

Metal uptake, antioxidant status and membrane potential in maize roots exposed to cadmium and nickel

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Abstract: Root growth of the seedlings of maize cultivars Premia and Blitz exposed to 2 μM cadmium (Cd), nickel (Ni) or both metals acting simultaneously (Cd + Ni) for 72 h was significantly reduced but not ceased. The effect was more pronounced in the seedlings of the cv. Blitz. The heavy metals (HMs) contents increased significantly in the roots. Simultaneous application of metals had an antagonistic effect on either Cd or Ni uptake in Premia but not in Blitz. In control roots the contents of ascorbic acid (AsA) and dehydroascorbic acid (DHA) were lower and glutathione (GSH) content was higher in Premia than in Blitz. A decrease of AsA content was induced by all metal treatments in Premia but only by Cd + Ni in Blitz while an increase was induced by single metals in this cultivar. All metal treatments increased DHA contents in both cultivars. GSH content decreased significantly in Premia treated with Cd or Cd + Ni, and in Blitz treated with Ni. Unlike the contents of AsA, DHA and GSH, the increased metal concentrations in root cells did not affect the membrane potential (E_M). The changes in antioxidant contents depended on both, maize genotypes and HMs treatments. Nevertheless, the results indicated a role of antioxidative system in minimizing the effects of oxidative stress and protecting cell membranes in both maize cultivars.

Key words: maize; antioxidative substances; heavy metals; membrane potential (E_M)

Introduction

Inhibition of plant growth and crop production by excess of HMs such as Cd and Ni in contaminated soils is a global agricultural problem leading to considerable losses in plant productivity. No doubt, the occurrence of HMs can be both beneficial and harmful for plants (Meuwly & Rauser 1992; Rauser & Meuwly 1995; Schützendübel & Polle 2002; Seregin & Kozhevnikova 2006). Unlike Ni, that is a component of urease and at low concentrations stimulates growth of some plants (Bai et al. 2006), Cd is not involved in any known physiological function within a plant body and has been reported to cause deleterious effects on plants (Tran & Popova 2013).

Several authors have highlighted the activation of reactive oxygen species (ROS) scavenging systems to ameliorate the oxidative burst induced upon exposure of plants to heavy metals (Baccouch et al. 2001; Arvind & Prasad 2005; Rodriguez-Serrano et al. 2006; Gajewska & Skłodowska 2008). Crucial role in plant adaptation to heavy metal toxicity is played by the pro-

TECTIVE antioxidant system (Jocsak et al. 2008; Liu et al. 2007; Maysa & Abdel-Aal 2008; Yadav 2010). The most important antioxidants that prevent excessive accumulation of ROS are the low-molecular antioxidants such as AsA (Arrigoni & De Tullio 2000; Gallie 2013), GSH (Freeman et al. 2004; Paradiso et al. 2008; Yadav 2010), thiols, α -tocopherol and protective pigments as well as antioxidative enzyme species (Sharma & Dietz 2009). Leading role in the elimination of oxygen radicals and termination of free radical reactions in plants is played by AsA (Gallie 2013). AsA is able to directly neutralize oxygen radicals and recover tocopherol thus protecting the membranes. Ascorbate also functions as co-substrate for plant oxidases, such as ascorbate peroxidase system that produces DHA (Maysa & Abdel-Aal 2008). All these activities cause large AsA consumption, leading to the generation of its oxidized forms, ascorbate free radical (AFR) and DHA. The endogenous level of AsA is thus determined by both de novo AsA biosynthesis and recycling of the oxidized forms of AsA (Arrigoni & De Tullio 2000). The ratios between the oxidized and the reduced forms of antioxidants have

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been suggested to play a key role in the oxidative stress response and the control of the antioxidant systems (Liu et al. 2007; Paradiso et al. 2008). Also, non-protein thiol groups, especially GSH, exert several important roles in protection of plants from environmental stress factors, particularly in the case of Cd and Ni (Freeman et al. 2004; Paradiso et al. 2008; Sharma & Dietz 2009; Yadav 2010).

The main entry point of HMs into the roots is the plasma membrane (PM) of root cells. Several reports on the effects of Cd and Ni suggested an increase in membrane permeability, rapid K^+ efflux and/or changes of E_M leading to membrane damage and disruption of ion homeostasis of the cells (Sanz et al. 2009). The metal tolerance of plants may involve the protection of PM integrity from HMs damage (Janicka-Russak et al. 2008).

The aim of the present study was to evaluate the roles of AsA, DHA and GSH in two cultivars (cv. Premia and cv. Blitz) of *Zea mays* L. responding to single and joint action of Cd and Ni stress. The relationship between the long term effects of both ions on the antioxidants and on E_M changes were studied. The tolerance of maize cultivars to HMs stress is also discussed.

Material and methods

Plant material and growth conditions

Seeds of two maize cultivars, Premia and Blitz, were soaked in running water for 1 h, surface sterilized with 5% NaClO for 10 min, washed in running water for 10 min and germinated in moist filter paper rolls at 25°C for 48h. For determining the content of HMs, AsA, DHA, GSH and E_M measurements the seedlings with the primary root length of 1–2 cm were exposed to the solutions containing 2 μ M CdSO₄, 2 μ M NiSO₄ or 2 μ M CdSO₄ + 2 μ M NiSO₄, or to distilled water (control) adjusted to pH 5.7, for 72 h. Length of the primary roots was measured in photographed seedlings using the image analysis software Olympus CellF.

Determination of Cd and Ni contents

Cd and Ni contents were measured in the whole root system. Plant tissues were dried at 80°C and used for HMs determination according to Angelova et al. (2004). The HMs concentration was determined using atomic adsorption spectrophotometer S-115 (Ukraine) and related to the fresh weight (Borzou & Azizinezhad 2012).

Extraction and analysis of AsA and DHA

Roots (1 g) were homogenized in 5% metaphosphoric acid at 4°C. The homogenate was centrifuged at 18,000 g and the supernatant was then used. The AsA and DHA contents were determined according to Kampfenkel et al. (1995) with modifications by de Pinto et al. (1999).

Content of GSH

The glutathione pool was assayed according to previously described methods of Beutler et al. (1963) modified by Gryshko & Syshchykov (2002) and the optical density was measured at 412 nm using spectrophotometer SF-2000 (Russia).

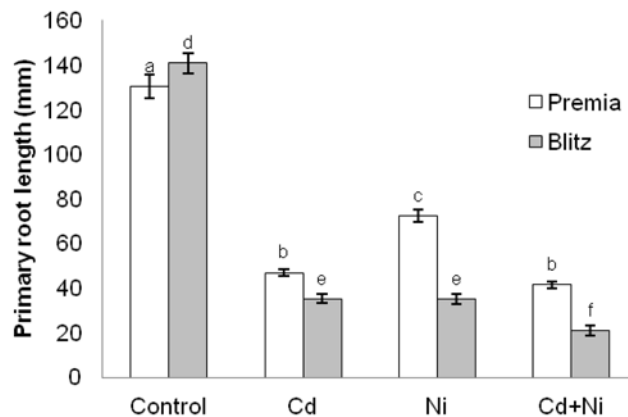


Fig. 1. Length of the primary roots of maize seedlings, cv. Premia and cv. Blitz after 3 days of exposure to 0 (control), 2 μ M Cd, 2 μ M Ni or 2 μ M Cd + 2 μ M Ni. Statistically significant differences ($P < 0.05$) among metal treatments or cultivars are indicated by different small letters (a–f).

Measurements of E_M

Measurements of E_M were performed on single outer cortical cells located 10 mm from the root tip of the 20 mm long apical segments of the primary root, using standard microelectrode techniques as described by Pavlovkin et al. (2006). The apical root segments were mounted to a Plexiglas holder with a soft rubber ring and mounted in a vertical 5 mL Plexiglas cuvette, which was perfused with a standard solution containing 0.1 mM KCl, adjusted to pH 5.7 using 0.1 M HCl at a flow rate of 5 mL/min.

Statistical analysis

Data were analyzed using one-way ANOVA with $P < 0.05$ or 0.01 (Prism 5, GraphPad Software Inc.). Means and standard deviations were calculated from three independent experiments ($n = 10$ apical root segments). Each experiment was repeated at least 3 times.

Results

Root growth

The length of primary roots was significantly reduced after all variants of metal treatments (Fig. 1). The root growth of the cv. Premia was significantly less affected with Ni than with Cd or Cd + Ni whereas it was reduced to a similar extent with single metal ions Cd or Ni and more severely with their joint action in the cv. Blitz. Differences between the cultivars appeared particularly in the stronger growth reduction of the roots of cv. Blitz.

Cd and Ni contents

The contents of Cd or Ni in control roots were similar in both cultivars. The experimental data allow us to state that Cd and Ni ions enter the root tissues of both maize cultivars and their contents in the roots of both cultivars increased within 72 h in comparison to the control (Table 1). The exposure of maize seedlings to 2 μ M Cd resulted in 6.5 and 4.5-fold increase of the Cd content in cv. Premia and Blitz, respectively. The intra-tissue contamination indices were significantly different

Table 1. Cd and Ni contents ($\mu\text{g/g}$ FW) in maize seedling roots exposed to either 2 μM Cd, 2 μM Ni or 2 μM Cd + 2 μM Ni for 72 h. Statistically significant differences ($P < 0.05$) among metal treatments or cultivars are indicated by different small letters (a–d).

	Cd ($\mu\text{g/g}$ FW)		Ni ($\mu\text{g/g}$ FW)	
	Premia	Blitz	Premia	Blitz
Control	0.71 ± 0.05^a	0.75 ± 0.03^a	0.14 ± 0.01^a	0.97 ± 0.01^a
Cd	4.50 ± 0.03^b	3.36 ± 0.18^d	0.14 ± 0.01^a	1.0 ± 0.20^a
Ni	0.82 ± 0.03^a	0.68 ± 0.02^a	23.66 ± 1.20^b	18.46 ± 0.79^c
Cd + Ni	3.60 ± 0.04^c	3.23 ± 0.20^d	16.38 ± 0.63^c	16.60 ± 0.35^c

for 2 μM Ni treatments. The Ni contents in the roots treated with Ni or Cd + Ni exceeded the contents in control roots 171 and 118-times, respectively in cv. Premia, while it was 17 and 19-times in cv. Blitz. After the simultaneous treatment with 2 μM Cd + 2 μM Ni, we observed antagonistic effects of Cd to Ni and vice versa in the cv. Premia. This was manifested in significantly lower uptake of Cd by the seedlings growing on medium with both metals (Cd + Ni) than in the seedlings growing on medium with Cd only. Similarly, the uptake of Ni was significantly lower in the seedlings exposed to both metals compared to those treated with Ni.

Contents of AsA and DHA

The absolute pools of AsA and DHA in control roots were similar and the concentration of DHA exceeded that of AsA in both cultivars (Fig. 1A, B). There were significant differences in the changes of AsA and DHA contents between the cultivars induced by each metal treatment, although the absolute amounts of DHA in Cd or Cd + Ni treated seedlings of both cultivars differed statistically.

The most intense decrease of AsA content in both cultivars was caused by the combined Cd + Ni treatment, although in cv. Premia, there were only slight differences between the single metal and the combined treatments. The contents of AsA in the roots of cv. Blitz seedlings varied significantly among the treatments. Comparing to control, opposite changes of AsA contents occurred following the treatments with Cd or Ni: there were statistically significant decrease in cv. Premia and increase in cv. Blitz.

The content of DHA increased significantly ($P = 0.05$) after any treatment in both cultivars. In the roots of cv. Blitz, the lowest increase of DHA content occurred after Ni and the highest after the combined treatment. Similar trend was observed also in the roots of cv. Premia, but the difference between Cd and combined treatment was not significant.

Contents of GSH

The obtained experimental data on the GSH in the root system of maize seedlings indicate different tendencies of HMs effects. Thus, the Cd ions, single as well as in combination with Ni, led to increase of the tripeptide concentration by 20–35% in the roots of the cv. Blitz, while it led to 10–30% reduction of the GSH in the root tissues of the cv. Premia seedlings (Fig. 2). The action of Ni ions led to about 50% decrease in GSH concentra-

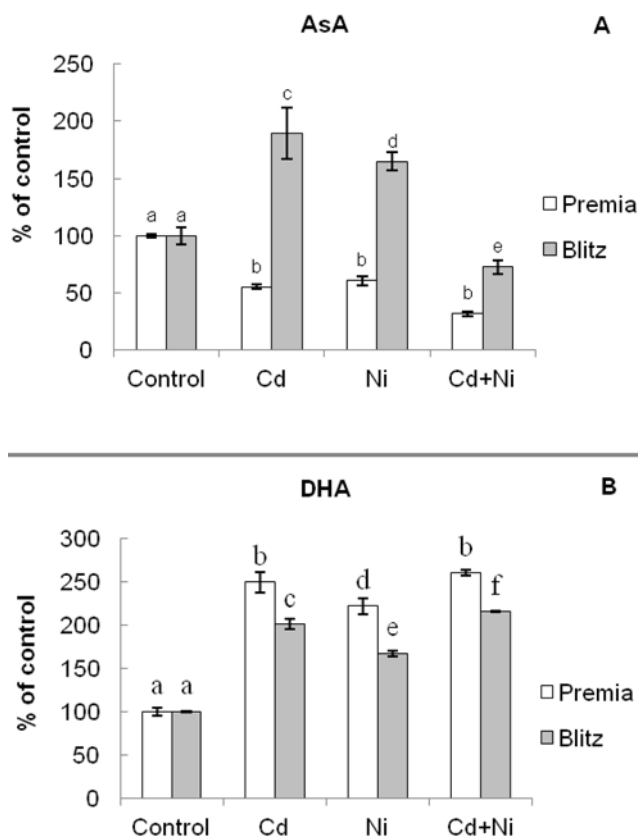


Fig. 2. Content of ASC (A) and DHA(B) in maize roots treated with 2 μM Cd, 2 μM Ni or 2 μM Cd + 2 μM Ni for 72 h. The means with the same letter are not significantly different from each other ($P < 0.05$).

Table 2. Membrane potential (E_M) changes of outer cortical root cells upon addition of 2 μM Cd, 2 μM Ni or 2 μM Cd + 2 μM Ni in perfusion solution within 72 h. The means with the same letter are not significantly different from the values of control cells ($P < 0.05$).

	Premia E_M (mV)	Blitz E_M (mV)
Control	-137 ± 7.8^a	-124 ± 7.1^b
Cd	-139 ± 7.6^a	-126 ± 6.8^b
Ni	-139 ± 7.9^a	-128 ± 6.5^b
Cd + Ni	-137 ± 8.0^a	-123 ± 6.1^b

tion in the roots of Blitz seedlings whereas differences between the control and Ni-treated roots were not significant in the cv. Premia.

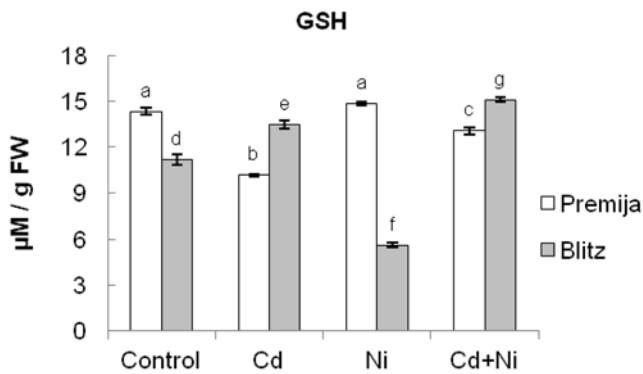


Fig. 3. GSH pool in maize roots treated with 2 μM Cd, 2 μM Ni or 2 μM Cd + 2 μM Ni for 72 h. The means with the same letter are not significantly different from each other ($P < 0.05$).

E_M changes

Under our experimental conditions the E_M of control maize outer cortical roots was more negative in cv. Premia in comparison to cv. Blitz (Table 2). The treatments of seedlings within 72 h with 2 μM Cd and 2 μM Ni or with 2 μM Cd + 2 μM Ni did not induce statistical changes in plasma membrane properties of the root cells of both cultivars.

Discussion

This is the first comparison of the toxic effects of Cd, Ni and Cd + Ni on the roots of maize cultivars, indicating that genetic variability in the extent of the protective responses can exist within one plant species. The 2 μM concentration of both metals was relatively low when compared to that used in great amount of experimental work reported in the literature. Nevertheless, the growth of the primary roots responded significantly in the seedlings of maize cvs Premia and Blitz as shown previously (Fiala et al. 2013). High sensitivity of maize plants subjected to even lower, 1 μM Cd concentration was reported (Široká et al. 2004). In our experiments, the high sensitivity of the maize seedlings might have been influenced by the proximate effects of the metal ions dissolved in distilled water in comparison with root media containing metal ions dissolved in nutrient solutions, and/or by the differences between maize cultivars in tolerance of Cd (Wang et al. 2007; Ekmekçi et al. 2008), or Ni (L'Huillier et al. 1996). In general, the differences in root growth responses between the cultivars indicate a higher sensitivity of Blitz than of Premia seedlings. There were also differences concerning the efficiency of either single or combined Cd + Ni treatments. The cv. Premia was significantly less affected with Ni than Cd or Cd + Ni. For this cultivar the presence of Cd in the root medium might have been crucial. The most toxic for the cv. Blitz, was the combined Cd + Ni treatment suggesting a cumulative effects of both metal ions.

The present observations of the higher accumulation of HMs in roots of cv. Premia in comparison to cv. Blitz, agree with a similar genetic variability in Cd

tolerance in pea genotypes (Belimov et al. 1999; Rivera-Becerril et al. 2002). The amounts of Cd and Ni in the roots of both cultivars characterize maize as a relatively highly metal-accumulating plant within the classification proposed previously (Reeves & Baker 2000) and, consequently, a possible candidate for vegetation cover and phytoextraction of contaminated soils.

In our experiments, the HMs induced changes in the pools of antioxidative compounds AsA, DHA and GSH. In the roots of cv. Premia treated with Cd, Ni or Cd + Ni, a significant decrease of AsA content and increased amount of DHA suggest that free radicals were neutralized by oxidation of AsA.

In the roots of cv. Blitz seedlings treated by Cd or Ni an increase in the content of AsA and DHA was observed. Similar enhancing of AsA and DHA contents induced by Cd was described by Paradiso et al. (2008) in durum wheat roots. In our opinion this can be explained by activation of the synthesis of AsA and a high speed of its utilization. Liu et al. (2007) also reported an increase in the content of AsA in Cd-treated plants of *Bechmeria nivea*.

By the combined action of Cd + Ni there was a tendency to decrease the content of AsA while increasing the amount of DHA in all variants, what could be explained by an intensification of the utilization of AsA for hindering free radical-induced lipid oxidation. Our results are consistent with those of other authors. The decrease of the total ASC pool (AsA plus DHA) was shown in Cd-treated durum wheat (Paradiso et al. 2008), in the roots of *Kandelia candel* and *Bruguiera gymnorhiza* treated with a mixture of Cd, Pb and Hg (Huang et al. 2010), and in the seedlings of pigeonpea in response to Zn and Ni (Rao & Sresty 2000).

Based on our experimental data on the GSH contents it is possible to assume that the response to Cd and Ni ions depended on the genotype. Thus the seedlings of the cv. Blitz seemed to be more sensitive to Ni ions as in their roots the lowest levels of GSH were recorded. In our set of experiments there seemed to be no expression of glutathione synthesis and the pool of the antioxidant available in cells was utilized similarly as reported in pigeonpea (Rao & Sresty 2000). The changes in GSH concentration induced by action of Cd were recorded also in maize (Rellán-Álvarez et al. 2006), wheat (Paradiso et al. 2008) and sunflower (Hatata & Abdel-Aal 2008) and could be caused by active functioning of enzymatic system of its reutilization. For the cv. Premia a higher sensitivity to Cd can be suggested as practically in all variants the concentration of GSH decreased significantly compared to control values while the treatment with nickel did not influence the tripeptide level. Most likely, in this case the obtained data can testify preferential functioning of other antioxidant systems (in particular ascorbate-dependent) for neutralization of the HM negative influence. These data indicate that cv. Blitz seemed to be more sensitive to Ni, whereas the cv. Premia to Cd. Our results show that AsA may play more important role in the fight against HM-induced oxidative damage in the roots of

the cv. Blitz than it does in the cv. Premia.

Since the PM of root cells is the main entry point of HMs into the roots, our measurements were performed to determine whether HMs induced any effect at this level. It is well known that changes resulting in disruption of membrane integrity and ion homeostasis of cells may subsequently have negative impact on specific biochemical processes and the vigor of plant cells to survive the stress conditions. Thus, the plant tolerance may involve the protection of PM integrity from HMs damage and maintaining ionic balance that may play a crucial role in adaptation of plants to stress conditions (Janicka-Russak et al. 2008). The data reported here show that HMs did not induce long-term effects on E_M and were in agreement with previous observations with much higher concentrations of HMs in barley and rice roots (Sanz et al. 2009). Our results confirmed the findings of Sanz et al. (2009) that the distinct effects caused by Cd and Ni were due to differences in cellular responses, triggered upon metal entering the cytoplasm (i. e. the activation of ascorbate-gluthatione cycle as an efficient detoxifying mechanism for HMs), rather than to differences in direct effects on PM.

Taken together, on the basis of the results presented here and previously (Freeman et al. 2004), it seems reasonable to assume that genetic manipulation of AsA and GSH synthesis may be used to increase heavy metal tolerance in maize. It remains to be cleared, however, if such manipulation could sufficiently improve the survival of maize seedlings under field conditions in order to become of agronomic interest.

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