DE GRUYTER OPEN

Acta Bot. Croat. 75 (1), 25–30, 2016
DOI: 10.1515/botcro-2016-0017

CODEN: ABCRA 25
ISSN 0365-0588
eISSN 1847-8476

Impact of nickel on grapevine (*Vitis vinifera* L.) root plasma membrane, ROS generation, and cell viability

Ján Pavlovkin[1]*, Roderik Fiala[1], Milada Čiamporová[1], Michal Martinka[1,2], Vladimír Repka[1]

[1] Institute of Botany, Slovak Academy of Sciences, Dúbravská cesta 9, SK-84523, Bratislava, Slovak Republic
[2] Department of Plant Physiology, Faculty of Natural Sciences, Comenius University, Mlynská dolina B-2, SK-84215 Bratislava, Slovak Republic

Abstract – The present study investigated the impact of nickel (Ni^{2+}) on trans-membrane electrical potential (E_M) and permeability properties of plasma membrane (PM) in epidermal cells of adventitious grapevine roots. The relationship between disturbances of membrane functionality and the production of superoxide anion, hydrogen peroxide and cell viability after the exposure of roots to Ni^{2+} was also studied. Treatments with 0.1–5 mmol L^{-1} $NiCl_2$ induced a concentration-dependent transient PM depolarization, which was recovered to the initial resting potential within 50–70 min in the presence of Ni^{2+}. Longer (up to 24 h) exposure of roots to 1 mmol L^{-1} of Ni^{2+} hyperpolarized the E_M by approximately 17 mV. Application of the highest 5 mmol L^{-1} concentration of Ni^{2+} during longer treatments (up to 48 h) resulted in the increase of membrane permeability; however the E_M, cell viability, and superoxide content remained unaffected. The increase in the formation of hydrogen peroxide was time- and concentration- dependent and maximum production was recorded after 180 min of Ni^{2+} treatment. We can conclude that oxidative stress resulting from an imbalance in the generation and/or removal of hydrogen peroxide in the root tissues of grapevine was the major cause of Ni^{2+} toxicity.

Keywords: cell viability, grapevine, nickel trans-membrane electrical potential, oxidative stress, roots

Introduction

Among different environmental heavy-metal pollutants, nickel (Ni^{2+}) has gained considerable attention in recent years, because of its rapidly increasing concentrations in soil, air, and water in different parts of the world. Most agricultural soils contain Ni^{2+} in average of 25 mg kg^{-1} soil dry weight (DW) but its content is often substantially increased up to 26,000 mg kg^{-1}, by human activities such as mining, emission of smelters, coal and oil burning, sewage, phosphate fertilizers and pesticides (Holmgren et al. 1993). Many plants that naturally grow on such contaminated soils contain Ni^{2+} in concentrations exceeding 1000 mg g^{-1} DW in their tissues (Gonnelli et al. 2001) but they generally possess mechanisms allowing them to tolerate Ni^{2+} and to develop without phytotoxic problems (Gabbrielli et al. 1990). However, many of agriculturally important crops contain less than 5 µg of Ni g^{-1} DW, and the symptoms of phytotoxicity often become apparent at soil Ni^{2+} concentrations as low as 25–30 µg g^{-1} (Khalid and Tinsley 1980).

Nickel is now considered an essential mineral nutrient (Brown et al. 1987, Seregin and Kozhevnikova 2006), which in low concentrations fulfils a variety of essential roles in plants. Therefore, Ni^{2+} deficiency produces an array of effects on growth and metabolism of plants, including reduced growth, and induction of senescence, leaf chlorosis, alterations in nitrogen metabolism, and reduced iron uptake (Ahmad and Ashraf 2011). According to Bollard (1983) iron deficiency could explain part of the symptoms induced by Ni^{2+}. In addition, Ni^{2+} is a constituent of several metalloenzymes such as urease (Brown et al. 1987). On the other hand excess of Ni^{2+} in the medium alters various physiological processes, resulting in detrimental effects on plants and causing diverse toxicity symptoms (Sharma and Dhiman 2013). Among these, iron deficiency that leads to chlorosis and foliar necrosis and inhibition of nutrient absorption by roots have been widely found in different plant species (Pandey and Sharma 2002, Chen et al. 2009). Ouzounidou et al. (2006) working with wheat plants reported, that long exposure with 1 mM Ni^{2+} reduced iron content leading to iron and manganese deficiency. Additionally, excess Ni^{2+} also can retard shoot and root growth, impair plant metabolism, inhibit photosynthesis and transpiration, and cause ultrastructural modifications, which are well documented in the review by Sharma and Dhiman (2013).

* Corresponding author, e-mail: jan.pavlovkin@savba.sk

Other symptoms observed in Ni^{2+}-treated plants are related to oxidative stress (Gajewska et al. 2006, Sharma and Dietz 2009). When generation of reactive oxygen species (ROS) such as superoxide and peroxide exceeds their removal, injuries may occur in the cells (Agrawal et al. 2013). The most common indicator of oxidative stress is lipid peroxidation resulting in disturbances of membrane integrity and consequently its enhanced permeability (Lukatkin et al. 2013). Baccouch et al. (2006) and Hao et al. (2006) showed enhanced lipid peroxidation in Ni^{2+}-treated wheat plants. By contrast, Ni^{2+} effects on wheat plants were not caused by lipid peroxidation (Gajewska et al. 2006). The changes in lipid and protein composition might disturb plasma membrane (PM) functions and, consequently the ion balance in the cytoplasm (Llamas et al. 2008). Several authors have shown that impairment of nutrient balance may result not only from the changes of plasmalemma lipid composition, but also that Ni^{2+} affected plasma membrane H^+-ATPase activity (Morgutti et al. 1981). Llamas et al. (2008) and Sanz et al. (2009) reported that Ni induced a concentration-dependent PM depolarization in rice and barley but the activity of the PM H^+-ATPase was not affected. However, in the long term experiments a drastic loss of potassium was recorded.

The first plant organ facing the elevated Ni^{2+} concentrations is the root system and especially the root cell plasma membrane being not only the site of Ni^{2+} entry but also a target of its toxic impact. The use of electrophysiological technique allowed us to record instant changes in electrophysiological parameters of individual epidermal cells of the adventitious grapevine roots during Ni^{2+} treatment. The trans-membrane electrical potential (E_M) and permeability measurements have been supplemented with determination of superoxide and hydrogen peroxide generation as well as cell viability in the roots exposed to various Ni^{2+} concentrations for up to 24 h.

Material and methods

Plant material and growth conditions

Grapevine (*Vitis vinifera* L., cv. Limberger) shoot cuttings were taken from the production vineyards of the region Rúbaň, Slovakia. After stratification in cold room (4 °C) for one month, nodal explants (10 cm) with single axillary bud were used for hydroponic cultivation. The explants were grown in magenta jars filled to 60 mL with aerated half strong MS medium (Murashige and Skoog 1962) at 25 ± 1 °C under 14 h photoperiod.

Ni^{2+} treatments

Based on published research of other authors (Pandey and Sharma 2002, Llamas et al. 2008), and our preliminary experiments, the concentrations of $NiCl_2$ that have caused the clear effects within short (48 h) treatments were chosen herein (1–5 mmol L^{-1}). Although such concentrations are not common in vineyards soils, they were not lethal for *Vitis* root cells. Two-month old explants with adventitious roots were transferred to aerated solutions containing 0.1 mmol L^{-1} KCl and 0.1 mmol L^{-1} $CaCl_2$, pH 5.8 supplemented with 0 (control), 1, 2 or 5 mmol L^{-1} $NiCl_2$ for 24 h in the concentration-dependence experiments, and with 0 (control) and the highest concentration 5 mmol L^{-1} $NiCl_2$ for 10, 30, 60 or 180 min in the time-dependence experiments. The roots were then stained for confocal microscope analyses as described later.

Electrophysiology

Measurements of trans-membrane electrical potential (E_M) were performed on single epidermal cells within the root zone located 0.5–0.9 mm from the root tip of 20 mm long root segments. The root epidermal cells being in direct contact with the environment are more sensitive comparing to the internal root tissues as shown in our previous research on grapevine root cells exposed to cadmium (Fiala et al. 2015). The E_M was measured using standard microelectrode technique as described in detail by Kenderešová et al. (2012). After rinsing with 0.5 mmol L^{-1} $CaSO_4$, the roots were mounted in a 5-mL volume Plexiglas chamber and constantly perfused (5 mL min^{-1}) with bathing solution containing 0.1 mmol L^{-1} KCl and 0.1 mmol L^{-1} $CaSO_4$. The initial maximum depolarization (ΔE_M) induced by 0.1–5 mmol L^{-1} Ni^{2+} concentrations 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 the 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. The E_M of roots treated for several hours (up to 24 h) with Ni^{2+} was also measured using the same solution, containing 1 and 5 mmol L^{-1} Ni^{2+}.

Membrane permeability

Changes in electrical conductivity of the solution surrounding the adventitious roots of 12 nodal explants were measured to assess the changes of membrane permeability caused by Ni^{2+} treatments. The 0.5 cm long apical segments of approximately 2 cm long roots (0.4–0.5 g) were transferred to 0.5 mmol L^{-1} $CaSO_4$ solution for 2 h in order to wash out the nutrient solution from the apoplast. After this time the segments were transferred into 10 mL of distilled water or 1 and 5 mmol L^{-1} $NiCl_2$ and incubated in a shaking water bath in the dark at 25 °C. Efflux of electrolytes from roots was determined within 48 h by conductivity meter OK-109-1 (Radelkis, Hungary). The conductivity was expressed in μS cm^{-1} g^{-1} fresh weight (FW). The changes in conductivity were expressed as differences between the values of particular conductivity measured, and the initial conductivity.

Confocal laser scanning microscopy

Propidium iodide (PI, Fluka, Switzerland) was used to counterstain the cell wall and nuclei of ruptured cells. 2', 7'-dichlorodihydrofluorescein diacetate (H_2DCFDA, Serva) was used as indicator for hydrogen peroxide accumulation in cells. To monitor real time superoxide production in the root tips we used superoxide detection kit (Enzo Life Sciences, USA). Apical 0.5 cm long root segments were

stained 2 min with 10 μg mL^{-1} PI in water, 15 min with 50 μmol L^{-1} H_2DCFDA in 50 mmol L^{-1} phosphate buffer pH 7.5 or 15 min with superoxide staining solution (following the manufacturer's manual), washed for 2 min in distilled water (for superoxide just briefly) and observed in confocal microscope Olympus FV1000 (Olympus, Japan). PI and H_2DCFDA were excited at 488 nm and fluorescence was detected using emission barrier filters 560–660 nm for PI or 505–550 nm for H_2DCFDA. The superoxide stain excitation wavelength was 543 nm and the emission was detected using 560–660 nm barrier filter. The confocal microscopy images represent at least three roots and were selected from at least three different images of each root. Fluorescence signal intensity (CTCF, corrected total cell fluorescence) was measured and calculated with the open source analysing software Image-J2/Fiji (http://imagej.net/Fiji).

Statistical analysis

Each experiment was repeated at least three times. Data on E_M and membrane conductivity were expressed as mean ± standard deviation (SD) with the number of samples (n). Data on cell viability, hydrogen peroxide accumulation and superoxide production were analysed using one-way ANOVA with $P < 0.05$ (Prism 5, GraphPad Software Inc.). Means and standard deviations were calculated from three independent experiments (n = 10 apical root segments). As for confocal microscopy, only representative images are shown.

Results

Trans-membrane electrical potential

Under control conditions the E_M values of epidermal cells located within the distance of 0.5–0.9 mm from the root tip varied between –121 and –133 mV (–126 ± 5.9 mV, mean ± SD, n = 39). In short term experiments (up to 70 minutes) the application of different Ni^{2+} concentrations induced immediate changes in the E_M values of root epidermal cells (Fig. 1). Both ions, Ni^{2+} and Cl^-, contributed equally to the E_M depolarization. Thus, for adequate balancing of high concentrations of Ni^{2+} and Cl^-, the resting potential was first measured at corresponding concentrations of $CaCl_2$, which were then replaced by the same concentrations of $NiCl_2$, with simultaneous presence of 0.1 mmol L^{-1} $CaSO_4$. The Ni^{2+}-induced rapid and transient depolarization was concentration-dependent (Fig. 2). The initial E_M depolarization induced with different Ni^{2+} concentrations repolarized to the initial resting potential values within 50–70 min (Fig. 1).

After transient depolarization, the Ni^{2+} applied at final 1 mmol L^{-1} concentration caused a slow membrane hyperpolarization. Its magnitude reached the maximum value 8 h after the metal application and, in comparison with the values of control cells it was more negative (by ΔmV 17.6 ± 3.3 mV, mean ± SD, n = 13). After withdrawal of Ni^{2+} from the perfusion solution the E_M repolarized to the value of control cells. Compared to control, the E_M was more negative after 1 mmol L^{-1} Ni while there was no difference after 5 mmol L^{-1} Ni^{2+} treatment (Fig. 3).

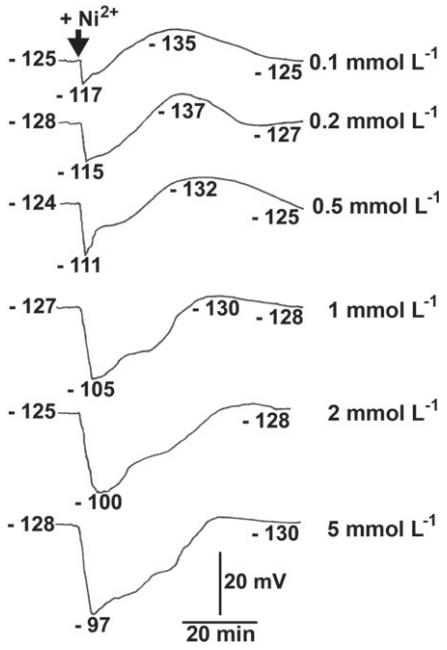

Fig. 1. Changes of the trans-membrane electrical potential (E_M) induced by increasing Ni^{2+} concentrations in epidermal cells of grapevine adventitious roots. The time of Ni^{2+} application to the perfusion solution is indicated by arrow. Representative curves of individual cells (n = 2–4) are shown.

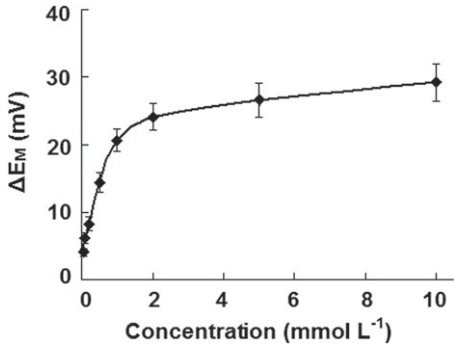

Fig. 2. Initial trans-membrane electrical potential depolarization (ΔE_M, mV) induced in epidermal cells of grapevine adventitious roots, by increasing concentrations of $NiCl_2$ added to the perfusion solution bathing the roots. Results are shown as mean values ± standard deviations, n = 3.

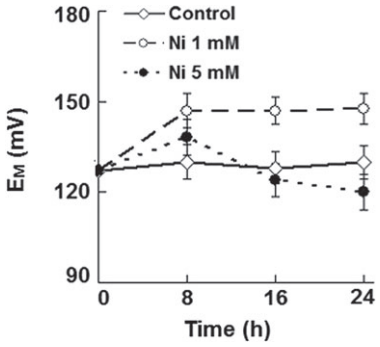

Fig. 3. Transmembrane potential difference (ΔE_M, mV) recorded in epidermal cells of grapevine adventitious roots treated with 1 and 5 mmol L^{-1} $NiCl_2$ for 0–24 h. Results are shown as mean values ± standard deviations, n = 3–8.

Membrane permeability

The treatment of roots with 1.0 mmol L^{-1} Ni^{2+} concentration did not change the root cell membrane permeability. Only the exposure to the highest Ni^{2+} concentration 5 mmol L^{-1} for 24 and 48 h resulted in membrane permeability increase comparing to control roots (Fig. 4).

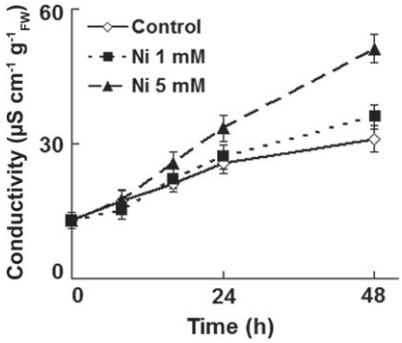

Fig. 4. Time-course of electrolyte leakage, measured as electrical conductivity, from the segments of grapevine adventitious roots treated with 1 and 5 mmol L^{-1} $NiCl_2$. Results are shown as mean values ± standard deviations, n = 3.

Cell viability, superoxide, hydrogen peroxide

In all experiments only a weak PI fluorescence was localized in the walls of the root tip cells indicating that the cells were viable, with intact cell membranes. Superoxide-specific staining did not reveal an increased orange fluorescence at any Ni^{2+} concentration or time used in the experiments (Figs. 5A, 6A). Only slightly elevated superoxide level occurred in the roots treated with 5 mmol L^{-1} Ni for 30 min (Fig. 6A). The dynamics of hydrogen peroxide accumulation monitored in the grapevine root cells with H_2D-CFDA showed concentration-dependence of the Ni-induced hydrogen peroxide formation as indicated by green cytoplasmic staining (Figs. 5A, B). The time course of hydrogen peroxide accumulation in grapevine root cells treated with 5 mmol L^{-1} Ni demonstrated that the highest hydrogen peroxide production occurred mainly after 180 min (Figs. 6A, B).

Discussion

Structural, physical and chemical properties of the PM itself as well as any effects of metal ions at the cell surfaces in general have an impact on transport processes. Alterations of the PM-H-ATPase activity can be assessed by

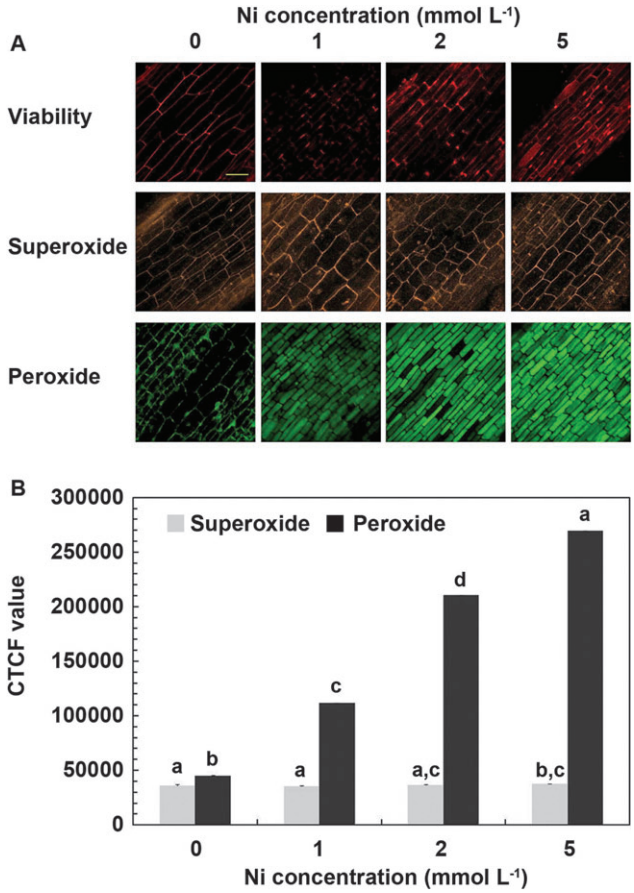

Fig. 5. Concentration-dependent effects of Ni-treatment on cell viability, superoxide and hydrogen peroxide accumulation in grapevine 0.5 cm apical root segments demonstrated with confocal microscope (A) and expressed using fluorescence signal intensity (CTCF) value (B). Bar represents 1 mm. Different letters indicate significant differences at 5% level. Results are shown as mean values ± standard deviations, n = 3.

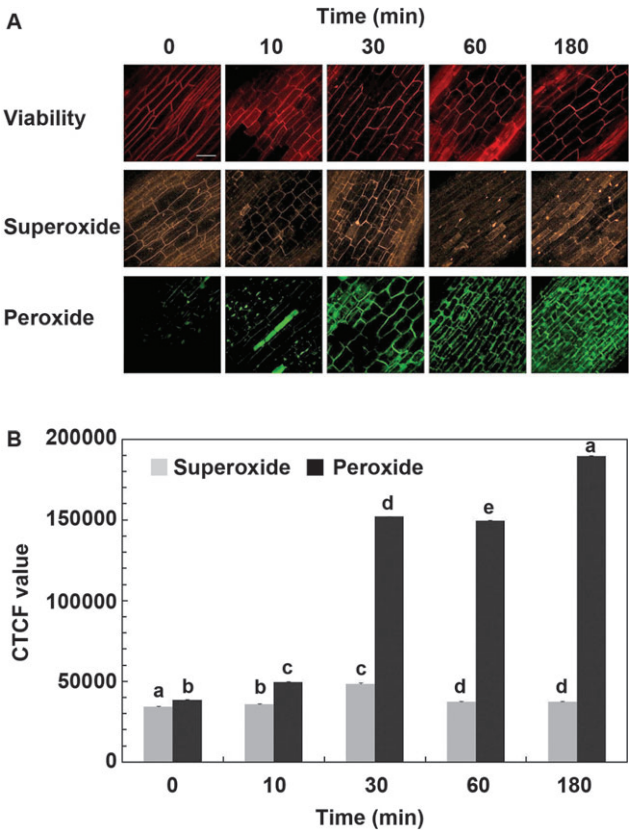

Fig. 6. Time-dependent responses to 5 mmol L^{-1} $NiCl_2$ of cell viability, superoxide and hydrogen peroxide accumulation in grapevine 0.5 cm apical root segments demonstrated with confocal microscope (A) and expressed using fluorescence signal intensity (CTCF) value (B). Bar represents 1 mm. Different letters indicate significant differences at 5% level. Results are shown as mean values ± standard deviations, n = 3.

studying changes in the E_M. According to our results, the effect of Ni on E_M of grapevine adventitious root epidermal cells differed from that reported for the other divalent cation, cadmium (Llamas et al. 2000, Pavlovkin et al. 2006, Fiala et al. 2015) and mercury (Repka et al. 2013). In short-term experiments, Ni induced rapid and concentration-dependent transient depolarization of the PM in the grapevine roots indicating its entry into the cells. But after this initial depolarization the E_M of Ni-treated roots reached the values similar or slightly higher than those of the control in less than 70 min. Llamas et al. (2008) working with rice plants suggested that such effect may be due to a stimulation of H^+ efflux, as demonstrated for Ni in maize roots (Morgutti et al. 1981). According to these authors, the entry of Ni into the cells occurs downhill the electrochemical gradient by a mechanism of uniport, inducing an immediate H^+ efflux for charge compensation, followed by K^+ efflux. Their results may explain the more negative values of E_M in comparison to the control, in the grapevine roots treated 24 h with 1 mmol L^{-1} Ni.

In addition to the initial effect on the active component of E_M, the effect of Ni^{2+} on the passive component of E_M cannot be ruled out. There is evidence that the PM permeability alterations might be involved in plant heavy metal tolerance (Llamas et al. 2008) and its disruption can be a consequence of increased peroxidation of unsaturated fatty acids in the cell membranes (Lukatkin et al. 2013). According to our results, no significant changes of the membrane permeability comparing to controls were found in grapevine roots treated with 0.5 mmol L^{-1} Ni^{2+} up to 24 h. However, when the roots were treated with 5 mmol L^{-1} Ni^{2+}, a progressive increase in membrane permeability was measured after 16 h of treatment.

Taken together, the changes induced by Ni^{2+} stress were not significant in E_M (Figs. 1–3), superoxide production or cell viability (Figs. 5B, 6B). However, hydrogen peroxide concentration significantly increased in the adventitious roots of grapevine exposed to 1, 2, and 5 mmol L^{-1} Ni^{2+} (Figs. 5B, 6B). At the highest 5 mmol L^{-1} concentration of Ni^{2+} a significant increase in membrane permeability occurred (Fig. 4), which could be responsible for the changes in water content, as was determined by Llamas et al. (2008). According to Gajewska et al. (2006), Ni^{2+} stress in roots is more related to the accumulation of hydrogen peroxide in root tissues than to enhanced lipid peroxidation. Furthermore, the elevated levels of hydrogen peroxide may be a consequence of its inappropriate removal. In line with this statement, a significant decrease of catalase activity was observed in Ni-treated wheat leaves (Gajewska and Sklodowska 2007). Our results suggest than Ni^{2+} does not directly affect plasma membrane ATPase and superoxide production. However, it increases plasma membrane permeability and hydrogen peroxide production, which can be related with Ni^{2+} toxicity in grapevine roots.

Acknowledgement

The work was supported by Slovak Grant Agency VEGA, Project 02/0023/13.

References

Agrawal, B., Czymmek, K. J., Sparks, D. L., Bais, H. P., 2013: Transient influx of nickel in root mitochondria modulates organic acid and reactive oxygen species production in nickel hyperaccumulator *Alyssum murale*. Journal of Biological Chemistry 8, 7351–7362.

Ahmad, M. S., Ashraf, M., 2011: Essential roles and hazardous effects of nickel in plants. Review of Environmental Contamination and Toxicology 214, 125–167.

Baccouch, S., Chaoui, A., El Ferjani, E., 2006: Nickel toxicity induces oxidative damage in *Zea mays* roots. Journal of Plant Nutrition 24, 1085–1097.

Bollard, E. G., 1983: Involvement of unusual elements in plant growth and nutrition. In: Läuchli, A., Bieleski R. L. (eds.), Encyclopedia of plant physiology, New Series, Vol. 15B, Inorganic plant nutrition, Springer, pp. 695–744.

Brown, P. H., Welch, R. M., Cary, E. E., 1987: Nickel: a micronutrient essential for higher plants. Plant Physiology 85, 801–803.

Chen, C., Huang, D., Liu, J., 2009: Functions and toxicity of nickel in plants: Recent advances and future prospects. Clean Soil, Air, Water 37, 304–313.

Fiala, R., Repka, V., Čiamporová, M., Martinka, M., Pavlovkin, J., 2015: Early cadmium-induced effects on reactive oxygen species production, cell viability and membrane electrical potential in grapevine roots. Vitis 54, 175–182.

Gabbrielli, R., Pandolfini, T., Vergnano, O., Palandri, M. R., 1990: Comparison of two serpentine species with different nickel tolerance strategies. Plant and Soil 122, 271–277.

Gonnelli, C., Galardi, F., Gabbrielli, R., 2001: Nickel and copper tolerance and toxicity in three Tuscan populations of *Silene paradoxa*. Physiologia Plantarum 113, 507–514.

Gajewska, E., Slaba, M., Andrzejewska, R., Skłodowska, M., 2006: Nickel-induced inhibition of wheat root growth is related to H_2O_2 production, but not to lipid peroxidation. Plant Growth Regulation 49, 95–103.

Gajewska, E., Skłodowska, M., 2007: Effect of nickel on ROS content and antioxidative enzyme activities in wheat leaves. BioMetals 20, 27–36.

Hao, F., Wang, X., Chen, J., 2006: Involvement of plasma-membrane NADPH oxidase in nickel-induced oxidative stress in roots of wheat seedlings. Plant Science 170, 151–158.

Holmgren, G. G. S., Meyer, M. W., Chaney, R. L., Daniels, R. B., 1993: Cadmium, lead, zinc, copper and nickel in agricultural soils of the United States of America. Journal of Environmental Quality 22, 335–348.

Khalid, B. Y., Tinsley, J., 1980: Some effects of nickel toxicity on rye grass. Plant and Soil 55, 139–144.

Kenderešová, L., Staňová, A., Pavlovkin, J., Ďurišová, E., Nadubinská, M., Čiamporová, M., Ovečka, M., 2012: Early Zn^{2+}-induced effects on membrane potential account for primary heavy metal susceptibility in tolerant and sensitive *Arabidopsis* species. Annals of Botany 110, 445–449.

Llamas, A., Ullrich, C. I., Sanz, A., 2000: Cd^{2+} effects on transmembrane electrical potential difference, respiration and

membrane permeability of rice (*Oryza sativa* L) roots. Plant and Soil 219, 21–28.

Llamas, A., Ullrich, C. I., Sanz, A., 2008: Ni^{2+} toxicity in rice: Effect on membrane functionality and plant water content. Plant Physiology and Biochemistry 46, 905–910.

Lukatkin, A. S., Kashtanova, N. N., Duchovskis, P., 2013: Changes in maize seedlings growth and membrane permeability under the effect of epibrassinolide and heavy metals. Russian Agricultural Sciences 39, 307–310.

Morgutti, S., Ferrari-Bravo, P., Marre, M. T., Cocucci, S. M., 1981: Effects of Ni^{2+} on proton extrusion and related transport processes and on the transmembrane electrical potential in maize roots. Plant Science Letters 23, 123–128.

Murashige, T., Skoog, F., 1962: A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15, 473–97.

Ouzounidou, G., Moustakas, M., Symeonidis, L., Karataglis, S., 2006: Response of wheat seedlings to Ni stress: Effects of supplemental calcium. Archives of Environmental and Contamination Toxicology 50, 346–352.

Pandey, N., Sharma, C. P., 2002: Effect of heavy metals Co^{2+}, Ni^{2+}, and Cd^{2+} on growth and metabolism of cabbage. Plant Science 163, 753–758.

Pavlovkin, J., Luxová, M., Mistríková, I., Mistrík, I., 2006: Short- and long-term effects of cadmium on transmembrane electric potential (E_M) in maize roots. Biologia 61, 109–114.

Repka, V., Fiala, R., Čarná, M., Pavlovkin, J., 2013: Membrane potential differences and viability of grapevine root cells treated with $HgCl_2$. Plant, Soil and Environment 59, 353–358.

Sanz, A., Llamas, A., Ullrich, C. I., 2009: Distinctive effects of Cd and Ni on membrane functionality. Plant Signalling and Behaviour 4, 980–982.

Seregin, I. V. Kozhevnikova, A. D., 2006: Physiological role of nickel and its toxic effects on higher plants. Russian Journal of Plant Physiology 53, 257–277.

Sharma, S. S., Dietz, K. J., 2009: The relationship between metal toxicity and cellular redox imbalance. Trends in Plant Science 14, 43–50.

Sharma, A., Dhiman, A., 2013: Nickel and cadmium toxicity in plants. Journal of Pharmaceutical and Scientific Innovation 2, 20–24.