Effect of nickel on root growth and the kinetics of metal ions transport in onion (Allium cepa) root

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Received 14 October 2008; revised 25 June 2009

The effect of different concentrations of nickel nitrate (0.25, 0.50, 1.00 and 2.00 mM) uptake by the roots, on root growth of onion (Allium cepa) and the transport of Ni2+, Fe2+, Mn2+, Zn2+, K+, Na+ and H+ ions were investigated spectrophotometrically. The uptake of Ni2+, Fe2+, Mn2+ and Zn2+ was monitored by flame atomic absorption spectrometry with a 24-h period for 7 days and the amounts of K+ and Na+ were determined in solutions by flame photometer. The mineral content of the solution, instead of the root material was measured. Ni2+ ions showed inhibitory effect on the root growth at all concentrations during the entire treatment. The EC50 (effective concentration that reduced root growth by 50%) was found at 0.25 mM Ni2+. No significant change in inhibitory effect was observed after at 0.50 mM Ni2+ concentrations. A large amount of Ni2+ was translocated into the roots. The kinetics of metal ion transport followed a pseudo-first order reaction in all metal ion concentrations. Ni2+, Zn2+, Fe2+, Mn2+ and H+ ions transferred together into plant, but Na+ and K+ ions transferred to the solution from the plant. The amount of H+ in the solution decreased at all Ni2+ concentrations.

Keywords: Allium cepa L, Atomic absorption spectrometry, Flame photometer, Nickel, Phytotoxic effect

Nickel is a constituent of urease and in small quantities (0.01 to 5 µg/g dry wt) is an essential trace element for some plants and many bacteria1-3, but, higher its concentrations may be toxic to plants4 and humans5. Growth inhibition, chlorosis and reduction of tissue water content have been commonly observed in the plants, exposed to phytotoxic amounts of nickel5,6. However, Ni2+ tolerance is found in certain plants, particularly those inhabiting Ni2+-rich soils, e.g. serpentine soils7. In some plants, Ni2+ is accumulated in the leaves, where it is believed to be sequestered in the vacuole as citrate and malate complexes8. Nickel accumulation in the cell walls and vacuoles in the cortical cells of onion roots is also established with the electron microscopy9. Presence of a nickel transporter protein is also reported in the vacuolar membrane of Arabidopsis thaliana10.

Although studies about the mechanisms of heavy metals uptake by different plant species11-13 have been reported, little work has been done on mechanism of Ni2+ uptake1. There is a competition between various metals during their uptake by the roots. While some metals are absorbed in insufficient quantities, the uptake of the other metals is excessive. This indirectly predetermines the effect of heavy metals on the various facets of metabolism1. Despite the increasing knowledge about the interaction of Ni2+ with diverse biochemical processes in the plants7, the specific mechanisms of Ni2+ uptake have not been reported yet14,15. There is less information on the exchange of these heavy metals and trace elements ions such as K+, Na+, Mn2+, Zn2+, Fe2+ and H+ between root and medium.

In this study, the phytotoxic effects of Ni2+ on root growth of onion (Allium cepa), the amount of Ni2+ absorbed by the plant and the Ni2+/H+, Ni2+/K+, Ni2+/Na+, Ni2+/Fe2+, Ni2+/Mn2+ and Ni2+/Zn2+ transport have been investigated.

Material and Methods

Plant material and plant culture

All experiments were performed on adventitious roots of the onion (Allium cepa). The onions were grown16 to obtain equal growth of adventitious roots and healthy and equal-sized bulbs were used. Before starting the experiment, outer dry scales of the bulbs were removed and the ring of root primordial was left intact. Twenty-eight onions comprised an experimental set, and 7 onions within each set were selected for control. At the beginning of the
experiment, onions were placed in distilled water 60 ml vessels. All samples were kept in laboratory with a 16:8 h photoperiod under 1500 luxes. As temperature is an important factor affecting root growth, the temperature of laboratory was kept at 22°C (± 0.5). When the roots reached a length of 1.5-1.7 cm, 28 onions were transferred to culture tanks in 60 ml Hoagland’s nutrient solution (Table 1), supplemented with some important microelements and grown in opaque containers. Ni(NO₃)₂ was used as a source of Ni²⁺ ions. Hoagland’s nutrient solution was prepared at 0.25, 0.50, 1.00 and 2.00 mM Ni²⁺ concentrations. Seven onions were left in previous solutions and treated as control groups. Experiments were replicated in 3-times.

Phytotoxic effect of Ni²⁺ on root elongation

The plants were grown for 7 days and the root elongation toxicity test was performed. Root length was measured using a millimeter ruler starting at the onset of incubation, then after 1, 2, and 3, 4, 5, 6, and 7 days and at the end of 7 days, the total root lengths were measured. Means and standard deviations of the length of onion root tips were determined. The phytotoxicity results based on the EC₅₀ (the effective concentration that reduced root growth by 50%) were extrapolated.

Mineral content of solution and estimation of pH

The mineral content of solution was measured, instead of the root material. The uptake of Ni²⁺, Fe²⁺, Mn²⁺ and Zn²⁺ by the plants was monitored by flame atomic absorption spectrometry (AAS) with a 24-h period for 7 days. The kinetics was worked out to check, whether it followed first or second order reaction. Fe²⁺, Mn²⁺, Zn²⁺, K⁺ and Na⁺ ions were also analyzed and pH value was measured. The concentration of Fe²⁺, Mn²⁺, Zn²⁺, K⁺ and Na⁺ ions in the solution was read directly, while Ni²⁺ was diluted 1/4 and 1/20 times. A Unicam model 929 flame atomic absorption spectrometer with deuterium lamp and air-acetylene burner was used for metal ions determination. In atomic absorption, sensitivity is defined as concentration of the element in ppm (or µg/ml or mg/l) in aqueous solution. The hollow cathode lamps were operated at 20 mA for Ni²⁺ and Fe²⁺ and at 10 mA and 12 mA for Zn²⁺ and Mn²⁺. The wavelengths for Ni²⁺, Fe²⁺, Zn²⁺ and Mn²⁺ were set at 232.1, 248.3, 213.9 and 279.5 nm, respectively. Jenway PFP 7 model flame photometer was used for determination of K⁺ and Na⁺ ions; sensitivity of photometer was at 3-100 ppm for reading of Na⁺ and K⁺ ions. All pH measurements were performed with a Jenway 3040 ion analyzer with a combination glass electrode.

Statistical analysis

The significant difference between the treated and control samples was analyzed by Student's-test. The values of roots and medium mineral contents were means of 20 and 3 measurements, respectively.

Results

The results of the study were evaluated in two steps: the effect of Ni²⁺ concentrations on root growth and the transport of Ni²⁺, Fe²⁺, K⁺, Na⁺, Mn²⁺, Zn²⁺ and H⁺.

Phytotoxic effect (root elongation)

Figure 1 shows the effect of various Ni²⁺ treatments on roots elongation after 7-days of exposure. Ni²⁺ inhibited root growth in a dose-
dependent manner. Although Ni\(^{2+}\) inhibited root growth at all concentrations, there was no significant change in inhibitory effect after 0.50 mM Ni\(^{2+}\) concentrations. The phytotoxic effect (EC\(_{50}\)) of Ni\(^{2+}\) on roots was found at 0.25 mM.

**Mineral content of the solution and pH**

Figure 2a shows Ni\(^{2+}\) concentration in nutrient solution after 7 days of exposure to various Ni\(^{2+}\) treatments. At the beginning, Ni\(^{2+}\) showed a sharp decrease, especially at 2.00 mM Ni\(^{2+}\) concentration; the decrease was due to Ni\(^{2+}\) ion binding and adsorption on the membrane surface, and Ni\(^{2+}\) ions transportation to the vesicle cavity.

From the changes in Ni\(^{2+}\) movement (Fig. 2a), kinetics of the process could be calculated for each set of measurements at the specific Ni\(^{2+}\) concentrations. A reaction rate constant can be written as:

\[
A = \frac{dA}{dt} = -k[A]^n
\]  

… (1)

where \([A]\) is the metal ion concentration (mM) in the nutrient solution; \(t\) is the time of exposure to various metal treatments (day); \(k\) is the rate constant and \(n\) is the reaction order. Since the metal ion concentration decreased in the course of time, the term \(dA/dt\) for a pseudo first order reaction (\(n = 1\));

\[
\ln A = \ln A_0 - kt
\]  

… (2)

To calculate the value of \(k\), plots of \(\ln A\) vs time were drawn and found to be linear, as seen in Fig. 3. The kinetics of Ni\(^{2+}\) ion transport was described by a pseudo-first order reaction for all Ni\(^{2+}\) ion concentrations. The reaction rate constants (\(k\)) were calculated from the slopes of the straight lines and given in Table 2. The results showed that rate constant was independent of the concentrations; there was only a slight deviation at 0.25 and 0.50 mM Ni\(^{2+}\) concentrations (Table 2).

The relationship between the Fe\(^{2+}\) and K\(^+\) concentration of the solutions to various Ni\(^{2+}\) treatments is shown in Figs 2b and c, respectively. As seen in Fig. 2b, amount of Fe\(^{2+}\) in solution decreased at all the concentrations of Ni\(^{2+}\) with time and the decrease was independent of Ni\(^{2+}\) concentration. However, K\(^+\) concentration in nutrient solutions increased with time for all studied Ni\(^{2+}\) treatments (Fig. 2c) and the increase was dependent of Ni\(^{2+}\) concentration. Na\(^+\) ions in solution showed no significant change at lower Ni\(^{2+}\) concentrations (0.25 and 0.50 mM), but increased at
higher Ni$^{2+}$ concentrations (1.00 and 2.00 mM). The increase of K$^+$ in solution was higher than Na$^+$. Furthermore, Mn$^{2+}$ and Zn$^{2+}$ decreased with increasing external Ni$^{2+}$ concentration (data not shown).

Fig. 4 represents the effect of $\Delta$pH (the difference between pH of control and nickel solution) variation in nutrient solution after 7-days of exposure to various Ni$^{2+}$ treatments. As seen as from Fig. 4, the pH values of the solutions decreased for first 2-3 days, due to H$^+$ ions transferred to solution from plants. In following days, the values of $\Delta$pH increased for all the Ni$^{2+}$ concentrations, due to H$^+$ ions transferred to plant from solution. Results showed that H$^+$ ions transferred to plants were higher than transferred to the solution. At the end of 7 days, H$^+$ ions transferred to plant from solution. The H$^+$ ion transport was lower at higher Ni$^{2+}$ concentrations (1 and 2 mM).

The concentration of metal ions transferred to the roots or solution was obtained from the total metal ion concentration in nutrient solution and control solutions (without Ni$^{2+}$). Ion transport (% mM) after 7 days at 2.00 mM Ni$^{2+}$ concentration is shown in Fig. 5. It was noted that at the beginning, nutrient solution had 0.002 mM Mn$^{2+}$ and Zn$^{2+}$, 0.02 mM Fe$^{2+}$ and Na$^+$, 0.65 mM K$^+$ and 0.003 mM H$^+$ ion concentrations (pH = 5.5), but Ni$^{2+}$ concentration was 2.00 mM.

**Discussion**

The tolerance index [the ratio (%) between the root/shoot length of heavy metal-stressed plant and control plant] and LC$_{50}$ (the concentration that inhibits root growth by 50%) are the indices of plant tolerance toward heavy metals. LC$_{50}$ indices in plant species have been classified into more tolerant (Cucumis sativus and Panicum miliaceum) and less tolerant (Chloris gayana, Lactuca sativa, Lolium perenne, Panicum maximum, and Zea mays). The strong toxic effects of Ni$^{2+}$ are reported at 0.225 and 0.5 µM in Alnus glutinosa$^{18}$ and 250 µM in Zea mays$^{19}$. Increasing Ni$^{2+}$ levels up to 100 mg/kg soils result in visible symptoms of leaf chlorosis in Petroselinium crispum$^{20}$. In our study, the root growth started to inhibit by 0.25 mM Ni$^{2+}$.

The root elongation is relative to cell metabolism. The diverse effects of heavy metals on cell metabolism are brought by direct and indirect activities of these heavy metals$^{1,21}$. In Zea mays, inhibition of shoot growth is detected after lipid peroxidation$^{19}$. The same mechanism is observed in primary leaves of Phaseolus vulgaris under Cu$^{2+}$ and Zn$^{2+}$ stress condition$^{22,23}$.

There have been few studies on transport of Ni$^{2+}$ and trace element like Fe$^{2+}$, Zn$^{2+}$, K$^+$, Na$^+$ and Mn$^{2+}$. The rate of metal transport is highly specific for varying plant sensitivity. In the presence of Ni, the contents of mineral nutrients in plant organs may increase, decrease, or stay even$^1$. In present study, it was observed that Ni$^{2+}$, Fe$^{2+}$, Zn$^{2+}$ and Mn$^{2+}$ were transferred together into plant like in rye grass$^{24}$ and Mn$^{2+}$, Zn$^{2+}$ and Fe$^{2+}$ contents of roots were increased. Whereas in Lolium perenne$^{24}$ and Triticum aestivum$^{25}$, Mn$^{2+}$ and Zn$^{2+}$ content and in T. aestivum$^{25}$ and Hordeum vulgare$^{26}$, Fe$^{2+}$ uptake are found to decrease and in P. vulgaris$^{27}$, Mn$^{2+}$ uptake remains even. In clover, Cd$^{2+}$ decreases both uptake and transport of Zn$^{2+}$, Fe$^{2+}$, Mn$^{2+}$, Ca$^{2+}$ and Mg$^{2+}$ to higher extent than in cabbage$^{28}$. In our previous study$^{29}$, it was seen that Cd$^{2+}$ displaced with Fe$^{2+}$ and Mn$^{2+}$.

In this study, Ni$^{2+}$ concentrations decreased of K$^+$ content of roots, as in Oryza sativa$^{30}$ and T. aestivum$^{35}$, while it was increased in P. vulgaris$^{27}$ and Thlaspi montanum$^{31}$. In addition, Na$^+$ content was also decreased in A. cepa.
The mechanism of Ni^{2+} ions penetration has not been studied in detail. Two well-discriminated mechanisms have been reported about the decrease of the uptake of macro and micronutrient by Ni^{2+}. The physical and chemical mechanisms depend on the size of metal ion radii. According to the hypothesis, a divalent metal ion transport protein in the membrane is capable of transporting a broad range of metal ions, including Mn^{2+}, Fe^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+}. Also, the transport of transition metal ions such as Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} is shown to be protein-mediated, similar to the Fe^{2+} transport and the experiments indicate that Fe^{2+} and Zn^{2+} compete for the same transport protein. Symptoms of growth depression, early senescence, grain development inhibition and in viability and reduction of tissue Fe levels have been noticed in cereal such as barley, oat and wheat with Ni^{2+} deficiency. The present study also showed that Fe^{2+} and Ni^{2+} ions can be transported with the same transport protein. The other mechanism relies on metal-induced disorder in membrane enzyme activities and membrane structure.

In this study, it was observed that Ni^{2+} and H^+ transferred together into the plant, as observed in the case of Cd^{2+} (ref 29). Gries and Wagner tested the possibility whether Ni^{2+} ions are transported across the tonoplast by Ni^{2+}/H^+ antiport mechanism in oat roots and have shown the absence of a Ni^{2+}/H^+ antiport in oat root tonoplasts. This study supported their results and showed the absence of a Ni^{2+}/H^+ antiport in the onion root.

In conclusion, the study showed that Ni^{2+} plays a negative role in growth of onion roots. Moreover, the root growth was inhibited by all studied doses of Ni^{2+}. The effective concentration (EC_{50}) of Ni^{2+} for roots was found as 0.25 mM. The kinetics of metal ion transport followed a pseudo-first order reaction in all metal ion concentrations. Ni^{2+}, Zn^{2+}, Fe^{2+}, Mn^{2+} and H^+ ions transferred together into plant, but Na^+ and K^+ transferred to the solution from plant. The amount of H^+ in the solution was found to decrease, resulting in a higher pH.

Acknowledgment
The study was supported by The Scientific Research Fund of Trakya University (Project no: TUBAP-495).

References
24. Khalid Y & Tinsley J (1980) Plant Soil 55, 139-144