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Plasma membrane ATPase and H^+ transport activities of microsomal membranes from wheat roots under Ni deficiency conditions as affected by exogenous histidine

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ABSTRACT

ATPase activity and ATP-dependent H^+ transport have been analyzed in membrane vesicles isolated from a winter wheat cultivar (*Triticum aestivum* cv. Back Cross Rushan) which was exposed to different concentrations of nickel (Ni) and histidine (His). Seedlings were grown in a modified Johnson nutrient solution and then exposed to the uptake solution containing 10 μM Ni to which either 100 μM His or no amino acid was added. Control plants were transplanted to nutrient solutions free of Ni and His. According to our results, using Ni alone or together with His enhanced the activity of plasma membrane (PM) H^+-ATPase and resulted in a higher pH gradient across the plasma membrane. The highest initial rate of H^+-pumping by PM H^+-ATPase also achieved in the Ni alone treatment which was roughly 2.2 times higher than the control treatment. His applied together with Ni to the nutrient solution resulted in higher Ni uptake but lower H^+-ATPase activity. Our results suggest that the (PM) H^+-ATPase in wheat roots plays an important role in Ni uptake but using His in combination with Ni can moderate the pump activity. Undissociated Ni-His may directly taken up via His transporters which seems to be less dependent on ATPase activity.

1. Introduction

To achieve the goal of optimizing the efficiency of micronutrient use and maximizing crop productivity, a better consideration of the processes involved in acquisition and utilization of micronutrients by plants is necessary. Nickel is the most recently defined plant essential element (Brown et al., 1987) that its deficiency can cause yield reductions. Several studies have investigated the positive effects of Ni nutrition on plant growth and urease activity in hydroponically grown crops (Khoshgoftarmanesh et al., 2011; Khoshgoftarmanesh and Bahmanziari, 2012; Kutman et al., 2013). Moreover, Ni deficiency was shown to disrupt amino acid and/or organic acid homeostasis in several crops including tomato (Shimada et al., 1980), cowpea (Walker et al., 1985), and barley (Brown et al., 1990).

Although such evidences indicate that Ni deficiency has a wide range of effects on plant growth and metabolism, relatively little is known about the role of Ni in plant physiology and metabolism at low concentration especially in crop species. There is, therefore, a need to more precisely verify the uptake and long distance transport mechanisms of Ni in plants.

Metals can be absorbed by plant roots both passively and actively. Passive uptake involves diffusion of ions in the soil solution into the root endodermis along a chemical potential (concentration) gradient. Such movement occurs across cell wall and intercellular spaces which are fully preamable (the apoplast path), while active ion uptake takes place against the concentration gradient with high selectivity of ions and energy-consuming mechanism (Marschner, 1995). Targets of the symplastic pathway are the transporters in cell membranes where living protoplasm involved (White et al., 2002). Passive transport of solutes may also occur across the membrane transport proteins. Channel proteins carry out passive transport but carrier proteins can carry out either passive or active transport (Cooper, 2000). The main role in the active transport process is mediated by proton-pump ATPase (H^+-ATPase) which acts as a primary transporter by pumping protons out of the cell. The principal function of this 'master enzyme' is to create and maintain a negative membrane potential and transmembrane pH gradient (acidic outside) for nutrient uptake (Sakano et al., 1992; Elmore and Coaker, 2011). Carrier proteins

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for example, use this energy to facilitate ion and solute exchange across the membrane (Sondergaard et al., 2004).

Several transporters usually work in antiport or symport mechanism. Antiport often indicates a counterflow of substrate (s) and H^+, whereas in the symport mechanism, substrate(s) and H^+ move in the same direction across the membrane (Legan et al., 1997). Although the increase in the (PM) H^+-ATPase under P and Fe deficiency conditions has been demonstrated (Zhang et al., 2011; Vansuyt et al., 2003; Ligaba et al., 2004), there is a lack of information about the PM-H^+-ATPase response under Ni deficiency and the role of amino acids in its regulation.

Plants have mechanisms of metal homeostasis which involve coordination of metal ion transporters for uptake, translocation and compartmentalization. However, very little metal in plants is thought to exist as free ions (Haydon and Cobbett, 2007). Several plant metal ligands have been identified in root exudate such as organic acids and amino acids (Bais et al., 2006). Amino acids are heterocyclic nitrogen donors that have high affinity for metal ions (Rauser, 1999). However, stability constants for complexes between nickel and amino acids and carboxylic acids demonstrate that nickel complexes with amino acids are considerably more stable than those with carboxylic acids (Homer et al., 1995), and hence offer better transport of this metal within the system.

The involvement of amino acids in the uptake of Ni^{2+} by plants has also been suggested. For example, it has been found that free His acts as an important Ni binding ligand in hyperaccumulator species of *Alyssum* and increases root uptake and xylem Ni concentration (Kerkeb and Kramer, 2003). Richau et al. (2009) also found much higher Ni uptake in the roots of the hyperaccumulator, *Thlaspi caerulescens* in the case of Ni–His supply than with $NiSO_4$ supply.

The main mechanisms by which Ni is taken up by plants are passive diffusion and active transport. At low concentrations, free Ni ions are absorbed by plants both passively and actively (Dalir and Khoshgoftarmanesh, 2015). Chelated Ni compounds are taken up through secondary active-transport-mediated means, using transport proteins such as permeases which can specifically bind to the Ni (Eitinger and Mandrand-Berthelot, 2000). On the other hand, amino acids can be absorbed by plant roots either by active or passive transport mechanisms. Passive uptake occurs by bulk flow, and diffusion probably involves carriers or channel proteins, while active import of the organic nitrogen into the root cell is probably mediated by proton-coupled amino acid transporters in a secondary active transport step generated by the H^+-pumping ATPase (Bush, 1993).

There are electrogenic carriers that can be driven by either the ΔpH or $\Delta\psi$ component of the proton-motive force ATPase (Boorer and Fischer, 1997). A better understanding underlying acquisition and utilization of micronutrients by plants is beneficial to optimize the efficiency of micronutrient use and improve the crop productivity. In the present study, we investigated the responses of wheat root plasma membrane H^+-ATPase and proton transport activity to Ni deficiency with or without histidine. We tried here to clarify the possible role of H^+-ATPase in active uptake of Ni, as well as the changes in its activity in the presence of histidine as a metal-ligand complex. For this purpose, plasma membrane was isolated and hydrolytic and transporting activities of H^+-ATPase were measured.

2. Material and methods

2.1. Plant material and growth conditions

Wheat seeds (*Triticum aestivum* cv. Back Cross Rushan) were surface-sterilized in 10% H_2O_2 for 10 min, rinsed thoroughly with sterile distilled water. After being soaked in distilled water for 24 h, they were pre-germinated for 2d in the dark at 25 °C. The germinated seeds were transferred on quartz sand moistened with deionized water. One week later, seedlings were selected and transplanted to 12 L beakers containing half Johnson nutrient solution which was prepared in double-deionized water (18 MΩ resistivity) 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 $MnSO_4$ xH_2O, 1 μM $ZnSO_4$ $7H_2O$, 0.25 μM $CuSO_4$ $5H_2O$, 0.25 μM H_2MoO_4, and 25 μM Fe(III)-EDTA (ethylene diamine-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 adjusting with 0.1 N HCl or KOH solution. Nutrient solutions were continuously aerated with pumps and replaced twice per week by fresh solutions. After two weeks, the most uniformly-sized plants were transferred to 4 L plastic pots of fresh culture solution buffered at pH 5.5 with 2 mM MES-TRIS. Almost all wheat plants grown in Ni–free solution showed typical symptoms of Ni deficiency with necrosis initiating from the tip of fully expanded leaves which resolved at this level. The treatments were: control (without nickel and histidine), 10 μM Ni, 100 μM His, and 100 μM His + 10 μM Ni. Ni concentration was selected from a wide range of Ni exposure levels, used in a pre-test experiment. Treatments were replicated 3 times, and four plants in the same pot were treated as one replicate. After exposure to amended solutions for 8 h the seedling were harvested. Shoot and root fresh weights were determined. Another experiment was also performed in parallel to assess the symplastic and apoplastic Ni contribution in the root sink and to measure apoplastic pH in response to external treatments.

2.2. Determination of tissue Ni fractionation

For the determination of apoplastic and symplastic Ni fractions in the root, a desorpsion method described by Harrison et al. (1979) was used. Prior to the determination of the symplastic Ni fraction, after 8 h exposure to Ni and His treatments, the roots were washed with 5 mM $PbCl_2$ at 0 °C for 30 min, in order to remove the extracellular bound Ni. The apoplastic Ni was calculated from the differences between the total and the symplastic Ni level. At harvest, root and shoot were separated, washed three times with pure water and oven-dried at 72 °C for three days. Dry matter weights (DWs) and Ni concentrations of all tissue samples were determined. About 0.50 g of the tissue samples was digested in an APCU-40 75 mL TFM Teflon vessel of microwave (Milestone Srl, START D, Sorisole, Italy) using 5 mL of HNO_3 and 3 mL of H_2O_2, and then filtered through Whatman no. 42 filters, transferred to 25-mL volumetric flasks, and diluted with deionized, distilled water. Ni concentrations were then measured using graphite furnace atomic absorption spectrometry (GFAAS) (Perkin-Elmer 3030). The detection limit (LOD) by graphite furnace AAS was 2 $\mu g\,L^{-1}$ for Ni.

2.3. Measurements of the pH in root apoplastic and symplastic sap

The root apoplast and symplast sap extracted according to the method of Yu et al. (1999) with slight modification. The root systems were harvested after 8 h exposure to Ni and His treatments. About 1 cm lateral root tips were cut off with a razor blade, washed in distilled water and blotted dry. About 30 root tips were arranged in a 1.5-mL microcentrifuge tubes with the cut ends facing down, fixed through one layer of cheese cloth and centrifuged from 600 to 7000g at 4 °C for 15 min and 3–6 time centrifuge for each replicates with the same RCFs were used to produce a reasonable volume. After centrifugation, root segments were frozen at 20 °C for 24 h and then thawed at room temperature. Symplastic sap was collected from frozen-thawed