BIOLOGIA PLANTARUM 50 (4): 653-659, 2006

Effect of nickel on antioxidative enzyme activities, proline and chlorophyll contents in wheat shoots

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Abstract

Effect of two Ni concentrations (10 and 200 μM) on growth, Ni accumulation, chlorophyll and proline contents, relative water content (RWC) as well as the activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and glutathione S-transferase (GST) were studied in shoots of wheat plants. Treatments caused a considerable accumulation of Ni in the shoots. However, exposure of plants to 10 μM Ni did not lead to significant alterations in shoot growth except for a slight increase in fresh mass. The other parameters studied were not affected by treatment of plants with 10 μM Ni. In contrast, 200 μM Ni caused inhibition of shoot growth, a decline in RWC and chlorophyll content, accumulation of proline and occurrence of visible symptoms of Ni toxicity. The activities of SOD and CAT decreased in response to 200 μM Ni. Conversely, several-fold enhancements of POD and GST activities were observed following the 3rd day of 200 μM Ni treatment.

Additional key words: catalase, glutathione S-transferase, heavy metal, peroxidase, superoxide dismutase, *Triticum aestivum* L.

Introduction

Nickel is considered to be an essential micronutrient for plants (Eskew *et al.* 1983), however at excess concentrations this metal becomes toxic for majority of plant species. The most common symptoms of nickel toxicity in plants are inhibition of growth, chlorosis, necrosis and wilting (Madhava Rao and Sresty 2000, Pandey and Sharma 2002, Nakazawa *et al.* 2004). Toxicity of this metal has been attributed to its negative effect on photosynthesis (Tripathy *et al.* 1981), mineral nutrition (Atta-Aly 1999, Parida *et al.* 2003), sugar transport (Samarakoon and Rauser 1979) and water relations (Pandey and Sharma 2002). However, influence of Ni on biochemical reactions of plants, including antioxidative responses still remains poorly understood.

Overproduction of reactive oxygen species (ROS) is a common response of plants to different stress factors. To maintain metabolic functions under stress conditions, the balance between generation and degradation of ROS is required, otherwise oxidative injuries may occur. The level of ROS in plant tissues is controlled by an antioxidative system that consists of antioxidative enzymes and nonezymatic low molecular mass antioxidants. Superoxide dismutase (SOD, EC 1.15.1.1) is a key antioxidative enzyme that catalyzes disproportionation of superoxide anion ($O_2^{\bullet -}$) to H_2O_2 and O_2. Catalase (CAT, EC 1.11.1.6) scavenges H_2O_2 by converting it to H_2O and O_2. Peroxidase (POD, EC 1.11.1.7.) reduces H_2O_2 using several reductants, *e.g.* phenolic compounds. POD is also the key enzyme in lignin biosynthesis participating in the formation of radicals of lignin units before their polymerization (Gaspar *et al.* 1991). Induction of POD activity has been observed in response of plants to different stress factors, including heavy metals (Díaz *et al.* 2001). This enzyme

Received 17 February 2005, *accepted* 22 July 2005.
Abbreviations: CAT - catalase; CDNB - 1-chloro-2,4-dinitrobenzene; GSH - reduced glutathione; GST - glutathione S-transferase; NBT - nitroblue tetrazolium; POD - peroxidase; ROS - reactive oxygen species; RWC - relative water content; SOD - superoxide dismutase.
Acknowledgements: This work was partly supported by University of Łódź Grant No. 505/429. The authors are grateful to Dr. Z. Nita (Plant Breeding Strzelce, Poland) for supplying wheat seeds.
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has been proposed to be a potential biomarker for sublethal metal toxicity in plants (Mocquot *et al*. 1996).

Glutathione transferase (GST, EC 2.5.1.18) catalyzes the conjugation of endogenous or exogenous electrophilic substrates to reduced glutathione (GSH). The resulting conjugates are transported to vacuoles for further processing or degradation (Marrs 1996). Therefore GST has been suggested to play an important role in the removal of toxic products of lipid and protein peroxidation. It has been reported that GST may be involved in resistance of plants to diverse environmental stresses (Davis and Swanson 2001, Sudhakar *et al*. 2001). However, relatively little is known about participation of GST in response of plants to heavy metal toxicity.

Proline is one of the most widespread metabolites produced in plant tissues under stress conditions. Protective role of proline has been ascribed to its ability to act as osmoprotectant (Hartzendorf and Rolletschek 2001), membrane stabilizer (Bandurska 2001), and ROS scavenger (Matysik *et al*. 2002).

Results of several studies indicate that excess Ni concentrations may cause oxidative stress in plants and affect activities of antioxidative enzymes (Baccouch *et al*. 1998, Madhava Rao and Sresty 2000, Gonnelli *et al*. 2001, Boominathan and Doran 2002). However, data concerning effect of Ni on these enzymes are contradictory, since both increases (Baccouch *et al*. 1998) and decreases (Boominathan and Doran 2002, Pandey and Sharma 2002) in their activities have been found.

The purpose of the present study was to contribute to a better understanding of the biochemical responses of plants to Ni stress. We investigated the influence of low and high Ni concentrations on the activities of antioxidative enzymes (SOD, CAT, POD), the activity of GST and proline content in shoots of wheat plants. In parallel, growth parameters, chlorophyll and water contents as well as Ni uptake were monitored. Concentrations of Ni for this experiment, chosen on the basis of the results of our preliminary studies were: 10 μM - not inducing symptoms of toxicity and 200 μM - causing visible morphological symptoms of toxicity in wheat plants.

Materials and methods

Plants: Wheat (*Triticum aestivum* L. cv. Zyta) seeds supplied by Plant Breeding, Strzelce, Poland were sown (20 per pot) in polyethylene pots filled with perlite (volume 300 cm^3). The seedlings were grown in a controlled climate room at 24 °C with 16-h photoperiod with irradiance of 175 μmol m^{-2} s^{-1}. They were regularly watered with half-strength Hoagland's solution. Four days after sowing 30 cm^3 of a $NiSO_4$ solution was applied to the perlite at the final concentrations of 10 and 200 μM. Plants treated with distilled water were referred to as control. Samples of shoots were taken at 3, 6 and 9 d and used for analyses.

Estimation of growth parameters and relative water content: Length and fresh mass (f.m.) of shoots were measured immediately after harvesting. Dry mass (d.m.) was measured after oven-drying of the shoot sample for 48 h at 105 °C. Relative water content (RWC) was determined according to Smart and Bingham (1974) and calculated as: RWC = [(f.m. - d.m.)/(f.s.m. - d.m.)] × 100. Fully saturated mass (f.s.m.) was determined after floating of shoot sample on distilled water for 4 h at 20 °C in darkness.

Enzyme extraction and assays: Shoots were homogenized (1:10 m/v) in an ice cold mortar using 50 mM sodium phosphate buffer pH 7.0 containing 1 mM EDTA and 0.5 M NaCl. After centrifugation (20 000 *g*, 20 min) the supernatant was used for determination of SOD, CAT, POD and GST activities.

Total SOD activity was assayed spectrophotometrically (*Helios Gamma*, *Thermo Spectronic*, Cambridge, UK) according to the method of Minami and Yoshikawa (1979) based on pyrogallol autooxidation. The reaction mixture consisted of 50 mM Tris-cacodylic buffer (sodium salt), pH 8.2, 0.1 mM EDTA, 1.4 % Triton X-100, 0.055 μM NBT, enzyme extract (approximately 20 μg protein) and 16 μM pyrogallol, which started the reaction. The reaction mixture was incubated in 37 °C, for 5 min. The reaction was stopped by addition of 3.5 cm^3 of mixture consisting of 0.35 M formic buffer pH 3.5, 0.6 % Triton X-100 and 3.5 % formaldehyde and absorbance was measured at 540 nm. According to McCord and Fridovich (1969) one unit of SOD activity is defined as the amount of enzyme that causes inhibition of NBT reduction by 50 %. Enzyme activity was expressed in units min^{-1}. CAT activity was measured spectrophotometrically according to Dhindsa *et al.* (1981). The assay mixture contained 50 mM sodium phosphate buffer pH 7.0, 15 mM H_2O_2 and enzyme extract (approximately 5 μg protein). Decomposition of H_2O_2 (coefficient of absorbance, ε = 45.2 M^{-1} cm^{-1}) was measured at 240 nm. Enzyme activity was expressed in units, each representing 1 mmol (H_2O_2 decomposed) min^{-1}. POD activity was measured by the method of Maehly and Chance (1954). The assay mixture contained 50 mM sodium acetate buffer pH 5.6, 5.4 mM guaiacol, 15 mM H_2O_2 and enzyme extract (approximately 5 μg protein). The increase in absorbance due to the oxidation of guaiacol to tetraguaiacol (ε = 26.6 mM^{-1} cm^{-1}) was monitored at 470 nm. Enzyme activity was expressed in units, each representing 1 mmol (tetraguaiacol formed) min^{-1}. Total GST activity was measured by the method of Habig *et al.* (1974) using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. The reaction mixture contained 100 mM potassium phosphate buffer pH 6.25, 0.75 mM

CDNB, 30 mM GSH and enzyme extract (approximately 150 μg protein). The increase in absorbance due to the formation of the conjugate (ε = 9.6 mM^{-1} cm^{-1}) between GSH and CDNB was monitored at 340 nm. Enzyme activity was expressed in units, each representing 1 μmol(S-conjugates formed) min^{-1}.

Determination of Ni, chlorophyll, proline and protein contents: Nickel content in shoots was determined by atomic absorption spectrometry (*SpektrAA 300*, *Varian*, Mulgrave, Australia) following wet digestion of oven dried tissue in HNO_3 : $HClO_4$ (4:1, v/v) solution at 140 °C. Chlorophyll content was determined in 80 % acetone extract. After centrifugation (20 000 *g*, 20 min) absorbance of clear supernatant was measured at 663 and 645 nm. Total chlorophyll as well as chlorophyll *a* and *b* concentrations were calculated according to Arnon (1949). Free proline content was determined using the ninhydrin method of Bates *et al*. (1973). 0.8 cm^3 of 3 % sulphosalicylic acid was added to 0.2 cm^3 of extract prepared as described above. The tubes were placed at 4 °C for 10 min. Than 1 cm^3 of glacial acetic acid and 1 cm^3 of acid ninhydrin were added. After boiling the mixture in water bath at 100 °C for 60 min the reaction was stopped by cooling the tubes in ice bath for 5 min. The chromophore formed was extracted with 3 cm^3 of toluene by vigorous shaking and the tubes were placed in the dark for 50 min. Absorbance of the resulting organic layer was measured at 520 nm. The concentration of proline was estimated by referring to a standard curve for L-proline. Protein was determined by the method of Bradford (1976), with standard curve prepared using bovine serum albumin.

Statistical analysis: The results presented are the means of at least 5 independent experiments. Sample variability is given as the standard deviation of the mean. The significance of differences between control and treatment mean values was determined by Student's *t*-test. Differences at $P < 0.05$ were considered significant.

Results

Ni accumulation and growth responses: Shoots of control plants contained up to 0.542 μg(Ni) g^{-1}(d.m.) (Table 1). Exposure of the seedlings to excess Ni caused a rapid increase in this metal content in the shoots. Already 3 d after Ni application shoot tissue accumulated 2.433 and 251.033 μg(Ni) g^{-1}(d.m.), at 10 and 200 μM treatments, respectively. Ni concentration in the shoots only slightly increased with duration of exposure to the metal.

Treatment of wheat seedlings with 10 μM Ni did not affect shoot length (Table 1). However, in plants subjected to 200 μM Ni a significant inhibition of shoot elongation was observed following the 3rd day after metal application. At the end of experiment shoot length in plants treated with 200 μM Ni was reduced by 44 % ($P < 0.001$) in comparison with control. In plants exposed to 10 μM Ni shoot fresh mass did not change significantly up to the 9th day of experiment, when 22 % ($P < 0.05$) increase over control level was noticed (Table 1). In contrast, application of 200 μM Ni caused a strong decrease in shoot fresh mass, up to 61 % ($P < 0.001$) below the control level at the end of experiment.

Apart from the reduction in growth parameters, in case of plants treated with the higher Ni concentration symptoms characteristic for this metal toxicity were noticed. Chlorosis and necrosis manifested as colourless longitudinal stripes were observed on the first leaves following the 3rd day after metal application. Chlorotic and necrotic areas were extending in the course of experiment. Appearance of the newly developed second leaves was only slightly altered by Ni treatment. They

Table 1. Effect of Ni treatment on wheat shoot Ni accumulation, length and fresh mass, and chlorophyll (Chl) content. Data are means ± SD, n = 5 for Ni content, n = 20 for growth parameters, n = 6 for chlorophyll content. *, **, *** indicate values that differ significantly from control at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

Time	Treatment	Ni content [μg g^{-1}(d.m.)]	Shoot length [mm]	Shoot fresh mass [mg]	Chl *a*+*b* content [mg g^{-1}(f.m.)]	Chl *a*/*b*
3 d	control	0.432 ± 0.053	111.4 ± 10.3	96.4 ± 15.2	0.710 ± 0.268	2.08 ± 0.22
	10 μM Ni	2.433 ± 0.503*	109.4 ± 8.0	93.6 ± 14.4	0.717 ± 0.262	2.06 ± 0.30
	200 μM Ni	251.033 ± 7.408***	83.0 ± 19.3***	61.5 ± 13.3***	0.457 ± 0.184*	1.98 ± 0.37
6 d	control	0.437 ± 0.087	171.1 ± 16.4	135.1 ± 24.1	1.134 ± 0.147	2.08 ± 0.05
	10 μM Ni	2.505 ± 0.473*	173.3 ± 19.4	139.6 ± 17.4	1.047 ± 0.104	2.09 ± 0.10
	200 μM Ni	276.457 ± 17.84***	110.9 ± 13.7***	69.3 ± 19.7***	0.636 ± 0.181***	2.19 ± 0.23
9 d	control	0.542 ± 0.099	200.3 ± 16.6	189.2 ± 35.8	1.034 ± 0.109	1.99 ± 0.08
	10 μM Ni	3.406 ± 0.740*	204.5 ± 13.7	230.6 ± 29.3*	1.066 ± 0.163	2.02 ± 0.09
	200 μM Ni	339.623 ± 38.20***	112.4 ± 10.4***	73.9 ± 14.2***	0.678 ± 0.097***	2.14 ± 0.07**

were shorter and narrower than those of control plants, but chlorosis occurred only occasionally. No visible symptoms of Ni injury were observed in the case of plants treated with 10 μM Ni.

Chlorophyll content: Total chlorophyll content in shoots did not change significantly after treatment of wheat plants with 10 μM Ni (Table 1). In plants exposed to 200 μM Ni chlorophyll *a*, chlorophyll *b* (data not shown) and total chlorophyll contents were markedly reduced starting from the 3rd day of experiment. Total chlorophyll content was decreased by 36 % ($P < 0.05$), 44 % ($P < 0.001$) and 34 % ($P < 0.001$) on days 3, 6 and 9, respectively. Additionally, treatment of wheat plants with 200 μM Ni for 9 d resulted in a slight increase (8 % over the control level, $P < 0.01$) in chlorophyll *a*/*b* ratio.

RWC and proline content: No significant changes in RWC of shoot tissue were observed in plants subjected to 10 μM Ni (Fig. 1*A*). In the case of 200 μM Ni treatment shoot RWC was decreased beginning from the 3rd day after metal application (6.6 % below the control level, $P < 0.001$). The strongest reduction in shoot RWC, by almost 10 % ($P < 0.001$) in comparison with control was found on the 6th day.

Treatment of wheat plants with 200 μM Ni resulted in a significant, time-dependent accumulation of proline in the shoots (Fig. 1*B*). As early as 3 d after 200 μM Ni application a 4-fold ($P < 0.01$) increase in proline

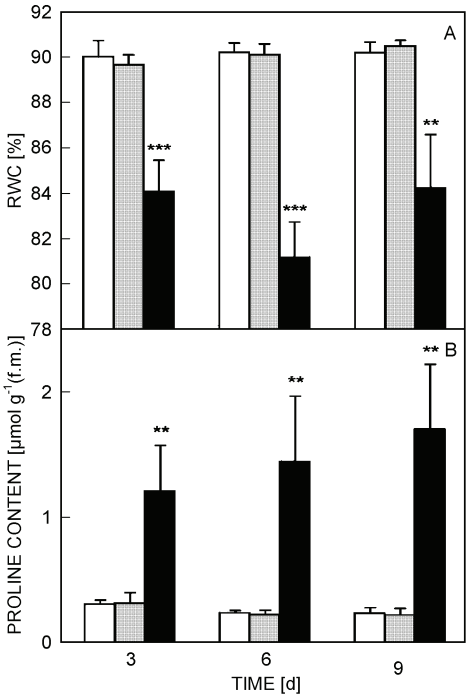

Fig. 1. Relative water content (*A*) and proline content (*B*) in shoots of wheat plants treated with Ni. *Bars* represent SD of means, $n = 5$. **, *** indicate values that differ significantly from control at $P < 0.01$ and $P < 0.001$, respectively; *white columns* - control, *grey columns* - 10 μM Ni, *black columns* - 200 μM Ni.

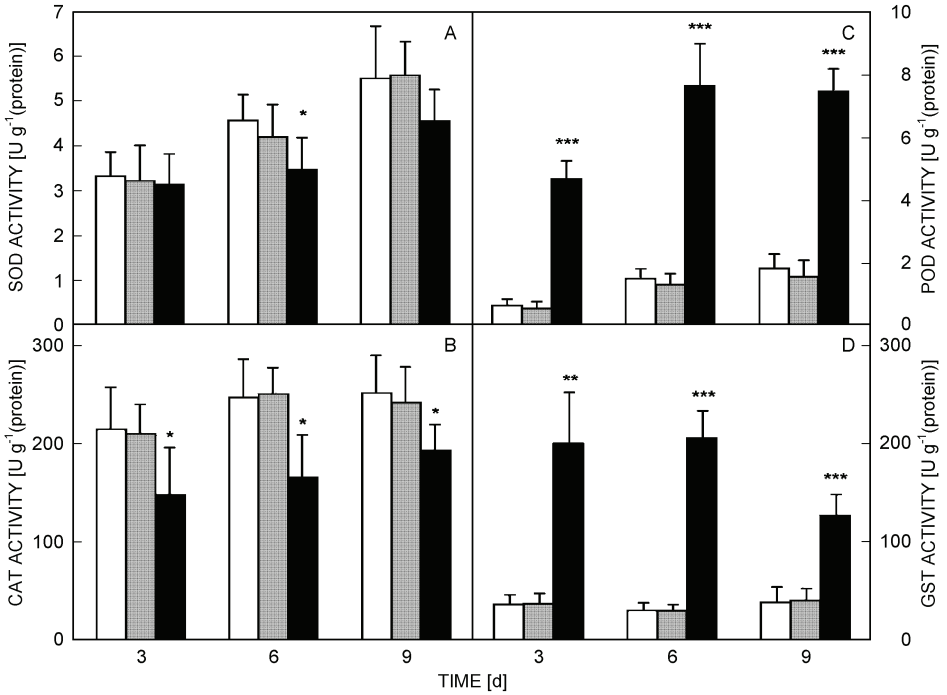

Fig. 2. Superoxide dismutase (*A*), catalase (*B*), peroxidase (*C*) and glutathione S-transferase (*D*) activities in shoots of wheat plants treated with Ni. *Bars* represent SD of means, $n = 5$. *, **, *** indicate values that differ significantly from control at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively; *white columns* - control, *grey columns* - 10 μM Ni, *black columns* - 200 μM Ni.

concentration was observed. After 9 d shoots of plants exposed to 200 μM Ni contained over 7 times ($P < 0.01$) more proline than control. In contrast, treatment with 10 μM Ni did not alter proline content in wheat shoots.

Enzyme activities: Total SOD activity remained unchanged in plants exposed to 10 μM Ni (Fig. 2*A*), while in response to treatment with 200 μM Ni a significant decrease (24 % below the control level, $P < 0.05$) in this enzyme activity was observed on the 6th day. Treatment of wheat plants with the higher Ni concentration resulted also in the inhibition of CAT activity (Fig. 2*B*). The activity of this enzyme in shoots was reduced by 31 % ($P < 0.05$), 33 % ($P < 0.05$) and 23 % ($P < 0.05$) on days 3, 6 and 9, respectively.

As early as 3 d after metal application a 7-fold increase ($P < 0.001$) in POD activity was noticed in shoots of plants exposed to 200 μM Ni (Fig. 2*C*). Enhanced activity of this enzyme was observed to the end of experiment and it was 5 and 4 times higher ($P < 0.001$) than in control, on days 6 and 9, respectively. Induction of GST activity in shoots of plants subjected to 200 μM Ni was found following the 3rd day of experiment (Fig. 2*D*). The activity of this enzyme was increased by 454 % ($P < 0.01$), 584 % ($P < 0.001$) and 231 % ($P < 0.001$) in comparison with control on days 3, 6 and 9, respectively. Similarly to other enzyme activities, POD and GST activities in shoots were not affected by treatment of wheat plants with 10 μM Ni (Fig. 2*C*,*D*).

Discussion

Inhibitory effect of Ni on plant growth has been reported by many authors (Ewais 1997, Parida *et al*. 2003, Vinterhalter and Vinterhalter 2005). In the present study neither reduction in growth parameters nor visible symptoms of Ni toxicity were observed in shoots of wheat plants exposed to 10 μM Ni. Conversely, a slight increase in shoot fresh mass was found in this group of plants after 9 d. Beneficial effect of Ni on growth has also been shown for other plants (Atta-Aly 1999). Treatment of wheat plants with 10 μM Ni led to a 5-fold increase in this metal concentration in the shoot. However, chlorophyll and proline contents as well as the activities of the studied enzymes were not altered by exposure of wheat plants to this concentration of Ni.

The higher Ni concentration (200 μM) caused a considerable inhibition of shoot growth, which was evidenced by the decrease in length and fresh mass. It might result from Ni-induced alteration of fundamental metabolic processes (Samarakoon and Rauser 1979, Tripathy *et al*. 1981). Reduction in shoot fresh mass in wheat plants exposed to 200 μM Ni might be at least partly due to the decrease in water content which was observed in our experiment. Lowering of RWC in wheat shoot was accompanied by a rapid accumulation of free proline. Decrease in RWC by almost 10 % might indicate that shoots of plants treated with 200 μM Ni suffered a mild osmotic stress (Hsiao 1973). It can be therefore assumed that increase in proline content found in wheat shoots may be associated with the mechanisms of osmoregulation. Our results are in accordance with the findings of Schat *et al*. (1997), who proved that accumulation of proline in the leaves of *Silene vulgaris* treated with Cu, Cd and Zn was related to the metal-induced water deficit in leaf tissues. Although enhancement of proline content in response to heavy metal toxicity has been described by several authors (Alia and Pardha Saradhi 1991, Pandey and Sharma 2002), the physiological significance of this amino acid accumulation under metal stress is not well understood. It has been suggested that besides osmoregulatory properties antioxidative activity of proline could be involved in protection of plants against injury caused by heavy metals (Matysik *et al*. 2002).

Chlorosis is the common symptom of toxicity of heavy metals, including Ni (Pandey and Sharma 2002). Decrease in chlorophyll content in tissues of metal-treated plants has been attributed to disturbances in the synthesis of this pigment (Stobart *et al*. 1985) as well as its increased degradation (Somashekaraiah *et al*. 1992). It can be suggested that decline in chlorophyll content in shoots of Ni-treated wheat plants may result mostly from its enhanced degradation, since severe chlorosis was visible on the older leaves and very scarcely appeared on the newly developed ones. Increase in chlorophyll *a*/*b* ratio observed at the end of experiment indicates that chlorophyll *b* is more sensitive to Ni toxicity than chlorophyll *a*. Our results are in agreement with the findings of Fargašová (1998) and Gopal *et al*. (2002). In contrast, Pandey and Sharma (2002) showed that in leaves of Ni-treated cabbage concentration of chlorophyll *a* was more reduced than that of chlorophyll *b*.

Exposure of wheat plants to 200 μM Ni caused a several-fold increase in POD activity in shoots. Induction of this enzyme activity after treatment of plants with heavy metals including Ni has previously been reported (Pandolfini *et al*. 1992, Díaz *et al*. 2001, Šimonovičová *et al*. 2004). Enhancement of POD activity under metal stress has been explained by its role in building up physical barrier against toxic metals entering the cell (Díaz *et al*. 2001) as well as in scavenging H_2O_2 (Tewari *et al*. 2002). In the present study induction of POD activity was correlated with the decrease in shoot length, which is in accordance with the results obtained by Díaz *et al*. (2001). They postulated involvement of POD in the reduction of the cell-wall plasticity by POD-catalyzed lignification and consequently in the inhibition of growth of Cu-exposed pepper seedlings. Additionally, a several-fold increase in POD activity observed in our experiment

might be associated with Ni-promoted senescence in plant tissues, which has been reported by Gabbrielli *et al.* (1999).

Treatment of wheat plants with higher Ni concentration resulted in a decrease in the activities of SOD and CAT. Decline in these enzyme activities after Ni application has also been found in hairy roots of *Alyssum bertolonii* and *Nicotiana tabacum* (Boominathan and Doran 2002) but the opposite effect has been observed in shoots of *Zea mays* (Baccouch *et al.* 1998). SODs are metalloenzymes containing Fe, Cu/Zn or Mn in their prosthetic groups, depending on the enzyme type and CAT is an iron-porphyrin. Since high concentrations of Ni have been shown to decrease Fe (Pandey and Sharma 2002), Cu and Zn (Parida *et al.* 2003) contents in plant tissues it can be speculated that reduction in SOD and CAT activities in shoots of wheat plants subjected to excess Ni may result from deficiency of metals essential for biosynthesis of these enzyme molecules. On the other hand, POD which also contains Fe in its structure was activated in response to Ni stress. Therefore mechanisms different from Ni-induced disturbance in iron-porphyrin synthesis were probably involved in the inhibition of CAT activity in wheat shoots. Catalase molecule might be cleaved by proteases induced by oxidative stress, which has been found by Distefano *et al.* (1999) in senescent pea leaves. Decrease in antioxidative enzyme activities could be also due to disruption of enzyme molecules by toxic ROS (Gallego *et al.* 1996, Sandalio *et al.* 2001).

Decline in SOD activity might lead to enhancement of $O_2^{\bullet -}$ level in wheat shoots. However, despite the inhibition of CAT activity, accumulation of its substrate, H_2O_2 might not occur since it could be utilized in POD-catalyzed reaction. Rapid and strong induction of POD activity suggests that in shoots of Ni-treated wheat plants this enzyme, besides its involvement in stiffening of cell walls, probably plays an important role in the removal of H_2O_2.

In shoots of wheat plants exposed to higher Ni concentration a significant increase in GST activity was observed. To our knowledge stimulation of GST activity in plants subjected to Ni stress has not been reported previously. Enhancement of this enzyme activity has been found after Cd (Dixit *et al.* 2001) and Cu (Nagalakshmi and Prasad 2001) application, but it was less pronounced than in our experiment. The role of GST in plant response to metal toxicity has not been elucidated yet. Since heavy metals, including Ni, have been shown to induce peroxidation of membrane lipids (Baccouch *et al.* 1998) it can be suggested that in plants subjected to metal stress GST may be involved in the removal of toxic products of lipid peroxidation. Alternatively, in heavy metal-exposed plants GST may participate in transport of phytochelatin-metal complexes to a vacuole, which has been described by Marrs and Walbot (1997).

In conclusion, the results of the present work showed that Ni at the concentration of 10 μM did not cause changes in the studied parameters in shoots of wheat plants. Thus low doses of this metal seem to be non-toxic to wheat cv. Zyta and may even stimulate its growth. Toxic effect of the high concentration of Ni might be related to the inhibition of antioxidative enzyme activities. It can be postulated that in shoots of Ni-stressed wheat plants POD may be responsible for the removal of H_2O_2. Decline in chlorophyll content and several-fold enhancement of POD activity imply acceleration of senescence by excess Ni concentrations. Our results suggest that induction of POD and GST activities can play an important role in the response of wheat plants to Ni toxicity. Increased values of these parameters may be considered as biomarkers of intensity of Ni stress.

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