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Dissimilar Responses of Membrane Potential (E_M), Permeability Properties and Respiration to Cadmium and Nickel in Maize Root Cells

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The short-term treatment with Cd^{2+} and Ni^{2+} triggered transient depolarization of trans-plasma membrane potential (E_M) in the outer cortical root cells of two maize cultivars (cv. Premia and cv. Blitz), however, both metals changed the E_M in a quantitatively different way. The magnitude and duration of E_M depolarization were concentration dependent and were greater in the metal susceptible cv. Blitz. The highest E_M depolarization was recorded with simultaneous application of Cd^{2+} + Ni^{2+} in both maize cultivars. The E_M depolarization induced by Cd^{2+} or Cd^{2+} + Ni^{2+} but not Ni^{2+} alone was accompanied with a tremendous increase of membrane conductivity, but it was not accompanied with the effect of heavy metals (HM) on respiration. Simultaneous application of fusiccocin (FC) with Cd^{2+} or Cd^{2+} + Ni^{2+} during the E_M depolarization, inability of FC to stop the depolarization by FC-enhanced proton extrusion and rapid restoration of E_M, suggested a transient inhibition of the plasma membrane H^+-ATPase by these toxic metals. Our data support the opinion that differences in the effects of the studied ions were not the result of their direct action on PM, but rather of their different influence on intracellular processes within root cells.

Keywords: maize roots, heavy metals (HM), trans-plasma membrane electrical potential (E_M), membrane permeability, respiration

Introduction

Metals are ubiquitous in soil environment and most of them are essential not only for plants but for all living forms. In spite of their necessity they are usually required in very low amounts and some of them can be toxic in excess. Plants have natural ability to extract elements from soil and in addition to the uptake of nutrient elements toxic compounds such heavy metals are taken up, too. When present in soil, HM can be absorbed, predominantly by plant roots using membrane transporters localised at the plasma membrane. To exert its biological effect a metal ion must cross the membrane and enter the cell where it

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can be bound to a cellular structure or alter specific biochemical processes. Several reports on the effects of Cd^{2+} and Ni^{2+} suggested an increase in membrane permeability, rapid K^+ efflux and/or changes of trans-plasma membrane electrical potentials (E_M) leading to membrane damage and disruption of ion homeostasis of the cells (Kennedy and Gonsalves 1987; Aidid and Okamoto 1992; Llamas et al. 2000, 2008; Pavlovkin et al. 2006; Karcz and Kurtyka 2007; Sanz et al. 2009; Kurtyka et al. 2011). All these changes result in disruption of membrane integrity and ion homeostasis of cells and subsequently may have negative impact on specific biochemical processes and the vigour of plant cells to survive the stress conditions. Thus, tolerance may involve the protection of PM integrity against HM damage and maintaining ionic balance. It is well known that PM H^+-ATPase plays a crucial role in adaptation of plants to stress conditions (Janicka-Russak 2008).

The co-existence of Cd^{2+} with other HM in the ecosystem can lead to various synergic and antagonistic interactions in their uptake and tissue distribution. Addition of Cd^{2+} significantly reduced the Zn^{2+} and Mn^{2+} levels in roots and shoots of barley (Wu et al. 2003) and durum wheat (Jalil et al. 1994). Similarly, the uptake and toxicity of Cd^{2+} can be moderated significantly by the presence of excess of certain essential metals like Zn^{2+}, Ca^{2+}, Fe^{2+}, Cu^{2+} or Mn^{2+} (Das et al. 1997; Aravind and Prasad 2003). On the other hand, Ni^{2+} in concentrations 4 and 40 μg/L enhanced Cd^{2+} uptake and ascorbate peroxidase activity in maize roots (Artyushenko and Gryshko 2010). The uptake of Cd^{2+} by intact seedlings of two contrasting ecotypes of the hyperaccumulator *Thlaspi caerulescens* was not inhibited by Ni^{2+} (Zhao et al. 2002).

Our previous results showed that Mn^{2+} clearly alleviated the toxic effect of Cd^{2+} on the root growth of maize seedlings and a total abolition of Cd^{2+} (10 μmol/L) toxicity was observed at Mn^{2+}:Cd^{2+} ratio of 20:1 (Paľove-Balang et al. 2006).

The primary goal of the work was to study combined effects of Ni^{2+}, one of the essential elements, which is needed for urea metabolism, iron absorption and nitrogen fixation (Welch 1995), and of Cd^{2+}, the highly toxic element without known biological function on the electrical parameters of root plasmalemma (E_M), membrane permeability and respiration in the roots of two maize cultivars. Relationship between the effects of the metal ions on the studied parameters and metal tolerance of maize cultivars to heavy metal stress is also discussed.

Materials and Methods

Plant material and growth conditions

Two maize cultivars, Premia 190 MB (metal tolerant) and Blitz 160 MB (metal susceptible) grown on the heavy metal-polluted soils in Ukraine were used in our experiments. Seeds were surface-sterilized with sodium hypochlorite (1% available chlorine) for 2 min and rinsed three times in sterile distilled water for 2 × 2 min. The seeds germinated in the rolls of moistened filter paper in thermostat in the dark at 21ºC for 3 days. Three-day-old seedlings were used for experiments.

Electrophysiological measurements

Measurements of EM were performed on single outer cortical cells located <1 mm from the root tip of 20 mm long apical primary root segments using standard microelectrode techniques as described in detail by Pavlovkin et al. (2006). After rinsing the roots with 0.5 mmol/L $CaSO_4$, the roots were mounted in a 4-mL volume plexiglass chamber and were constantly perfused (10 mL min^{-1}) with bathing solution containing 0.1 mmol/L KCl, 0.1 mmol/L $CaCl_2$ and 0.1 mmol/L $CaSO_4$ adjusted to pH 5.5 using 0.1 m HCl. Experimental HM-containing solutions consisted of 0.1 mmol/L KCl, 0.1 mmol/L $CaCl_2$ and 5–1000 μmol/L $CdCl_2$ or 0.01–5 mmol/L $NiCl_2$. The presence of HM as a dichloride salt in the perfusion solution induced immediate membrane changes of the root cells. Both Cd or Ni and Cl^- ions contributed to membrane depolarization, as previously described for Ni2+-induced effects on rice root cells (Llamas et al. 2008). Therefore, to separate and reset the effect of Cl^- subsequent experiments were performed by pre-treatment of plants with bathing solution according to Kenderešová et al. (2012). EM changes induced by HM (2–1000 μmol/L) in short-term treatments were measured continuously during the whole experiment (until 8 h) at 22°C. Fusicoccin (FC), a plasma membrane H+-ATPase stimulator, was used to monitor the functionality of the plasma membrane H+-pump (Marrè 1979), at a final concentration of 30 μm in 0.1% ethanol.

For the separation of diffusion potential (E_D) from energy-dependent component maintained by the PM-ATPase (E_P) of E_M, the perfusion solution was saturated with N_2 gas by flushing.

Membrane permeability

Samples of 20 apical 1 mm long root segments were incubated in 5 mL 0.1 mmol/L $CaSO_4$ for 2 h to eliminate ions from the apoplast and then transferred to 1 mmol/L of medium containing HM and the conductivity of the medium was measured by conductivity meter OK-109-1 (*Radelkis,* Hungary). The conductivity changes were expressed as a difference between the value of the particular conductivity measured, and the value of the initial conductivity. The values were related to the root fresh weight.

Respiration measurements

Primary roots, 2.5 cm long, of maize seedlings were used for measuring the total respiration rates (VT; nmol O_2. g^{-1}. DW. s^{-1}). Respiration was measured polarographically using an oxygen Clark-type electrode (YSI 5300, Yellow Springs Instrument, USA) at 25ºC. The root segments were sealed in a water-jacketed vessel containing 3 cm^3 of fully aerated 10 mM Na-phosphate buffer (pH 6.8). In order to minimize the problems of nonlinear O_2 depletion traces and to eliminate potential wound respiration, handling of roots was kept at a minimum and the uptake of O_2 was measured immediately after excision from the intact root. Linear traces that indicated no wound-dependent increase in the respiration rate were used for the calculations.

Statistics

Data were analyzed using 2-way ANOVA with $P < 0.05$ or 0.01 (Prism 5, GraphPad Software Inc.). Means and standard deviations were calculated from three and more independent experiments.

Results

Electrophysiological measurements

Under experimental conditions without HM treatment (control) the resting values of E_M and E_P in the outer cortical cells of cv. Premia, were generally more negative (–139 ± 5.3 mV, $n = 56$) than that of cv. Blitz (–123 ± 5.5 mV, $n = 63$). Higher E_P values were recorded in the outer cortical cells of cv. Premia (58 ±3.3 mV, $n = 5$) in comparison with cv. Blitz (42 ± 3.1 mV, $n = 5$), but the E_D values –84 ± 4.4 and –82 ± 4.1 mV for cv. Premia and Blitz, respectively, did not differ from each other.

In the short-term experiments HM induced an immediate depolarization of E_M of the outer cortical cells. After maximal depolarization in all measurements a tendency to complete repolarization of the resting potential could be observed (Fig. 1A, B, C). Our results also pointed out different effects of Cd^{2+} and Ni^{2+} on the E_M. Addition of 1 mM Ni^{2+} to the perfusion solution induced a transient membrane depolarization with a recovery of the resting potential within 40–60 min (Fig. 1A). In contrast, perfusion of maize roots with tenfold lower concentration of Cd^{2+} (Fig. 1B) or $Cd^{2+} + Ni^{2+}$ (Fig. 1C) resulted in a strong depolarization of cortical cells, down to values within the range of the E_D. Ni^{2+} at the 1:1 stoichiometry used in our conditions 20, 50 and 100 µmol/L, significantly enhanced the effect of Cd^{2+} on the E_M depolarization magnitude (Fig. 1C). After 90–120 min exposition to Cd^{2+} or $Cd^{2+} + Ni^{2+}$ the membranes repolarized attaining the initial E_M values only within 6–8 h (Fig. 1B, C).

Addition of FC to the control medium caused immediate E_M hyperpolarization by 24 to 30 mV in both cultivars (–168 ± 3 mV, $n = 2$ for cv. Premia and cv. Blitz –148 ± 3.1 mV, $n = 2$). On the other hand, FC applied to the perfusion solution following 90-min treatment with 100 µmol/L Cd^{2+} did not counteract the E_M depolarization that had decreased to values around E_D. Root cell membranes began to hyperpolarize after 60 min of the FC treatment and within 2 h the E_M hyperpolarized to the original values recorded in control cells (Fig. 2).

The extent of the membrane depolarization upon Cd^{2+} and Ni^{2+} addition was concentration dependent (Fig. 3) and was significantly higher in cv. Blitz than in cv. Premia. It is necessary to note that in short-term experiments the concentrations of $Cd^{2+} < 5$ µmol/L and $Ni^{2+} < 100$ µmol/L did not cause any significant changes of E_M. Addition of 2 µmol/L or 1 mmol/L Ni^{2+} to the perfusion solution containing various concentrations of Cd^{2+} enhanced the magnitude of E_M depolarization in comparison with Cd^{2+} alone (Fig. 3). Highly significant differences in E_M depolarization values were found not only among cultivars ($P = 0.01$) but also between the treatments with Cd^{2+} or $Cd^{2+} + Ni^{2+}$ ($P = 0.05$).

The magnitude of E_M depolarization decreased in the order $Cd^{2+} + Ni^{2+} > Cd^{2+} > Ni^{2+}$ in both hybrids and was greater in the cv. Blitz in comparison to cv. Premia ($P = 0.01$) in all measurements.

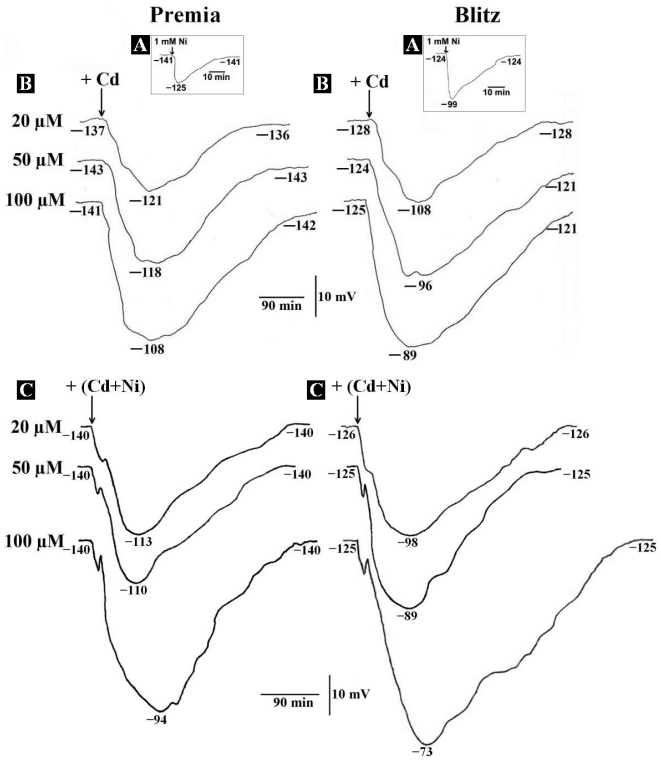

Figure 1. Comparison of dynamic changes of E_M upon Ni^{2+}– (A), Cd^{2+}– (B) or Cd^{2+}+Ni^{2+}– (C) treatment in the root tip outer cortical cells of maize. Cultivars and metal ions with their concentrations are marked; the time points of their addition are indicated by arrows. The bars denote time and milivoltage

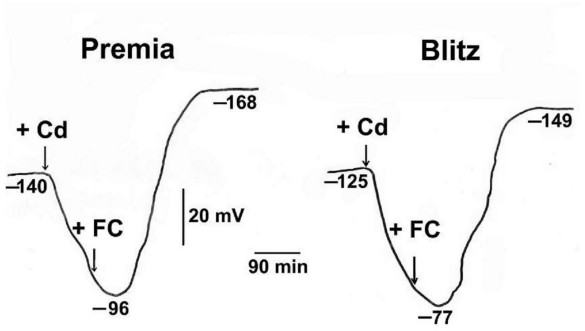

Figure 2. Addition of FC to perfusion solution contained 100 µmol/L of Cd^{2+}

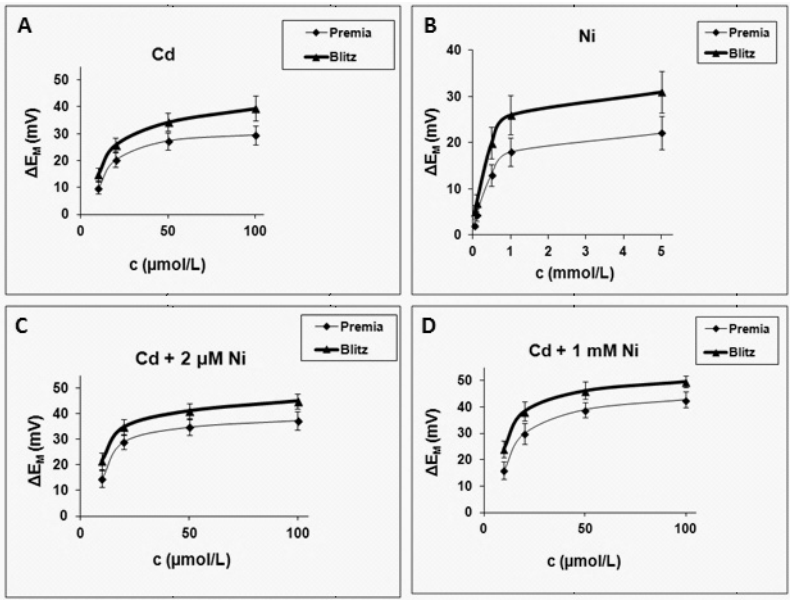

Figure 3. Concentration-dependence of the magnitude of the initial membrane depolarization (ΔE_M) induced in outer cortical cells of maize roots by different concentrations of Cd^{2+} (A), Ni^{2+} (B) or both (C, D) (mean ± SD, n = 4–8)

Table 1. Effect of 1000 μmol/L HM on the maize root membrane conductivity

Time (min)	Membrane conductivity (μS/g FW)							
	Premia				Blitz			
	Control	Cd	Ni	Cd + Ni	Control	Cd	Ni	Cd + Ni
30	2.9±0.23 ^{a}	30±3.4 ^{b}	7.4±1.9 ^{a,d}	33±3.22 ^{b,c}	2.7±0.23 ^{a}	38±3.6 ^{c}	12.3±2.5 ^{d}	39.3±4.1 ^{c}
60	3.3±0.34 ^{a}	36±3.8 ^{b}	8.2±2.3 ^{a,d}	40±3.7 ^{b,c}	3±0.34 ^{a}	45.4±4.1 ^{c}	12.8±2.7 ^{d}	44.1±3.96 ^{c}
90	3.7±0.35 ^{a}	39±3.3 ^{b}	9.3±2.7 ^{a,d}	42±3.6 ^{b,c}	3.3±0.36 ^{a}	49±4.3 ^{c}	12.2±2.3 ^{d}	46.3±4.37 ^{c}
120	3.9±0.41 ^{a}	40±4.5 ^{b}	9.3±2.5 ^{a,d}	41±5.8 ^{b,c}	3.5±0.31 ^{a}	50±4.27 ^{c}	12±2.1 ^{d}	44.5±4.07 ^{c}
180	4.2±0.37 ^{a}	38.9±4.2 ^{b}	7.2±1.9 ^{a,d}	39±5.8 ^{b,c}	3.7±0.33 ^{a}	49±4.17 ^{c}	11.7±2.6 ^{d}	42.3±3.65 ^{c}

Means ± SD. Statistically significant differences ($P < 0.05$) among metal treatments or cultivars are indicated by different small letters (a–d). The means with the same letter are not significantly different from each other ($P < 0.05$).

Membrane permeability

The effect of HM concentrations (1 mmol/L) on root cell membrane permeability was investigated in short-term experiments up to 3 h (Table 1). Membrane permeability of the root cells treated with Cd^{2+} and $Cd^{2+} + Ni^{2+}$ increased tremendously during the first 3 h in both cultivars with the same intensity, but the significant increase induced by Ni^{2+} treatment ($P = 0.05$) represented only 20% of the values recorded in Cd^{2+}-treated roots. After this rapid increase of the membrane permeability, the values remained stable for the next

10–15 h. Comparing both cultivars highly significant differences in membrane conductivity were registered only in Cd^{2+}-treated roots and the values were higher in cv. Blitz than in cv. Premia.

Respiration measurements

To study the possible relationships between transient E_M changes and respiration we measured the effects of HM (100 µmol/L) on O_2 production (Fig. 4) after 2 and 6 h when the maximum depolarization induced by Cd^{2+} and $Cd^{2+} + Ni^{2+}$ and following repolarization of E_M were recorded. In both cultivars HM did not induce significant differences compared to control after 2 h. Statistically significant differences ($P = 0.05$) were observed only after 6 h of the HM treatment. The magnitude of inhibition of root respiration was similar in both cultivars.

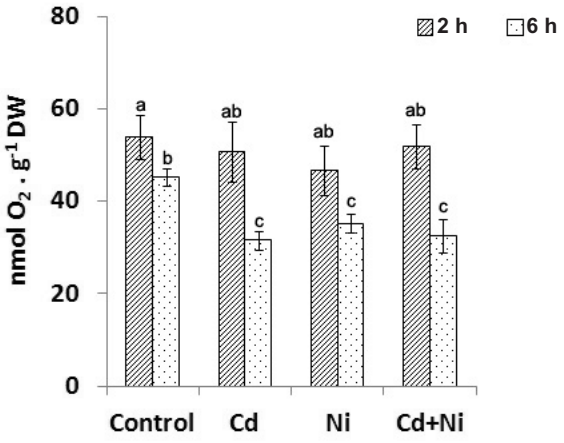

Figure 4. Respiration rates of maize roots cv. Premia grown in nutrient solution 2 and 6 h supplemented with 100 µmol/L of HM (mean ± SD, $n = 3$). The means with the same letter are not significantly different from each other ($P < 0.05$)

Discussion

There are several reports on the effect of Cd^{2+} or Ni^{2+} administered separately on membrane functionality of plant cells, but to date their simultaneous effect has not been studied. Data presented here show that in the short-term treatment Cd^{2+} or Ni^{2+} caused a transient depolarization of E_M, whereas both metals changed E_M in a different manner. While Ni^{2+} caused depolarization only at high concentrations (>0.5 mmol/L) (Fig. 1A), depolarization occurred at five- to tenfold lower concentrations of Cd^{2+} (50 and 100 µmol/L) (Fig. 1B). Our results seem to well agree with the data presented by Llamas et al. (2000, 2008) and Sanz et al. (2009) on barley and rice roots. The authors revealed that E_M depolarization

upon Cd^{2+} was followed by almost total repolarization within ca 6 h. Ni^{2+} in comparison with Cd^{2+}-induced smaller concentration-dependent depolarization of E_M (Fig. 3). Depolarization of E_M with 1 mM Ni^{2+}, indicating its uptake into the cells, followed by repolarization of E_M to the values similar to control (Fig. 1A) may be due to a stimulation of PM-H^+ ATPase, as has been demonstrated for Ni^{2+} in maize rootsegments by Coccuci and Morgutti (1986).

Llamas et al. (2000) suggested a direct effect of Cd^{2+} on PM-ATPases, and the subsequent recovery of E_M due to the induction of a Cd^{2+} detoxification system. For example it is well know that lipid peroxidation caused by HM stress induced some defence mechanisms in cells. The previous experiments with cv. Blitz showed that 6 h treatment with Cd^{2+} + Ni^{2+} statistically increased ascorbate peroxidase and TBA-active products in roots (Artyushenko and Gryshko 2010). In contrast to Cd^{2+}, the exposure to Ni^{2+} of rice and barley roots (Llamas et al. 2008; Sanz et al. 2009) caused PM depolarization but the activity of the PM H^+-ATPase was not inhibited and the initial resting potential recovered in less than 1 h.

These results are in accordance with our FC measurements (Fig. 2), which showed that FC did not counteract the toxic effect of Cd^{2+} in the early stages of the FC action because E_M depolarization continued declining to very low values obtained in experiments without FC and, only after 60 min the E_M began to increase faster in comparison to Cd^{2+} experiments (Fig. 1B, C).

Our data allow us assuming that HM effects on E_P of E_M through direct inhibition of the PM H^+-ATPase might be the most probable, as the inhibition of root cell respiration was recorded 6 h after the exposure of roots to HM treatment (Fig. 4). Kurtyka et al. (2011) showed that FC combined with Cd^{2+} at 100 μM decreased (by 50%) Cd^{2+} content in maize coleoptile segments. In case of the higher Cd^{2+} concentration (1 mM), FC was not effective in either recovering the Cd^{2+}-induced membrane depolarization, or decreasing Cd^{2+} content in maize coleoptile cells. On the other hand, FC accelerated E_M repolarization at 1 mmol/L Cd^{2+} in maize root cortical cells (Pavlovkin et al. 2006).

Apart from the separate effects of Cd^{2+} or Ni^{2+} on the membrane properties of root cells we have also analyzed their synergic effects. We also followed the response of E_M to equimolar concentrations of Cd^{2+} + Ni^{2+} (Fig. 1C). These metal combinations caused depolarization of the E_M, which was similar to that caused with Cd^{2+} alone (Fig. 1B), whereas addition of Ni^{2+} significantly enhanced Cd^{2+} effect on the E_M depolarization magnitude (Fig. 1C and Fig. 3).

The lower E_M values of HM-induced depolarization in cv. Premia, in comparison with cv. Blitz, were probably influenced by higher values of the resting E_M and in particular, by the energy-dependent component (E_P) of E_M maintained by the PM-H^+ ATPase in the cortex cells. In cv. Blitz these values reached about 30% of those recorded in cortex cells of the cv. Premia.

Taken together, HM stress signalling appears to be highly complex and specific for different metal ions. We showed differential response of two maize cultivars to Cd^{2+} and Ni^{2+}. The data presented in this paper clearly show that Cd^{2+} is much more phytotoxic than Ni^{2+}. Moreover, Ni^{2+} enhanced the phytotoxic effect of Cd^{2+}. In short-term treatment nei-

ther of the ions influenced the PM directly, but their toxicity appeared after their entering the root cells as it was demonstrated with electrophysiology and respiration of the root cells. Based on our results we can also conclude that cv. Premia was more tolerant to HM treatment than cv. Blitz in all parameters studied.

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