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Root uptake and translocation of nickel in wheat as affected by histidine

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ABSTRACT

The role of histidine (His) on root uptake, xylem loading and root to shoot transport of nickel (Ni) was investigated in a winter (*Triticum aestivum* cv. Back Cross) and a durum wheat (*Triticum durum* cv. Durum) cultivar. Seedlings were grown in a modified Johnson nutrient solution and exposed to 10 μM of Ni and 100 μM of histidine (His) as no His, Ni (10) + His (100) and Ni(His) in a 1:1 mole ratio (1:1) complex. In our study, the presence of vanadate (a metabolic inhibitor) resulted in a significant decrease of root Ni uptake, indicating that a part of Ni uptake by the plant root is energy-dependent. Addition of His significantly increased the Ni content in shoots and roots of both wheat cultivars. The data suggest that the Ni(His) is most likely to be taken up as a complex or receptors at the membrane are able to enhance Ni uptake from Ni(His) complex. This result was indirectly supported by using EDTA as a strong chelating reagent to reduce the uptake of Ni(His) complexes. By using this ligand, the xylem loading of Ni and His was disproportionately reduced. Cycloheximide (a translation inhibitor) strongly decreased the release of His and Ni from the root into the xylem of wheat, suggesting the significance of a symplastic pathway for Ni loading into the xylem.

Introduction

Nickel (Ni) has been listed as an essential micronutrient that has been shown to function in the active center of urease (Marschner, 1995). Due to its role in the metalloenzyme urease, Ni is considered to be an important nutrient for plants fed with urea as nitrogen source (Follmer, 2008; Witte, 2011). Several researchers have reported growth responses of plants to Ni (Khoshgoftarmanesh et al., 2011; Khoshgoftarmanesh and Bahmanziari, 2012; Eskew et al., 1983). However, in comparison with some other metals, the mechanisms of Ni accumulation in plants are not well understood.

In general, two processes other than bioavailability determine metal accumulation in plants: root uptake and translocation to shoots (Clemens, 2006). Root uptake is affected by the transporter affinity and by the factors that determine how much ion can be transported to the root, i.e. the concentration of labile species in solution, the degree of solid phase buffering and the root surface area (Degryse and Smolders, 2012). On the other hand, plants can significantly affect the uptake by modifying the pH of the rhizosphere, which affects the metal solution concentration and therefore the metal diffusion flux (Bravin et al., 2009). Plants also produce a number of chelating agents including organic acids and amino acids (Callahan et al., 2006) that may influence metal solubility in the rhizosphere and its uptake by roots. Internalization flux is also involved in uptake in addition to diffusive transport of metal ions to the roots. In cases of strong diffusion limitation, ion competition effects on the internalization are expected to have negligible effects on the uptake, as the uptake is controlled by diffusion and not by internalization (Degryse and Smolders, 2012).

Metals can be absorbed by plant roots both passively and actively. Energy-dependent (active) transport of many divalent cations has been demonstrated (Hart et al., 1998; Lu et al., 2008). On the other hand, apoplastic (passive) uptake is driven by diffusion through the cell walls and the inter-cell free spaces and adsorption of ions by the cell walls (Meychik and Yermakov, 2001). Passive uptake could be quantified by using vanadate which is an inhibitor of plasma membrane H^+-ATPase. By using this compound, the disappearance of metal from the incubation solution is viewed as either a leakage into the root or a binding to the apoplastic surfaces since active uptake, dependent on ATPase activity, is inhibited (O'neill and Soanswick, 1984). It is now well established that the primary active transport across the plasmalemma and tonoplast of higher plant cells involves processes driven by the H^+ electrochemical gradient generated by the plasma membrane H^+-ATPase, which pumps protons out of the cell (Serrano, 1989).

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It has been suggested that the heterocyclic nitrogen donors i.e., amino acids, affect the contribution of passive and active uptake of Ni^{2+} by plant roots (Still and Williams, 1980). Direct uptake of the metal-amino acid complex was estimated to be a major contribution only at mM concentrations of the complex or at very large ratios of complex to free ion concentration. Puschenreiter et al. (2005) also observed that Ni–histidine complexes did not contribute to Ni uptake by *Thlaspi goesingense* in a solution (pH 6) with 2.5 μM Ni^{2+} and 2.5 μM Ni–histidine. As noted by the authors, it is uncertain whether the complexes did not contribute because uptake was internalization limited or because the complexes were not labile.

In addition to root uptake, loading into the xylem and long-distance transport of metal ions toward aboveground organs is of high importance for plant nutrition (Tiffin, 1972). In this long-distance transport process, water and minerals are taken up from the root medium and predominantly translocated towards the upper plant parts by the vascular tissues of the xylem (Mengel and Kirby, 1982). Such transport is strongly regulated by metal–ligand complexes and by some proteins that specifically bind and transport Ni (Haydon and Cobbett, 2007). On the other hand, a number of possible ligands i.e., organic acids, amino acids, peptides and proteins, stimulate the root to shoot transport of mineral ions (Callahan et al., 2006). The transport rate of di- or trivalent cations has been reported to be enhanced significantly by complexation with organic acids (Senden et al., 1994; Yang et al., 1997), amino acids, or peptides (Mullins et al., 1986; Senden et al., 1994; Stephan et al., 1996; Clark et al., 1986). Only a small fraction of the metal content in plants is present in the form of free aqua ions. It is assumed that most ions in plants are bound to low molecular mass ligands or to proteins (Salt et al., 1999). Organic compounds in the roots (Collins and Reilly, 1968; Tonin et al., 1990) and amino acids and cation–organic complexes in xylem fluid (Tiffin, 1966; Bradfield, 1976; Wu et al., 2009) have been reported to greatly affect the movement of metals in the xylem, both in longitudinal and lateral directions. Amino acids have higher stability constants for Ni than do carboxylates (Homer et al., 1991), and thus offer better transport of this metal within the plant. Among the potential non-protein metal chelators, the amino acid histidine (His) has been suggested to be implicated in plant-internal transport of Ni in hyperaccumulator and non-hyperaccumulator plants (Kerkeb and Kramer, 2003; Richau et al., 2009; Kozhevnikova et al., 2014). In some hyperaccumulator plants, however, transport of Ni in the xylem sap is mainly as free hydrated cation and histidine has no significant role in xylem sap chelation of Ni during root-to-shoot transport (Tiffin, 1972; Alves et al., 2011; Centofanti et al., 2013).

There are relatively limited reports available concerning the effect of amino acids as chelating agents on root uptake and transport to shoots of Ni in crops. Such information will promote the metal use efficiency and may contribute to genetic modification of plants for improving plant nutrition. The aim of the study presented here was therefore to investigate the mechanism of Ni root uptake and transport to shoots in wheat as affected by external histidine. In the first step, we identified and quantified the active and passive uptake of Ni, and in the second step, the xylem loading and long-distance transport of Ni in the presence and absence of histidine was investigated.

Material and methods

Plant material and growth conditions

Seeds of one winter (*Triticum aestivum* cv. Back Cross) and one durum wheat cultivar (*Triticum durum* cv. Durum) were sterilized in a 1% aqueous solution of Na-hypochlorite for 10 min and washed thoroughly with distilled water. After soaking the seeds in distilled water for 24 h, they were allowed to germinate in the dark (2 days) at 25 °C. The seeds were sown on quartz sand and moistened with deionized water. Two weeks later, uniformly-sized seedlings were selected and transplanted to 12 l beakers containing half Johnson nutrient solution that was prepared in double-deionized water (18 MOhm cm^{-1} resistivity) that contained: 0.5 mM $Ca(NO_3)_2{\cdot}4H_2O$, 0.5 mM KNO_3, 0.5 mM $NH_4H_2PO_4$, 0.5 mM $MgSO_4$, 25 μM KCl, 12.5 μM H_3BO_3, 1 μM $MnSO_4{\cdot}H_2O$, 1 μM $ZnSO_4{\cdot}7H_2O$, 0.25 μM $CuSO_4{\cdot}5H_2O$, 0.25 μM H_2MoO_4, and 25 μM Fe(III)-EDTA (ethylenediamine-tetra acetic acid). Analytical-grade chemical reagents with a purity of >98% and pure water were used in the preparation of nutrient solutions. The pH was maintained in the range of 5.8–6.0 using 0.1 N HCl or KOH as pH buffers when needed. Nutrient solutions were continuously aerated with pumps and replaced twice per week by fresh solutions. Four weeks after germination, plants were removed from the nutrient solution and transferred to the treatment solutions.

Experimental design

Experiment 1: Assessment of active and passive uptake of Ni

To assess the active uptake of Ni, wheat plants, cultivar Backcross were grown for 4 weeks in non-metal amended nutrient solution and then transferred to 600 ml plastic pots (one seedling per pot) containing 2 mM MES-TRIS (pH 5.5) with different treatments including: free histidine control, 100 μM histidine and 10 μM Ni in the form of $NiSO_4$ and Ni(His) in a 1:1 mole ratio (1:1) complex in the presence and absence of 100 μM sodium orthovanadate as metallic inhibitor. Roots of the plants were immersed in the uptake solution for 2 h.

Synthesis of Ni(His) complex

For synthesis of Ni(His) complex, a solution of histidine (1 mmol) in 5 ml distilled water was slowly added to a solution of $NiCl_2$ (1 mmol) in 2 ml distilled water. The mixture was heated at reflux temperature for 2 h while being stirred vigorously. After evaporation of the solvent at room temperature, blue microcrystals of Ni(His) were recovered. The products were washed with cold ethanol, followed by diethyl ether and air-dried. The quantities of the carbon, nitrogen, hydrogen and oxygen in various operating modes (CHN and oxygen modes) were determined using the Perkin-Elmer 2400 CHN elemental analyzer. The concentration of Ni was determined by atomic absorption spectrometry (PerkinElmer 3030; PerkinElmer, Wellesley, MA, USA) and the FT-IR spectra were measured with a FT-IR JASCO 460 spectrophotometer over KBr pellet in 4000–400 cm^{-1} range. The results of elemental analysis, Anal. Calc. for $C_6H_8N_3O_2Ni$ (212.8457 g mol^{-1}): C, 33.86; H, 3.79; N, 19.74. Found: C, 33.38; H, 3.80; N, 19.55%, support the composition of Ni(His) complex with 1:1 ligand to metal molar ratio in consistent with their theoretical formulation, [Ni(His)].

Experiment 2: Collecting and measurement of xylem loading of Ni and His

To characterize the effect of amino acids on the Ni concentration in xylem sap, 4-week-old wheat plants with their roots were placed in a fresh solution containing the respective added compounds (10 μM Ni in the form of $NiSO_4$, 100 μM histidine, and 5 μM EDTA) and adjusted to pH = 5.5 with KOH, 4 h before the onset of xylem sap collection. To clarify the mechanism of Ni uptake and translocation in plants and the role of His in these processes, the root medium was supplemented with 5 μM cycloheximide (CHX), a translational inhibitor (Zinck et al., 1995), 1 h before further addition of 10 μM Ni^{2+} and/or 100 μM histidine. For further investigation of the role