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ScienceDirect
Plant Physiology and Biochemistry 46 (2008) 905–910
Plant Physiology and Biochemistry
www.elsevier.com/locate/plaphy

Research article

Ni^{2+} toxicity in rice: Effect on membrane functionality and plant water content

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Received 16 August 2007
Available online 24 May 2008

Abstract

The heavy metal nickel is an essential mineral trace nutrient found at low concentrations in most natural soils. However, it may reach toxic levels in certain areas and affect a number of biochemical and physiological processes in plants. Wilting and leaf necrosis have been described as typical visible symptoms of Ni^{2+} toxicity. The plasma membrane (PM) of root cells constitutes the first barrier for the entry of heavy metals but also a target of their toxic action. This work studies the relationship between disturbances of membrane functionality and the development of the typical symptoms of Ni^{2+} toxicity. Rice plants (*Oryza sativa* L. cv. Bahia) grown in nutrient medium containing 0.5 mM Ni^{2+} showed a significant decrease in water content as a consequence of the stress. Addition of Ni^{2+} to the solution bathing the roots induced a concentration-dependent PM depolarization but the activity of the PM-H^+-ATPase was not inhibited by the presence of Ni^{2+} and the initial resting potential recovered in less than 1 h. In the short term (hours), membrane permeability of root cells was not significantly affected by Ni^{2+} treatments. However, in the long term (days) a drastic loss of K^+ was measured in roots and shoots, which should be responsible for the changes in the water content measured, since stomatal conductance and the transpiration rate remained unaffected by Ni^{2+} treatment. The effects induced by Ni^{2+} were not permanent and could be reverted, at least in part, by transferring the plants to a medium without Ni^{2+}.

Keywords: Membrane permeability; Nickel toxicity; *Oryza sativa*; Stress alleviation; Water content

1. Introduction

Ni^{2+} is a transition metal that, except in ultramafic or serpentinic soils, is found in natural soils at trace concentrations. However, the Ni^{2+} concentration is increased in certain areas by human activities such as mining works, emission of smelters, burning of coal and oil, sewage, phosphate fertilizers and pesticides [9]. Ni^{2+} concentrations in polluted soils may reach 200–26,000 mg kg^{-1}, 20- to 30-fold higher than the overall ranges (10–1000 mg kg^{-1}) found in natural soils [11]. Though this metal is now considered an essential mineral nutrient [3,34], excess Ni^{2+} in the medium alters various physiological processes, resulting in detrimental effects on plants and causing diverse toxicity symptoms. Among these, chlorosis and necrosis have been widely found in different plant species [24,28,38,40], including rice [33]. According to Bollard [2], Fe deficiency could explain part of the symptoms induced by Ni^{2+} and other heavy metals. In accordance, Ouzounidou et al. [23] reported that long-term exposure of wheat plants to 1 mM Ni^{2+} reduces Fe contents and results in Fe and Mg deficiency symptoms. However, Parida et al. [26] observed that Fe contents in plants of *Trigonella* increased with the increase in the Ni^{2+}concentration applied. As for Fe, several authors have shown a decrease of other mineral nutrient contents in shoots and/or roots of Ni^{2+}-treated plants of various species while in others an increase was measured [28,30,34,39,40].

Impairment of nutrient balance may result from metal-induced disorders in the functionality of cell membranes. Thus, Ni^{2+} affected the lipid composition of the PM and the PM-H^+-ATPase activity in *Oryza sativa* shoots [29]. Pandolfini et al. [25] showed enhanced lipid peroxidation activity in wheat

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doi:10.1016/j.plaphy.2008.05.006

plants following Ni^{2+}-treatment. Moreover, Gonnelli et al. [10] reported that malondialdehyde (MDA) concentrations, a marker of lipid peroxidation, increased in shoots of plants from Ni^{2+}-sensitive but not from tolerant populations of *Silene*. All these changes might disturb membrane functionality, and therefore the ion balance in the cytoplasm, particularly of K^+, the most mobile ion across plant cell membranes. Gajewska et al. [8], however, indicated that Ni^{2+} effects on wheat plants are not caused by lipid peroxidation.

Other symptoms observed in Ni^{2+}-treated plants are related with changes in water balance. Thus it has been pointed out that Ni^{2+} induces a decline in plant water content of dicot and monocot species [6,7,24] and Jones and Hutchinson [12] used the decrease in water uptake as an indicator of the progression of Ni^{2+} toxicity in birch seedlings. Lin and Kao [14], however, reported that Ni^{2+} treatments did not modify the relative water content of detached rice leaves. Pandey and Sharma [24] emphasized the lack of information existing on development of water stress in response to excess supply of Ni^{2+}, compared with other metals.

The hypothesis of the present work is that one of the mechanisms of phytotoxicity from excess Ni^{2+} in rice plants is based on disturbances of cell membrane functions. If the capacity of ion influx (i.e. changes in membrane potential) and/or passive efflux increases as a result of Ni^{2+} treatment, the K^+ content would decrease, thus disturbing plant water balance. Therefore, our work focuses in the study of the plasma membrane as a target of Ni^{2+} effects. Changes in membrane electropotential induced by Ni^{2+} have been followed, as well as variations of membrane permeability and plant water content. Plant responses to alleviation of the stress after removing Ni^{2+} from the medium have also been measured to differentiate between transitory and permanent damage.

2. Materials and methods

2.1. Plant material and growth conditions

Rice seeds (*Oryza sativa* L. cv. Bahia) were grown in hydroponic cultures containing 1 mM KCl, 3 mM KNO_3, 1 mM $Ca(NO_3)_2$, 1 mM NaH_2PO_4, 0.75 mM $MgSO_4$, 2.4 mM NH_4NO_3, 5.9 μM $MnCl_2$, 15 μM H_3BO_3, 0.5 μM $CuSO_4$, 0.28 μM $ZnSO_4$, 0.014 μM $(NH_4)_6MoO_{24}$ and 3 mg l^{-1} Fe–EDDHA (ethylenediamine-*N*,*N*′bis[-hydroxyphenylacetic acid]), and kept in darkness. Six days after sowing, $NiCl_2$ was added to the nutrient medium with a final concentration of 0.5 mM, unless stated otherwise, and a 15/9 h photoperiod was provided (30 °C/light, 25 °C/dark). Plants were sampled periodically up to 10 days after the onset of the treatments. Putative recovery from the effects of metal stress was studied in some experiments by transferring the plants to fresh nutrient solution without heavy metal (+/− Ni^{2+} plants) after 5 days of treatment (control and treated).

2.2. Membrane permeability

Potassium efflux from duplicate samples of 10 plants was measured to assess the changes of membrane permeability caused by Ni^{2+} treatments. The roots of whole plants were thoroughly washed twice with cold 0.2 mM $CaSO_4$ to eliminate the nutrient solution from the apoplast, blotted on filter paper and submerged in 25 ml of 0.5 mM Ni^{2+} in 0.2 mM $CaSO_4$. They were then incubated for 2–8 h in a water bath (Selecta, Unitronic 320 OR) at 25 °C, and gently shaken (50 U min^{-1}). K^+ efflux from the roots to the external solution was measured by flame photometry (PFP7, Jenway, Dunmow, UK), as well as K^+ remaining in the roots. After each incubation time, the roots were washed, homogenized (Ultraturrax T25, Janke & Kunkel, Staufen, Germany) in 10 ml distilled water, boiled for 30 min and centrifuged for 10 min at 5000 × *g*, at 20 °C. Control experiments, without metal, were run simultaneously.

To study long-term effects of the metal, plants were grown in 0.5 mM Ni^{2+} and sampled at different time intervals (days). After washing the root apoplast with cold 0.2 mM $CaCl_2$, 100 mg (fresh weight; FW) aliquots of leaf segments or whole roots were incubated for 1 h in a water bath. K^+ efflux to the medium and K^+ content in the tissue were determined by flame photometry as described above.

2.3. Electrophysiological measurements

The effect of Ni^{2+} on the transmembrane electropotential difference (E_m) was measured as described by Llamas et al. [16]. Roots of whole plants were introduced into a Plexiglas chamber that was perfused at a flow rate of 7 ml min^{-1} with a standard solution consisting of 0.2 mM KCl and 0.2 mM $CaSO_4$, adjusted to pH 6.5 with NaOH. E_m was measured with glass micropipettes filled with 3 M KCl and reference salt bridges filled with 3 M KCl in 2% agar, connected via Ag/AgCl electrodes with an electrometer amplifier (LM-1, List, Göttingen, Germany) and a line recorder. The reference electrode was kept in the perfusion chamber, in the vicinity of the root, and the micropipette was inserted into the root with a micromanipulator at the beginning of the root hair zone (10–30 mm from the tip). The initial maximum depolarization (ΔE_m) induced by different Ni^{2+} concentrations (0.1–5 mM) was measured by addition of $NiCl_2$ to the perfusion solution. Subsequently, the effect of short-term treatments with Ni^{2+} was registered after the cells attained a resting potential with an equimolar $CaCl_2$ solution by exchanging $CaCl_2$ with $NiCl_2$ in the perfusion solution, to avoid the effect of the counterion. E_m of plants treated for several hours with Ni^{2+} was also measured using the same solution, containing 0.5 mM Ni^{2+}.

2.4. Gas exchange parameters

Stomatal conductance (g_s) and transpiration rates were determined with a portable infrared gas analyzer (Li-6200, Li-Cor Inc., Lincoln, NE). Measurements were performed by inserting into the chamber the second leaf of plants after 5 and 10 days of treatment with 0.5 mM Ni^{2+}.

2.5. Statistical analysis

The number of independent experiments performed in each case is indicated in the figure legends. The obtained data were