

Alleviation of nickel toxicity in wheat (*Triticum aestivum* L.) seedlings by selenium supplementation

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(Received on 28 February 2013; Accepted on 18 September 2013)

Abstract: Hydroponically grown wheat seedlings were treated with 50 μM Ni and/or 15 μM Se. After a 7-day culture period, their growth parameters, Ni, Se, Fe, and Mg contents, electrolyte leakage, photosynthetic pigment concentrations, and photochemical activity of photosystem II were determined. Exposure of wheat seedlings to Ni alone resulted in reduction in the total shoot and root lengths, by 22% and 50%, respectively. Addition of Se to the Ni-containing medium significantly improved the growth of these organs, compared to the seedlings subjected to Ni alone. Application of Se decreased the accumulation of Ni in shoots and roots and partially alleviated the Ni-induced decrease in Fe and Mg concentrations in shoots. Electrolyte leakage increased in response to Ni stress, but in shoots it was diminished by Se supplementation. Exposure to Ni led to a decrease in chlorophyll *a* and *b* contents and enhancement of chlorophyll *a/b* ratio, but did not influence the concentration of carotenoids. Enrichment of the Ni-containing medium with Se significantly increased chlorophyll *b* content, compared to the seedlings treated with Ni alone. Photochemical activity, estimated in terms of the maximum quantum yield of photosystem II, decreased in response to Ni treatment but was significantly improved by simultaneous addition of Se. Results of our study suggest that alleviation of Ni toxicity in wheat seedlings by Se supplementation may be related to limitation of Ni uptake.

Keywords: chlorophyll, electrolyte leakage, nickel stress, photochemical activity, selenium

INTRODUCTION

Nickel (Ni) is classified as a micronutrient and in small quantities is essential for plant growth (MARSCHNER 1995). However, its excess is toxic to most plant species, which is evidenced by growth reduction and occurrence of other symptoms,

such as leaf chlorosis and necrosis. Our earlier study indicated that Ni phytotoxicity may be at least partly attributed to its ability to induce oxidative stress (GAJEWSKA & SKŁODOWSKA 2007) and disturbance of nitrogen metabolism (GAJEWSKA & SKŁODOWSKA 2009). This heavy metal has also been reported to interfere with mineral nutrition (PANDEY & SHARMA 2002; OUZOUNIDOU et al. 2006), water relations (PANDEY & SHARMA 2002), respiration (LLAMAS & SANZ 2008), and photosynthesis (SHEORAN et al. 1990; DRAŹKIEWICZ & BASZYŃSKI 2010). Negative effects of Ni on physiological and metabolic processes may result in decreased biomass production and, consequently, considerably reduced yields of crop plants.

Selenium (Se), although not considered to be an essential nutrient for plants, at low doses has been shown to promote their growth. The beneficial effect of Se on plants is usually ascribed to its ability to enhance antioxidative capacity of their cells (XUE et al. 2001; HASANUZZAMAN et al. 2012). There is also some evidence that Se may increase the tolerance of plants to various abiotic stress factors, such as UV radiation (PENNANEN et al. 2002), salinity (KONG et al. 2005), and heavy metals (FILEK et al. 2008). Nevertheless, at high concentrations this metalloid is toxic to plants. This is related to the competition between selenium and sulphur, leading to synthesis of selenocysteine and selenomethionine, and subsequent production of proteins with disturbed function and structure (HASANUZZAMAN et al. 2010).

The aim of our study was to investigate the ability of Se to alleviate Ni stress in wheat seedlings. Our study was focused on the effect of Ni and Se used separately and in combination on growth parameters, membrane permeability, photosynthetic pigment concentrations, and photochemical activity of photosystem II (PSII). Since chlorophyll content and, therefore, photosynthetic process depend on the availability of Mg and Fe in plant tissues, we also measured concentrations of these elements.

MATERIAL AND METHODS

Plant material and growth conditions

Wheat (*Triticum aestivum* L., cv. Zyta) seeds were germinated in the dark at 24°C for 2 days. Next the seedlings were transferred into diluted (1:4) Hoagland nutrient solution containing 50 µM Ni (NiSO₄) and 15 µM Se (Na₂SeO₃) supplied individually or simultaneously. These concentrations were chosen on the basis of the preliminary experiment, showing that Se at the dose of 15 µM (but not lower) is able to ameliorate wheat seedling growth affected by 50 µM Ni, the dose that we used in our earlier studies (GAJEWSKA & SKŁODOWSKA 2009; GAJEWSKA et al. 2012). The seedlings grown in the nutrient solution without addition of Ni and Se were considered as the control. All the seedlings were grown in a growth room at 24°C with 16-h photoperiod (photosynthetic photon flux density 175 µmol m⁻² s⁻¹). After a 7-day culture period, the growth parameters, concentrations of Ni, Se, Fe, Mg, and photosynthetic pigments, electrolyte leakage as well as photochemical activity of PSII were estimated.

Growth parameters

Growth parameters were estimated by an automatic analysis of scanned images of shoots and roots. The images were acquired as JPEG files by using a standard

flatbed scanner. The shoots and roots were scanned separately at 300 dpi and 600 dpi resolutions, respectively, using highly saturated blue background. Such background facilitates distinguishing of plant parts, assuming that no blue colour can be observed in the plant tissue. The scanned images were processed digitally, using specialized software developed for this purpose in cooperation with the Institute of Applied Computer Science, Łódź University of Technology, Poland. The software includes MATLAB scripts calling appropriate library functions of image analysis (THE MATHWORKS INC. 2011a) implemented as C++ MEX files (THE MATHWORKS INC. 2011b). Mathematical description and validation tests of the applied computer measurement methods for wheat shoot and root systems were published previously (GOCLAWSKI et al. 2009; SEKULSKA-NALEWAJKO & GOCLAWSKI 2011). The total shoot length, lengths of the 1st and the 2nd leaves as well as the total root length and lengths of primary and secondary roots were determined.

Analysis of elements and estimation of electrolyte leakage

For determination of element concentrations, samples of shoots and roots (150 mg DW each) were wet-digested in a mixture (5:1, v/v) of 70% HNO₃ and 30% H₂O₂, using a microwave mineralizer. Concentrations of Ni, Fe, and Mg were determined by atomic absorption spectrometry (SpectrAA 300, Varian). Prior to Se analysis, Se⁶⁺ present in the samples was reduced to Se⁴⁺ by boiling the mineralizates with addition of 6 M HCl for 1.5 h. Selenium was determined using the method of WATKINSON (1966) based on measurement of fluorescence of cyclohexane-extracted selenite-2,3-diaminonaphthalene complex at 370.0 nm (excitation) and 520.0 nm (emission). Electrolyte leakage from shoot and root cells was evaluated conductometrically as described earlier (GAJEWSKA et al. 2012).

Photosynthetic pigment concentrations and photochemical activity of PS II

For determination of photosynthetic pigment concentrations, the 1st leaves were homogenized twice with 80% acetone and centrifuged (33 000 × g, 15 min). After measurement of absorbance of the supernatants at 663.2 nm, and 470.0 nm, chlorophyll *a*, chlorophyll *b*, and total carotenoid contents were calculated according to the method of WELLBURN (1994).

Photochemical activity of PSII was estimated by a chlorophyll fluorescence-based determination of the maximum quantum yield of PS II (equivalent to *F_v/F_m*) by using a portable fluorometer FluorPen FP 100 (Photon Systems Instruments). Prior to measurements the seedlings were dark-adapted for 20 min. Measurements were done on the 1st leaf, about 1 cm below its tip.

Statistical analysis

The results presented are means of 3 independent experiments. For growth parameters and quantum yield estimation, 10 seedlings were used per experiment (*n* = 30). For determination of electrolyte leakage and photosynthetic pigment content, 4 plants were used per experiment (*n* = 12). For estimation of element contents, a single pooled sample prepared from 20 seedlings was analysed per experiment (*n* = 3). Sample variability is expressed as the standard deviation (SD) of the mean.

The significance of changes between mean values for each treatment and the control as well as between mean values for Ni and Ni+Se treatments were determined by Student's *t*-test. Differences at $P < 0.05$ were considered significant.

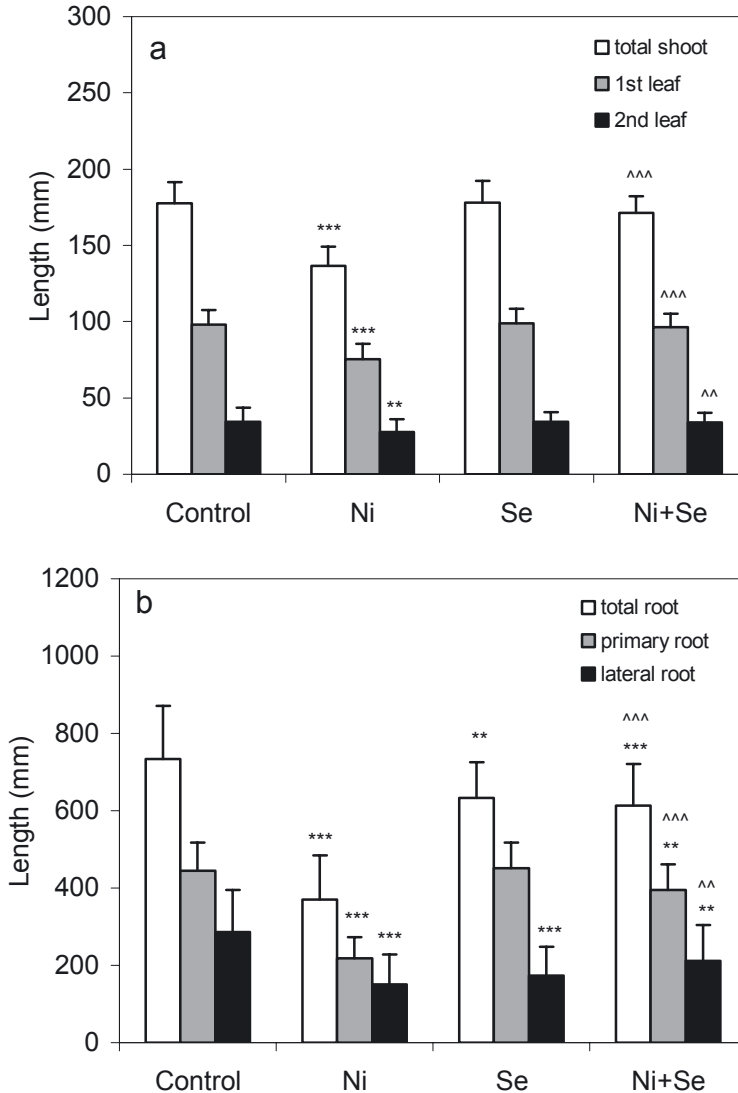


Fig. 1. Effect of Ni and Se treatments on total shoot length, lengths of the 1st and 2nd leaves (a) as well as the total root length and lengths of primary and lateral roots (b) of wheat seedlings. Data are means \pm SD; **, *** significantly different from the control at $P < 0.01$ and $P < 0.001$, respectively; ^, ^^, ^^ significantly different from Ni treatment at $P < 0.01$ and $P < 0.001$, respectively

RESULTS

Exposure of wheat seedlings to Ni resulted in 22% reduction in the total shoot length (Fig. 1a). A similar decrease in length was also found for the 1st and 2nd leaves. Compared to the aboveground part of the seedling, root growth was more affected by Ni stress. The total root length as well as lengths of primary and lateral roots were decreased after Ni treatment by about 50%, compared to the control (Fig. 1b). Treatment of the wheat seedlings with Se alone did not influence significantly the shoot growth parameters, but led to a 16% reduction in the total root length, compared to the control (Fig. 1a-b). Primary and lateral roots differed in their sensitivity to Se. The length of primary roots was not affected, while that of lateral roots was about 40% lower than in the control (Fig. 1b).

Compared to the seedlings subjected to Ni alone, those treated simultaneously with Ni and Se showed an improvement of the total shoot length and lengths of the 1st and 2nd leaves, by about 25%. In the seedlings treated with the combination of Ni and Se, shoot growth parameters did not differ from those of the control. Addition of Se to the Ni-containing medium resulted also in increased total root length as well as lengths of the primary and lateral roots (by 67%, 81%, and 41%, respectively), compared to the seedlings subjected to Ni alone (Fig. 1a-b).

Ni treatment of the seedlings led to considerable accumulation of this metal in the tissues (Table 1). Most of Ni (almost 94% of the total amount taken up by the seedlings) was accumulated in the root. Application of Se led to about 5-fold higher accumulation of this element in the roots, compared to the shoots. In the seedlings treated simultaneously with Ni and Se, Ni concentration in shoots and roots was 33% and 45% lower, respectively, than in the seedlings exposed to Ni alone, while Se uptake by the seedlings was not affected by Ni stress.

Table 1. Effect of Ni and Se treatments on their accumulation in shoots and roots of wheat seedlings. Data are means \pm SD; *** significantly different from the control at $P < 0.001$; ^^ significantly different from Ni treatment at $P < 0.001$

Treatment	Ni content [$\mu\text{g g}^{-1}$ DW]		Se content [$\mu\text{g g}^{-1}$ DW]	
	Shoots	Roots	Shoots	Roots
Control	2.75 \pm 0.79	2.84 \pm 0.66	0.89 \pm 0.20	0.87 \pm 0.15
Ni	82.10 \pm 5.4 ***	1239.75 \pm 160.09 ***	0.95 \pm 0.16	0.89 \pm 0.22
Se	3.04 \pm 0.43	2.57 \pm 0.25	102.57 \pm 6.12 ***	489.43 \pm 39.79 ***
Ni+Se	55.74 \pm 2.29 ***, ^^	677.67 \pm 118.04 ***, ^^	89.84 \pm 2.9 ***	497.51 \pm 25.39 ***

Exposure to Ni resulted in enhancement of electrolyte leakage from shoot and root cells, as it was 122% and 19% higher, respectively, than in the control (Fig. 2). Se applied individually did not influence electrolyte leakage, neither in the shoots nor in the roots. However, addition of Se to the Ni-containing medium significantly reduced electrolyte leakage from shoot cells, as compared to the seedlings treated with Ni alone. In contrast, such a protective effect of Se was not found in the roots.

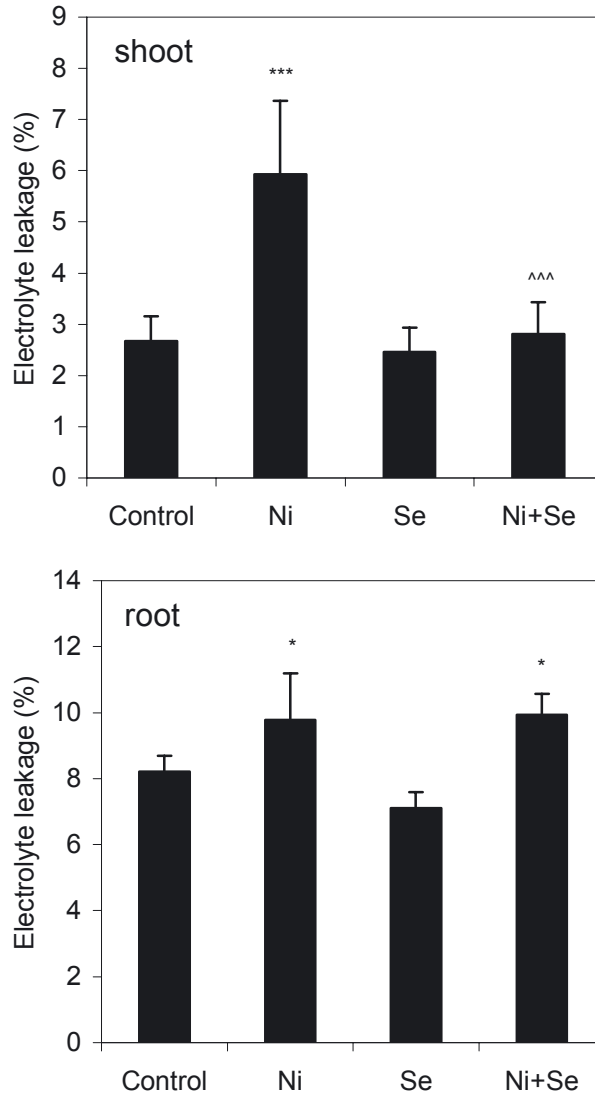


Fig. 2. Effect of Ni and Se treatments on electrolyte leakage from wheat shoot and root cells. Data are means \pm SD; *, *** significantly different from the control at $P < 0.05$ and $P < 0.001$, respectively; ^^^ significantly different from Ni treatment at $P < 0.001$

Application of Ni led to decrease in Fe and Mg concentrations in the wheat shoots, by 33% and 20%, respectively (Fig. 3a-b). In comparison to the seedlings treated with Ni alone, in those subjected to both Se and Ni the concentration of Fe was 23% higher, but still remained below the control value (Fig. 3a). Concentration of Mg in the shoots of the seedlings grown in the presence of Ni and Se applied simultaneously did not differ significantly from the control (Fig. 3b). When supplied individually,

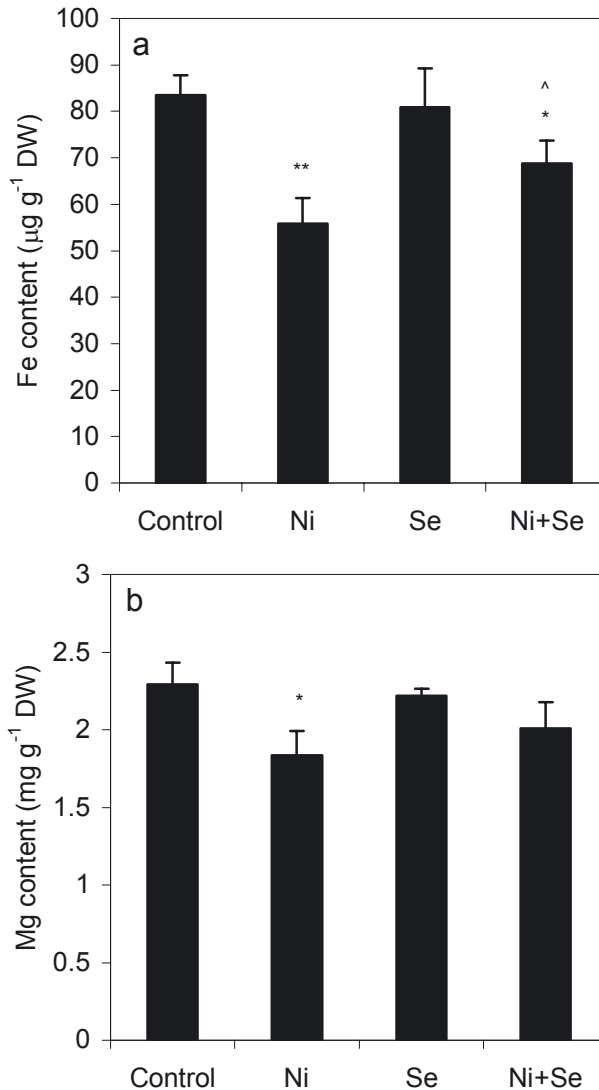


Fig. 3. Effect of Ni and Se treatments on Fe (a) and Mg (b) content of wheat shoots. Data are means \pm SD; *, ** significantly different from the control at $P < 0.05$ and $P < 0.01$, respectively; [^] significantly different from Ni treatment at $P < 0.05$

Se did not influence Fe and Mg concentrations in wheat shoots. In our experiment, Fe concentration in the roots was not affected by Ni and Se treatments. Mg concentration in the roots of Ni-exposed seedlings decreased by 43%, compared to the control, and was not improved by Se supplementation (data not shown).

Exposure to Ni resulted in decreased chlorophyll *a* and chlorophyll *b* concentrations in the 1st leaves, by 15% and 26%, respectively, as compared to the control (Fig. 4a). A more pronounced decrease in chlorophyll *b* than in chlorophyll *a* content was reflected by the increased value of chlorophyll *a/b* ratio, which in the seedlings treated with Ni was 14% higher than in the control (Fig. 4b). Se applied individually did not influence chlorophyll concentration in leaves, but its addition to the Ni-containing medium significantly enhanced the concentration of this pigment, which was accompanied by considerable reduction of total chlorotic area on the leaves (data not

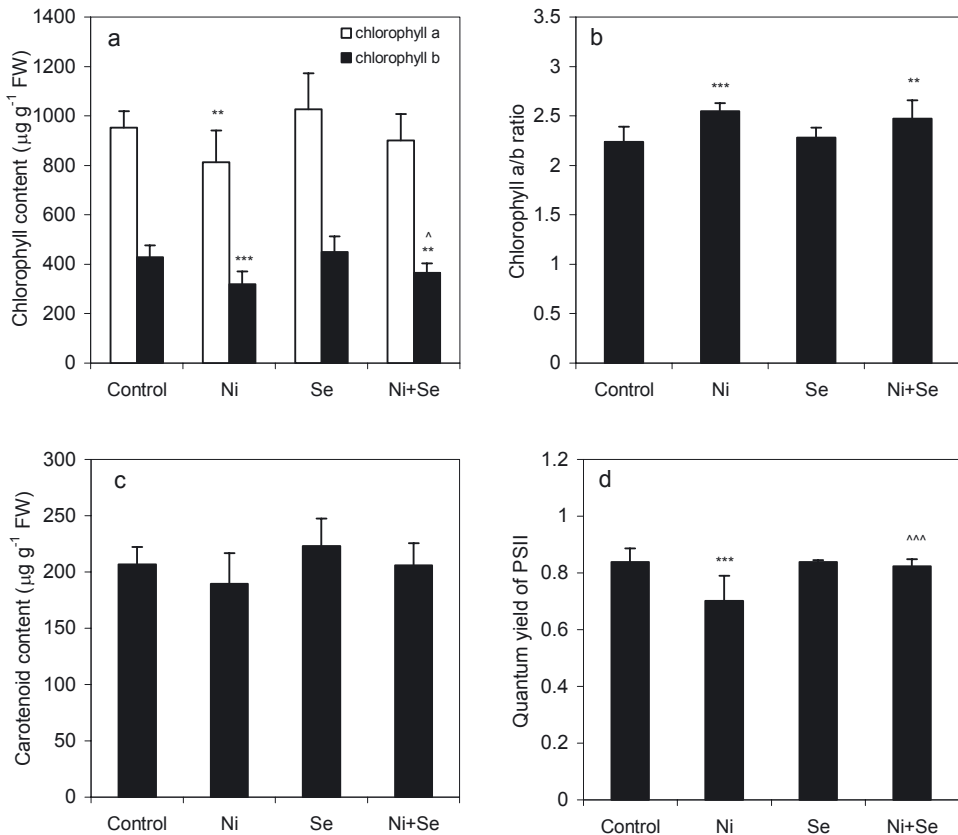


Fig. 4. Effect of Ni and Se treatments on chlorophyll content (a), chlorophyll *a/b* ratio (b), carotenoid content (c), and maximum quantum yield of photosystem II (d) in wheat leaves. Data are means \pm SD; **, *** significantly different from the control at $P < 0.01$ and $P < 0.001$, respectively; [^], ^{^^} significantly different from Ni treatment at $P < 0.05$ and $P < 0.001$, respectively

shown). In the seedlings grown in the presence of Ni and Se, chlorophyll *a* content did not differ from that in the control, while chlorophyll *b* content was increased by 14%, as compared to the seedlings treated with Ni alone (Fig. 4a). Nevertheless, compared to the control, chlorophyll *b* concentration in the leaves of seedlings exposed to the combination of Ni and Se was still significantly lower, and chlorophyll *a/b* ratio exceeded the control value by 10% (Fig. 4a-b). The total carotenoid concentration in wheat leaves was not affected by Ni (Fig. 4c).

In the leaves of Ni-treated wheat seedlings, the maximum quantum yield of PSII was significantly reduced, by 16% in comparison to the control (Fig. 4d). Enrichment of the Ni-contaminated medium with Se increased quantum yield by 17%, compared to the seedlings treated with Ni alone.

DISCUSSION

Growth inhibition is regarded as the most common symptom of heavy metal stress in plants. The heavy-metal-induced growth reduction has been suggested to result from restriction of cell division and cell elongation (CHO & PARK 2000) as well as from metabolic disturbances (GAJEWSKA & SKŁODOWSKA 2009). Consistently with previous reports concerning Ni toxicity in plants (PANDEY & SHARMA 2002; OUZOUNIDOU et al. 2006), we observed in the wheat treated with 50 μM Ni considerable decreases in shoot and root lengths, with roots showing greater sensitivity to Ni stress compared to aboveground parts of the tested seedlings. Addition of Se to the Ni-containing medium significantly improved the growth of wheat seedlings, which was evidenced by increases in total root and shoot lengths. In agreement with our results, amelioration of growth due to Se supplementation was also found in Ni-treated lettuce seedlings (HAWRYLAK et al. 2007). Similarly, Se-induced growth promotion has been reported for plants subjected to Cd stress (FILEK et al. 2008; ZEMBALA et al. 2010). Apart from protection against heavy metals, Se has also been shown to counteract the stress induced in plants by other abiotic factors. Published data suggest that the ability of Se to neutralize various environmental stresses can be ascribed to several different mechanisms, but they have not been fully elucidated yet (FENG et al. 2013).

In our study, Se applied individually did not influence shoot growth but reduced the length of lateral roots. Since our preliminary experiment revealed that Se doses lower than 15 μM were insufficient to alleviate the reduction of wheat seedling growth caused by 50 μM Ni, in the present experiment we decided to use Se at the concentration of 15 μM , despite its slight inhibitory effect on root growth.

Exposure to Ni resulted in a significant increase in Ni concentration in wheat seedlings, mainly in the roots. Greater accumulation of Ni in root tissues may explain stronger growth inhibition of this organ, compared to the shoot. Application of Se considerably reduced the accumulation of Ni, both in shoots and in roots of the wheat seedlings. In contrast, in Ni-stressed lettuce, supplementation with Se increased Ni accumulation in the leaves (HAWRYLAK et al. 2007). Similarly to our work, Se-evoked reduction in metal uptake has been reported for plants treated with Cr, Cd, and Pb (SHANKER et al. 1996; SRIVASTAVA et al. 1998; HE et al. 2004; FILEK et al. 2008). Limitation of heavy metal uptake by Se may be related to the formation of non-toxic Se-

metal complexes, which considerably reduces the availability of the metal to plants (SHANKER et al. 1996). The counteracting effect of Se on heavy metal uptake has also been explained by competition of the metal and Se ions for the binding sites in the protein carriers (KUMAR et al. 2012).

Membrane permeability, estimated in terms of electrolyte leakage from cells, has been reported to increase in plants subjected to heavy metals, including Ni (LLAMAS et al. 2008; POPOVA et al. 2009). Similarly, in the Ni-treated wheat seedlings, we found enhanced electrolyte leakage from shoots and roots. Our earlier study suggested that the destructive effect of Ni on membranes might be associated with increased lipid peroxidation as well as changes in fatty acid profile, resulting in decreased lipid unsaturation (GAJEWSKA et al. 2012). In addition, findings of OUZOUNIDOU et al. (2006) indicated that membrane structure and functioning might also be affected due to displacement of Ca^{2+} from the membrane ligands by Ni^{2+} ions. Our experiment revealed that supplementation of the Ni-containing medium with Se significantly reduced electrolyte leakage from shoot cells. A similar ameliorative effect of Se on membrane permeability was observed in the shoots of As-treated mungbean (MALIK et al. 2012). Results obtained by KUMAR et al. (2012) implied that improvement of the heavy-metal-disturbed membrane function by Se might be associated with enhancement of fatty acid unsaturation. We suggest that Se-induced protection of cell membranes under conditions of Ni stress is related mainly to augmentation of antioxidative capacity. Such a supposition is in agreement with the results of studies documenting decreases in lipid peroxidation and reactive oxygen species accumulation as well as increases in antioxidative enzyme activities and non-enzymatic antioxidant contents after supplementation of heavy-metal-stressed plants with Se (PEDRERO et al. 2008; HASANUZZAMAN et al. 2012; KUMAR et al. 2012).

Disturbed uptake of essential elements is considered to be one of the main reasons of heavy metal toxicity in plants. In accordance with earlier studies dealing with the effect of Ni on mineral composition of plant tissues (PANDEY & SHARMA 2002; OUZOUNIDOU et al. 2006), in the shoots of Ni-treated wheat seedlings we found decreased Fe and Mg contents. Impaired uptake of polyvalent cations due to Ni has been suggested to result from their competition for specific binding sites in the roots (OUZOUNIDOU et al. 2006). Application of Se significantly counterbalanced the Ni-induced decrease in Fe and Mg concentrations in wheat shoots, which might be related to the general beneficial effect of Se on membranes. To our knowledge, the ameliorative effect of Se on concentrations of these elements in Ni-stressed plants has not been reported so far. ZEMBALA et al. (2010) in the shoots of Cd-treated wheat found a slight Se-induced enhancement of Fe content, but they did not observe any improvement in Mg content.

Reduced chlorophyll concentration and the resultant leaf chlorosis have often been observed in plants subjected to Ni stress (FARGAŠOVÁ 1998; OUZOUNIDOU et al. 2006). In line with those reports, in the leaves of Ni-treated wheat seedlings we found a significant decrease in both chlorophyll *a* and chlorophyll *b* contents. Our results indicate that a decline in chlorophyll content might be at least partly due to Ni-evoked deficiency of Mg (a structural component of this pigment) and of Fe, which participates in its biosynthesis (MARSH et al. 1963). A deleterious effect of Ni on chlorophyll

concentration has also been suggested to be a consequence of the metal-induced inhibition of enzyme activities involved in its synthesis and stimulation of chlorophyllase activity (ABDEL-BASSET et al. 1995; SZYMAŃSKA & MATRASZEK 2005). Our study revealed a higher sensitivity of chlorophyll *b* to Ni stress, in comparison to chlorophyll *a*, which was consistent with the findings of FARGAŠOVÁ (1998) in white mustard. In contrast, HAWRYLAK et al. (2007) in leaves of Ni-treated lettuce observed a greater reduction of chlorophyll *a* than of chlorophyll *b* content. Results obtained by DRAŹKIEWICZ and BASZYŃSKI (2010) suggested that the effect of Ni on chlorophyll *a/b* ratio might depend on the time of exposure. Similarly to our study, in maize treated with Ni for 8 days those authors noticed an increase in chlorophyll *a/b* ratio, but after 13 days its value decreased. In contrast to the finding of HAWRYLAK et al. (2007), who reported a marked reduction in the total carotenoid concentration in lettuce leaves treated with 50 μ M Ni, we did not observe in the Ni-exposed wheat any decrease in concentration of these pigments.

Application of Se positively influenced chlorophyll concentration in leaves of Ni-treated wheat, so that in the seedlings exposed to the combination of Ni and Se, chlorophyll *a* concentration did not differ from that in the control. Chlorophyll *b* content, although significantly increased in comparison to the Ni-treated seedlings, was still lower than in the control. Partial alleviation of Ni-induced decline in chlorophyll content by Se application may be related to its protective effect on chloroplast enzymes involved in chlorophyll biosynthesis, mostly due to antioxidant properties of Se (PENNANEN et al. 2002).

In agreement with previous studies (DRAŹKIEWICZ & BASZYŃSKI 2010; VELIKOVA et al. 2011), the maximum quantum yield of PSII in wheat leaves was significantly reduced in response to Ni exposure. A decline in this parameter has been suggested to be associated with the limited reoxidation of plastoquinone A by a decrease or partial blocking of electron transport from PSII to PSI (VELIKOVA et al. 2011). Earlier reports provided evidence that the negative effect of Ni on photosynthesis is multidirectional. In addition to disturbance of photochemical reactions and reduction in photosynthetic pigment contents, decreases in the activities of the Calvin cycle enzymes (SHEORAN et al. 1990) and changes in chloroplast ultrastructure (MOLAS 1997; FILEK et al. 2010) were observed. In the present study we demonstrated that the application of Se significantly increased quantum yield in the leaves of Ni-treated wheat seedlings. Our results imply that improvement of photochemical activity of PSII by Se supplementation might be at least partly related to the beneficial effect of this element on chlorophyll concentration and integrity of chloroplast membranes. Literature data indicate that Se may also counterbalance the detrimental impact of heavy metals on photosynthesis by increasing unsaturation and fluidity of chloroplast envelope as well as by amelioration of chloroplast size and ultrastructure (FILEK et al. 2010).

CONCLUSIONS

Our study shows that Se application significantly alleviates toxic effects induced by Ni in wheat seedlings, which was evidenced by improved growth, increased Fe, Mg, and chlorophyll concentrations in shoots, enhanced photochemical activity of

PSII, and reduced electrolyte leakage from shoot cells. The results suggest that the beneficial effect of Se may be associated with limitation of Ni uptake by the seedlings. Further studies are needed to get better insight into the mechanisms of alleviation of Ni toxicity by Se.

Acknowledgements: This work was supported by the University of Łódź (grant No. 506/819).

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