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ARTICLE

Investigation of nickel stress induction in terms of metal accumulation and antioxidative enzyme activity in barley seedlings

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ABSTRACT Changes of the activity of antioxidative enzymes (APX, GPX) were investigated in barley roots and leaves during a one day and a one week setting of 0, 100, 500, 1000 µM nickel treatment parallel with the measurement of nickel accumulation in order to elucidate the mechanisms in terms of anitoxidative enzyme activity during the early phase of nickel exposure. We concluded that nickel also cause ROS formation in barely seedlings in the roots and also in the leaves. In the inactivation of ROS both APX and GPX play role although to different extent and for different duration. Moreover APX reacts rapidly to nickel stress, since its activity rises even after three hours of the nickel treatment. **Acta Biol Szeged 52(1):167-171 (2008)**

KEY WORDS

nickel
antioxidative enzyme activity
ascorbate peroxidase (APX)
guiacol peroxidase (GPX)

Heavy metals get into and accumulate in various plant organs (Tuba and Csintalan 1993; Prasad 2004a), and whenever the excess amount is beyond the constitutively present detoxifying capacity of plants, in order to maintain healthy conditions, there is a need for induction of new processes or the activity of the ongoing metabolic pathways should increase.

Nickel toxicity is mainly dealt with organic acids in plants in terms of transportation and detoxification (Jócsák et al. 2005), and similarly to the effects of other heavy metals, nickel toxicity also results in the induction of reactive oxygen species (ROS), such as $O_2^{\cdot-}$ and H_2O_2 (Gajewska and Sklodowska 2007). The detoxification of ROS is a constant phenomena in plants, since they always emerge during the normal metabolic processes of photosynthesis and respiration regardless their being under stressed conditions. Normally there is a balance in the formation and the destruction of ROS with the help of a complex antioxidative system present in plants. The most important elements are the antioxidative enzymes, such as superoxide dismutase (SOD), guiacol peroxidase (GPX), a catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) and lipoxigenase (LOX) (Elstner et al. 1988; Gora et al. 1989; Mehlhorn et al. 1995; Dipierro and De Leonardis 1997) and the non-enzymatic carotenoids, ascorbic acid or glutathione, operate in plants for the inactivation of ROS. Frequent defence phenomenon that is easy to measure is the initial activity rise of antioxidative enzyme activity, that is followed by a decrease of activity when the excesses of the metal is above the tolerance level of the plant (Iturbe-Ormaetxe et al. 1998).

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The literature of nickel toxicity and the following detoxification in plants is still poor compared to other heavy metals, such as aluminium or cadmium (Prasad 2004b) and there are few works on the ROS inducing capability of nickel (Boominathan and Doran 2002; Hao et al. 2005; Gajewska and Sklodowska 2007), so further investigation is still needed on the whole plant level, since most of the works dealing with ROS formation after nickel stress were only done on roots (Boominathan and Doran 2002; Hao et al. 2005) or on the above ground organs (Gajewska and Sklodowska 2007) of a plant species. Moreover, we did not find data about the effect of nickel on the activity of antioxidative enzymes in the initial phase of heavy metal stress, within a day. In our experiment we investigated the changes of guaiacol and ascorbate peroxidases under nickel stress on barley seedlings in a one day and a one week setting hoping to elucidate the mechanisms in terms of antioxidative enzyme activity during the primary stage of nickel exposure.

Materials and Methods

Growth conditions

Barley (*Hordeum vulgare* L. „Triangel") seeds germinated for three days then the seedlings grown on modified Hoagland solution (Hegedűs et al. 2001) for seven days.

$NiSO_4$ was added to the solution of ten days old seedlings with the final concentration of 0,1; 0,5 and 1mM respectively. The growth conditions were: 20°C; 120 $m^{-2} \cdot s^{-1}$ light intensity; 12-12 h light/dark period in a Conviron S10 type phytothrone. Seedlings were sampled for antioxidative enzyme activity and Ni content measurement. In the first type of the experi-

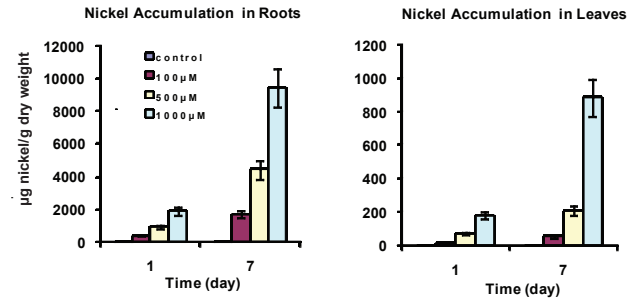

Figure 1. Ni accumulation in roots and leaves of barley seedlings.

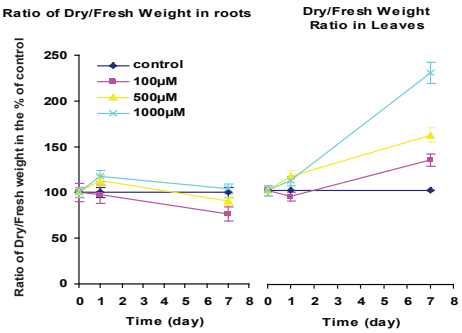

Figure 2. Ratio of dry and fresh weights of barley seedlings on the first and seventh day of 0, 100 µM, 500 µM, 1000 µM nickel treatment.

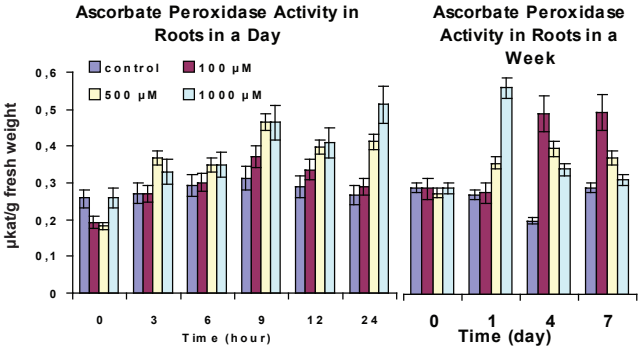

Figure 3. Changes in ascorbate peroxidase activity in roots during a one day and a one week nickel treatment.

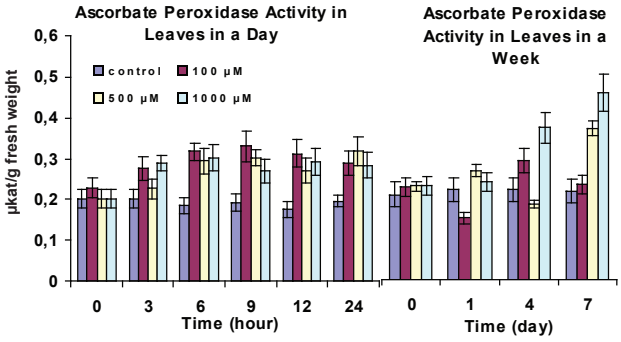

Figure 4. Changes in ascorbate peroxidase activity in shoots during a one day and a one week nickel treatment.

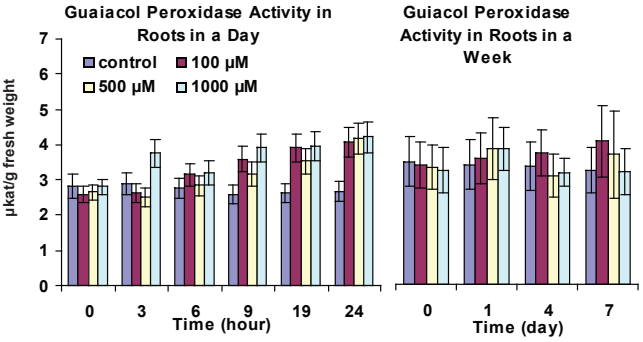

Figure 5. Changes in guiacol peroxidase activity in roots during a one day and a one week nickel treatment.

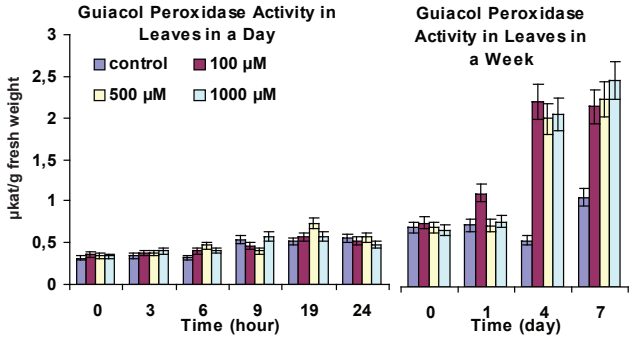

Figure 6. Changes in guiacol peroxidase activity in shoots during a one day and a one week nickel treatment.

ments, samples were taken in 0, 3, 6, 9, 12, 24 hours after Ni treatment. In the second type of the experiments samples were taken 0, 1, 4 and 7 days after metal treatment. Each experiment was repeated three times; averages and standard deviations were calculated and presented.

Ni content measurement

0.2 g of powdered dry material was dissolved in 2 cm^3 of HNO_3:H_2O_2 mixture (1:1 v/v). The nickel content of the filtered solution was determined by an ICAP 61E Plasma Spectrometer (Horváth et al. 1996).

Tissue extraction for antioxidative enzyme measurement

0.5 g of barley tissue (root, leaf) was ground in 1,5 ml isolating buffer (0,1M potassium-phosphate, pH 6,8). The mixture was centrifuged (4ºC, 20 minutes, 10000 g). The supernatant was used for enzyme activity measurement.

Antioxidative enzyme activity measurement

The GPX activity determination was from the extracted supernatant according to Chance et al. (1995), and the APX activity was according to Nakano and Asada (1981).The activity values were calculated as the average of three parallel measurements.

Results

Nickel accumulation

Barley seedlings accumulate remarkable amount of nickel from the hydroponic Hoagland solution during a week of treatment with different concentrations (0; 100 µM; 500 µM; 1000 µM). The nickel accumulation in the roots was an order of magnitude higher than that of in leaves (Fig. 1). In roots by the end of the experiment 100; 500 and 1000 µM nickel caused 1735; 4450; 9460 µg/g dry wt respectively compared to that of control (1.9596 µg/g dry wt). Whereas in leaves, the same duration and concentration of nickel treatment resulted in 52; 211; 885, µg/g dry wt compared to that of control. The accumulation pattern of nickel seems to be proportional to the applied concentration in the first day, but after a week long nickel exposure, although roots still seemed to remain around proportional; in leaves, 1000 µM nickel treatment resulted in ninety times more nickel accumulation than in case of 100 µM and five times more at 500 µM nickel treatment.

Ratio of dry and fresh weights

The ratio of dry and fresh weight did not change significantly in roots compared to that of leaves (Fig. 2), although some alteration in the ratios were observable during one week of nickel treatment. In leaves there was a constant rise and after 1 and 7 days of nickel exposure the highest concentration resulted an approximately 220 of increased compared to control.

Changes of enzyme activities

When comparing the activities of the two enzymes, GPX activity was higher than APX activity: in roots ten times more and five times more active in leaves, as it was found by Hegedűs et al (2001).

The extent of the activity of the two investigated enzymes was fairly different. The constitutive GPX activity in roots was two or three times higher, than in leaves (Fig. 5, 6). This was not true for APX, where roots and leaves show similar activity (Fig. 3, 4).

Changes of ascorbate peroxidase (APX- EC 1.11.1.11) activity

Considering roots, it is seen that the activity of ascorbate peroxidase increased slightly during the 24 hours of nickel treatments, although not significantly after 3 hours of treatment with 500 µM and 1000 µM nickel concentrations (Fig. 3). This rise in activity was more pronounced after 9, 12 and 24 hours and from the sixth hour of treatment, all of the applied concentrations resulted in enhanced activity values compared to that of the control. By the end of the one day experiment, seedlings treated with 1000 µM nickel had approximately twice as high APX activity (0.51 µkat/g fr wt), than that of control (0.26 µkat/g fr wt). By the fourth day, only 100 µM nickel treated seedlings showed enhanced activity and the seedlings treated with the higher concentrations had a gradual decrease in APX activity, 0.32 and 0.27 µkat/g fr wt, respectively.

The pattern of APX activity change was the similar in case of the leaves as it was observed with the roots that means the rise started after 3 hours of nickel treatment (Fig. 4). The main difference compared to the APX activity in the roots was that in the one week experiment there was a constant and well pronounced increase in the APX activity with all the applied concentrations 0.22, 0.24, 0.38 and 0.47 µkat/g fr wt, respectively that in case of 1000 µM nickel treatment means approximately a 208% rise.

Changes of guiacol peroxidase (GPX- EC 1.11.1.7) activity

The activity of GPX did not change significantly in our experiments, only on the first day of the nickel treatment increased slightly. In leaves by the fourth day of nickel treatment though, the GPX activity rose considerably: 394%, 363%, and 376% with the 100, 500 and 1000 µM nickel treatments respectively. In the following days these values kept rising further a bit, though not significantly.

Discussion

Barley seedlings accumulate remarkable amount of nickel during a week of treatment with different concentrations. The nickel accumulation in the roots was an order of magnitude higher than that of in leaves (Fig. 1). Nickel is known to be a heavy metal that distributes to all parts of the plants (Yantiang and Marschner 1995), and in accordance with their findings, our data further confirmed this, since even by the first day, the amount of nickel became 200 times higher in the leaves the 1000 µM nickel treated plants. These results are in accordance with the fact that nickel is transported and stored

in vacuole by organic acid chelates (Tatár et al. 2000) and it seems to be rapidly transported to the above ground organs of the barely compared to other metals, such as cadmium (Tuba and Csintalan 1993).

It is known that excess nickel alters the growth and water balance of plants (Molas and Baran 2004), so we considered the investigation of dry and fresh weight ratio to be necessary. According to our findings, the ratio of dry and fresh weight increased constantly in leaves during our experiments indicating that nickel indeed alters water balance in barley. This is a consequence of nickel stress induced lignification. Degenhardt and Gimmler (2000) found that in maize heavy metal toxicity resulted in enhanced rigidity of root cell walls that leads to disrupted water transportation processes towards leaves that manifested in higher dry/fresh weight ratio in our case.

The activity of APX started to rise after three hours of the treatment, but in the first day the activity rise was more pronounced in roots, than in leaves probably because most of the uptaken nickel remains and accumulates in roots, so the detoxification mechanisms are also activated earlier compared to that of leaves. Moreover from the fourth day of the 1000 μM nickel treatment the APX activity in roots declined that indicates a possible inactivation of the enzyme (Iturbe-Ormaetxe et al. 1998). This was not seen in leaves, where the activity constantly grew throughout the experiments.

GPX activity did not change in the initial, three hours phase after nickel treatment in roots or in leaves, either because GPX does not play a crucial role in the early detoxification, or by the constitutively high level of GPX activity or by other non-enzymatic antioxidants (Végvári and Brunori 2007), such as carotenoids, ascorbic acid or glutathione enable plants to detoxify the formed ROS. Nickel does not belong to those metals that directly induce oxidative stress, through Fenton-type reaction (Zhang et al. 2005), but induces lipid peroxidation later (Gajewska and Sklodowska 2007; Verma et al. 2008) and disturbs the electron transport chains (Smeets et al 2005). GPX is an abundant enzyme and its constitutive activity in roots is much higher than in leaves. Also GPX activity is higher than AXP activity both in roots and leaves. This fact can be the reason of the standard activity of the enzyme in the roots during the one week period of nickel treatment. In leaves however after the fourth day of nickel treatment there is an evident activity rise that is in accordance with the increase of nickel content.

We concluded and further convinced the few evidence of the known literature (Madhava and Sresty 2000; Boominathan and Doran 2002; Hao et al 2005; Gajewska and Sklodowska 2007) that nickel causes ROS formation in barely in the roots and also in the leaves of barley seedlings. In the inactivation of ROS both APX and GPX play role although to different extent and for different duration. We also found that APX particularly takes part in the inactivation of ROS with enhanced activity even after three hours of the nickel treatment. All these indicate a very rapid effect of this metal and also highlights that although nickel itself is mainly transported (Tatár et al. 2000) and detoxified with organic acids, after one day of exposure (Jócsák et al. 2005) the two antioxidative enzymes have importance in the elimination of the nickel toxicity induced ROS formation. Further investigations of other members (superoxide-dismutase, catalase, glutathione reductase) of the antioxidative enzyme system are needed for elucidating the exact sequence of the activation of different antioxidative enzymes with a desirably wider, more naturally occurring concentration range.

Acknowledgements

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