreased by 23% (Fig. 1). The addition of 2 or 6 μM Se as selenite to the NaCl-containing medium caused an increase in the root FW by 69 and 37%, respectively, compared to NaCl-alone treatment. The FW of shoots of NaCl-stressed plants supplemented with 2 μM Se IV was 24% higher than in the plants grown with NaCl alone. However, an increase in the selenite concentration to 6 μM did not have a positive effect on the shoot FW. The application of selenate was beneficial for salt-exposed plants only when selenate was applied at a concentration of 2 μM, since under these conditions the root FW increased by 32%, compared to NaCl-alone treatment. The addition of Se, regardless of its chemical form and concentration, did not positively affect the LA of the NaCl-exposed plants (Fig. 1). Thus, it seems that Se can positively affect NaCl-treated plants mainly by the root system growth stimulation.

Although the NaCl-induced stress did not impair the accumulation of photosynthetic pigments, the application of Se as selenite evoked significant increase in the concentration of chlorophylls and carotenoids, compared to both the NaCl-exposed and the control plants. Nonetheless, this phenomenon has not been observed in plants supplemented with selenate (Fig. 2).

TABLE 1. The effect of Se supplementation on H_2O_2, thiobarbituric acid reactive substances (TBARS), and proline concentrations in NaCl-stressed lettuce plants.

Treatment		H_2O_2 (nmol/g FW)		TBRAS (nmol/g FW)		Proline (µg/g FW)
NaCl	Se	root	leaf	root	leaf	
0	0	3.67^{b}	2.89^{d}	4.81^{b}	6.57^{c}	13.38^{b}
40 mM	0	5.25^{a}	3.66^{c}	6.25^{a}	8.81^{a}	21.60^{a}
40 mM	2 µM Se IV	3.92^{b}	3.64^{c}	4.81^{b}	7.76^{b}	22.76^{a}
40 mM	6 µM Se IV	5.21^{a}	4.30^{b}	6.57^{a}	6.25^{c}	22.84^{a}
40 mM	2 µM Se VI	4.93^{ab}	3.81^{c}	6.66^{a}	7.21^{bc}	25.11^{a}
40 mM	6 µM Se VI	4.83^{ab}	5.13^{a}	5.45^{ab}	6.25^{c}	22.42^{a}

Means (n = 6) sharing the same letter in a column do not differ significantly at $p < 0.05$

TABLE 2. The effect of Se supplementation on Na^+, Cl^-, K^+, and total Se concentrations in shoots of NaCl-stressed lettuce (on dry weight basis).

Treatment		Na^+ (%)	Cl^- (%)	K^+ (%)	Se (mg/kg)
NaCl	Se				
0	0	0.09^{b}	1.01^{b}	8.75^{a}	0.14^{e}
40 mM	0	2.46^{a}	3.91^{a}	6.38^{b}	0.05^{e}
40 mM	2 µM Se IV	2.44^{a}	3.70^{a}	6.33^{b}	7.00^{d}
40 mM	6 µM Se IV	2.63^{a}	3.90^{a}	5.60^{c}	17.10^{b}
40 mM	2 µM Se VI	2.54^{a}	3.88^{a}	5.70^{c}	9.10^{c}
40 mM	6 µM Se VI	2.55^{a}	3.95^{a}	5.90^{bc}	21.50^{a}

Means (n = 3) sharing the same letter in a column do not differ significantly at $p < 0.05$

NaCl stress induced accumulation of TBARS (an indicator of lipid peroxidation) in root and leaf tissues by 30 and 34%, respectively, compared to the control. The increase in lipid peroxidation was accompanied by an increase in the concentration of H_2O_2 in roots and leaves by 43 and 26%, respectively (Table 1). In the NaCl-exposed plants supplemented with 2 µM Se as selenite, a significant reduction in TBARS concentration was found in both roots and leaves, compared to the NaCl alone treatment. Under these conditions also H_2O_2 level was lowered, but only in roots. In the selenate supplied plants, the foliar TBARS concentration was also lower than that under NaCl alone treatment, whereas H_2O_2 level was not decreased, neither in leaves nor in roots (Table 1).

Under salt stress the concentration of proline was approx. 60% greater than that in the control plants. In the salt-exposed plants supplemented with Se, regardless of its form and concentration, the level of proline was still elevated and was not significantly different form the plants grown with NaCl alone (Table 1).

The foliar Na^+ and Cl^- contents notably increased after NaCl treatment, while the K^+ content was lowered. The level of Na^+ and Cl^- in the salt-stressed plants was unaffected by Se addition. Moreover, in the plants supplied with 6 µM Se IV and 2 µM Se VI the reduced K^+ level was noted, compared to the NaCl alone treatment (Table 2). In the NaCl-treated plants supplied with selenate, the concentrations of Se in the shoots were higher than in the plants supplied with selenite (Table 2).

DISCUSSION

Increased salinity of cultivated soils is expected to have destructive global effects, resulting in up to half land loss in the next couple of decades. The adverse impact of salinity has been attributed mainly to an increase in the accumulation of Na^+ and Cl^- ions. Over-accumulation of these ions generates many physiological disorders in plants, but Cl^- ions are considered to be more toxic than Na^+ (Hasanuzzaman et al., 2013). The results of previous studies indicated that exogenously applied Se both in the form of selenate (Kong et al., 2005; Hawrylak-Nowak, 2009; Hasanuzzaman et al., 2011) and selenite (Walaa et al., 2010; KeLing et al., 2013; Diao et al., 2014) can be beneficial for plants grown under salt stress. Nevertheless, in the above studies the effectiveness of selenate and selenite in alleviation of salinity stress was not compared. The

present results support the beneficial role of Se, and further indicate that the chemical form of Se applied (not concentration only) may be of significant importance for positive effect of Se on NaCl-exposed plants.

In this study, the NaCl alone treatment did not adversely affect the photosynthetic pigment concentration. On the other hand, the application of selenite (but not selenate) to the NaCl containing medium induced an increase in the concentration of chlorophylls and carotenoids. In the previous study (Hawrylak-Nowak, 2009), it was found that 5 μM Se applied as selenate enhanced accumulation of both chlorophylls and carotenoids in cucumber plants under salt stress. However, in cucumber, as opposite to lettuce examined in this study, the exposition of plants to NaCl provoked a decrease in the concentration of photosynthetic pigments. Diao et al. (2014) suggest that Se alleviates salt-induced stress in tomato seedlings through regulating the antioxidant defense systems in the chloroplasts which is associated with the improvement of the photochemical efficiency of PSII.

A wide range of environmental stresses cause overproduction of ROS which may lead to enhanced lipid peroxidation. One of the causes of lipid peroxidation is accumulation of H_2O_2 which, like other ROS, attacks lipid membranes (Hawrylak-Nowak, 2013; KeLing et al., 2013). These results, indicating antioxidant activity of Se in the salt-stressed plants, are in agreement with those obtained by Kong et al. (2005), Hawrylak-Nowak (2009), Walaa et al. (2010), Hasanuzzaman et al. (2011), KeLing et al. (2013) and Diao et al. (2014). However, in this study the highest growth-stimulating effect of exogenous Se noted at 2 μM Se IV was associated with simultaneous reduction of lipid peroxidation and H_2O_2 accumulation, mainly in the root tissues. This phenomenon may partly explain why the growth of roots was more stimulated under the influence of Se than the growth of shoots.

Proline accumulation is one of the adaptations of plants to salt stress and water deficit. Some researchers did not observe any considerable increase in free proline content, whilst others consider increased proline accumulation merely a stress effect, rather than a cause of stress tolerance (Kumar et al., 2003). In this study, an increase in proline level in the NaCl-exposed lettuce was found. In the salt-stressed plants supplemented with Se, the level of free proline was not significantly different form plants treated with NaCl alone. In contrast, supplementation of NaCl-stressed cucumber plants with selenate (Hawrylak-Nowak, 2009) or selenite (Walaa et al., 2010) caused an increase in the free proline level, compared to NaCl-alone treatment. However, the reasons for increased proline content in Se-supplied plants grown under salt stress have not been studied and are not known. Moreover, in the previous study on cucumber (Hawrylak-Nowak, 2009), exposition of plants to NaCl alone, as opposite to lettuce examined in this study, did not have any significant effect on free proline accumulation.

Although in the NaCl-exposed cucumber plants supplemented with Se the reduced Cl^- content was found (Hawrylak-Nowak, 2009), these studies did not confirm this phenomenon. In the experiments by Kong et al. (2005) and Hawrylak-Nowak (2009), Se supply generally neither blocked the uptake of Na^+, nor promoted the uptake of K^+. However, the reduced K^+ content in Se-treated lettuce noted in these studies is the first reported instance where Se negatively influenced K^+ level in the NaCl-exposed plants.

The NaCl-exposed plants supplemented with selenate contained higher concentrations of Se in the aboveground organs than plants supplied with selenite, which confirms the well-known better translocation of selenate than selenite (Kopsell and Kopsell, 2007). Comparing the foliar Se concentrations achieved in lettuce under the same conditions but without NaCl (Hawrylak-Nowak, 2013), it was found that in the NaCl-exposed lettuce the accumulation of Se was higher than in that grown without salt. Therefore, it may be suggested that NaCl can stimulate Se uptake under hydroponic conditions. Similar relationships were found by Wu et al. (1988), who revealed that Se uptake was increased by addition of NaCl, whereas Na^+ uptake was not affected by the presence of Se. In contrast, Diao et al. (2014) found that the Se contents in leaves of tomato grown under salt stress with Se addition were significantly lower than those under non-saline conditions with Se only. Therefore, it is difficult to clearly state how NaCl affects Se uptake, because it can be varied depending on plant species and experimental conditions.

CONCLUSIONS

Based on the above results, selenite can be recommended for supplementation of lettuce plants in the areas affected by excessive salinity. Application of 2 μM Se as selenite was found more effective in alleviating perilous effect of salt stress as compared to other treatments. Since these experiments were conducted under hydroponic conditions, and in the field many other factors appear, in the next step field experiments should complete those undertaken in this study.

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