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Symplastic and apoplastic uptake and root to shoot translocation of nickel in wheat as affected by exogenous amino acids

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ABSTRACT

This study investigated the effect of exogenous amino acids on apoplastic and symplastic uptake and root to shoot translocation of nickel (Ni) in two wheat cultivars. Seedlings of a bread (*Triticum aestivum* cv. Back Cross) and a durum wheat cultivar (*T. durum* cv. Durum) were grown in a modified Johnson nutrient solution and exposed to two levels (50 and 100 μM) of histidine, glycine, and glutamine. Application of amino acids resulted in increasing symplastic to apoplastic Ni ratio in roots of both wheat cultivars, although glutamine and glycine were more effective than histidine under our experimental conditions. The amino acid used in the present study generally increased the relative transport of Ni from the roots to shoots in both wheat cultivars. Higher amounts of Ni were translocated to wheat shoots in the presence of histidine than the other amino acids studied, which indicated that histidine was more effective in translocation of Ni from roots to shoots. Amino acids used in the present study largely increased root symplastic Ni, but shoot Ni accumulation was much lower than the total Ni accumulation in roots, indicating a large proportion of Ni was retained or immobilized in wheat roots (either in the apoplastic or symplastic space), with only a very small fraction of Ni being translocated from the root to the shoot. According to the results, glutamine and glycine were more effective than histidine in enhancing the symplastic to apoplastic Ni ratio in the roots, while more Ni was translocated from the roots to the shoots in the presence of histidine.

Introduction

Nickel (Ni) is an essential micronutrient for higher plant nutrition (Brown et al., 1987a,b). Whereas many proteins contain Ni (Thomson, 1982), the role of this metal in plant nutrition, physiology, and metabolism has received much less attention. There are several enzyme systems (e.g., NiFe-hydrogenase, superoxide dismutase, Ni-dependent glyoxylase, and methyleneurease) in bacteria that are activated by Ni (Walsh and Orme-Johnson, 1987; Mulrooney and Hausinger, 2003). However, the activation of urease currently appears to be the only enzymatic function of Ni in higher plants (Gerendas et al., 1999; Ciurli, 2001). The significance of Ni for plant growth and urease activation has been clearly established in hydroponically grown cereals (Brown et al., 1987a,b), lettuce (Khoshgoftarmanesh et al., 2011), cucumber (Khoshgoftarmanesh and Bahmanziari, 2012), and legumes (Eskew et al., 1984). Urea accumulation in foliage of soybean (*Glycine max*) and cowpea (*Vigna unguiculata*) under Ni deficiency has also been reported (Eskew et al., 1983, 1984; Walker et al., 1985). Kutman et al. (2013) reported that foliar applied urea-N was better utilized in soybean when adequate Ni was supplied to plants by seed reserves and/or externally. Although such reports of a growth response to Ni additions under controlled experimental conditions indicate that Ni deficiency has a wide range of effects on plant growth and metabolism, the agricultural and biological significance of Ni is still poorly understood, and the roles of this element in plant metabolism remain mostly unknown. This is largely because of the low levels thought to be needed by plants (about 1–100 ng g^{-1} dry weight) in relation to the relative abundance of Ni in essentially all soils (>5 kg ha^{-1}) (Bai et al., 2006).

There is, therefore, an increasing need to better understand the mechanisms of Ni in plants, starting with the entry of the metal into the organism and also plant Ni transfer from root to shoot, in which root uptake is a key process. Current data available for root uptake of most trace elements are needed. Root uptake of divalent cations typically exhibits two phases: apoplastic binding and symplastic uptake (Hart et al., 1998; Zhao et al., 2002). The apoplast is the space within the plant outside the symplast that includes the cell wall, intercellular and xylem–lumen spaces (Canny, 1995). The accumulation of cations takes place in the apoplast during the first stage of their uptake from an outer medium (Meychik and Yermakov, 2001). The apoplast serves as a cation reservoir if the amount of negative fixed charges in the cell wall matrix is high, and if the protoplasts have the capability to regulate pH and concentration of

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other cations in water free space (Freundling et al., 1988; Starrach et al., 1985). It is generally assumed that apoplastic uptake has no role in trace element uptake. Apoplastic trace elements are considered to be blocked in the roots behind the apoplastic barriers (Casparian band), and therefore not translocated into the shoots (Redjala et al., 2010). However, if there is a substantial adsorption of trace elements on the root apoplast, this might act as a driving force to move the trace element from the soil toward the roots, compete with the symplastic absorption and contribute to the element being taken up by the plant, at least into its roots (Redjala et al., 2010).

Metal adsorption on the apoplastic spaces of the root is dependent on the speciation of metal ion in media solution. In the root media, only a small fraction of the metals is present in the form of free ions and most ions are bound to low molecular mass ligands (Salt et al., 1999). Plants produce a number of chelating agents (Callahan et al., 2006) that form complexes with metals in the solution (Nowack et al., 2006). Among several substances identified in root exudates, sugars, amino acids (AA) and organic acids have drawn considerable interest due to their role in processes at the root–soil interface such as metal chelating (Jones et al., 2004; Fageria and Stone, 2006). However, it has been shown that some amino acids had little effect in mobilizing metal micronutrients in soils, due to their rapid microbial degradation (Jones et al., 1994; Jones and Hodge, 1999). On the other hand, concentrations of AA in the soil solution are often much higher than those of trace elements (Brynhildsen and Rosswall, 1995; Schwab et al., 2008). Therefore, AA may play a significant role in forming complexes with metals. Amino acids form complexes with metal cations mainly through carboxylate (—COO) and amine ($—NH_2$) groups, thereby affecting the bioavailability of metals to plants (Aravind and Prasad, 2005; Jones et al., 2004). Little attention has been paid to the effect of amino acids on apoplastic adsorption and symplasmic uptake of metal ions, although involvement of organic or amino acid chelation in enhancing the rate of root-to-shoot transport of transition metal ions has been reported. For example, histidine has a high affinity for binding metals both as the free amino acid and as metal coordination residues in proteins (Kerkeb and Kramer, 2003) and has a relatively high association constant (Log K: 8.7) for Ni^{2+} (Callahan et al., 2006). Metal–chelant complexes are taken up by plants through the apoplast (Tanton and Crowdy, 1971). Several reports have shown that chelating agents are indeed taken up into the shoots and that they can facilitate the uptake of metals that are not normally taken up to a larger extent at concentrations equal to their own accumulation (Vassil et al., 1998; Epstein et al., 1999; Schaider et al., 2006). There are limited reports on the effect of chelating agents on root uptake of Ni. Richau et al. (2009) found that the Ni uptake by roots of non-hyperaccumulator *Thlaspi arvense* was much greater from the Ni–histidine complex than the $NiSO_4$.

A better understanding of the characteristics and physiological mechanisms by which plants absorb nickel from the root media may provide additional basic information to aid in the development of better plant nutrition purposes. The aim of this research was to investigate the contributions of the apoplast and symplast uptake in the root sink as affected by exogenous amino acids and to characterize the role of these amino acids in root uptake and translocation to shoots of Ni in two wheat cultivars.

Materials and methods

Seeds of one bread (*Triticum aestivum* cv. Back Cross) and one durum wheat cultivar (*Triticum durum* cv. Durum) were surface sterilized with 1% H_2O_2 for 30 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, and the germinated seeds were transferred on to a sand culture moistened with deionized water. Two weeks later, the seedlings were transferred into the nutrient solution, which consisted of 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 mM KCl, 12.5 mM H_3BO_3, 1 mM $MnSO_4{\cdot}H_2O$, 1 mM $ZnSO_4{\cdot}7H_2O$, 0.25 mM $CuSO_4{\cdot}5H_2O$, 0.25 mM H_2MoO_4, and 25 mM Fe(III)-EDTA (ethylenediamine-tetra acetic acid). The nutrient solution in the growth containers was continuously aerated with pumps and renewed twice a week. The pH was maintained in the range 5.8–6.0 using 0.1 N HCl or KOH when needed. Four weeks after germination, plants were removed from the nutrient solution and transferred to the treatment solutions.

Uptake experiment

Seedlings (30-day-old) were transferred to 600 ml plastic pots (one seedling per pot) and grown for another two days in the basal uptake solutions. Three amino acids i.e., histidine, glycine and glutamine were chosen to be used in this experiment. The seedlings were exposed to 10 μM Ni in the form of $NiSO_4$ and two concentrations (50 and 100 μM) of amino acids resulting in amino acid/metal molar ratios of 5:1 and 10:1, respectively. These ratios could lead to various degrees of nickel complexation and amino acids in excess. The nickel alone treatment, in the absence of amino acids, was used as a control. Although there are some results that indicate negligible degradation of amino acids in nutrient solutions (Jamtgard et al., 2008), we tried to prevent amino acids from rapid degradation in solution as much as possible by sterilizing the containers with 5% NaClO prior to treatment and by using pure water in the preparation of nutrient solutions.

Harvest and analysis

After exposure to amended solutions for 2 days (without aeration), the seedlings were harvested by cutting with a sterilized razor blade at the stem point leveled to the upper surface of plant supporting plates and separated into root and shoot tissues. The shoot part was first rinsed in tap water and then washed three times with pure water and oven-dried at 72 °C for three days. The root part was further divided into two aliquots. One was oven-dried as above, and the other was washed with an EDTA solution and then oven-dried as above. Dry matter weights (DWs) and Ni concentrations of all tissue samples were determined. About 0.50 g of the tissue samples were 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 an atomic absorption spectrophotometer (AAS) (PerkinElmer 3030, PerkinElmer, Wellesley, MA). One gram of fresh root was placed in a 100 mL centrifuge tube, and 50 ml 10 mM EDTA (pH 6.0) pre-cooled in a refrigerator was added. The tube was stirred for 30 min on a rotation bed at a gentle speed of 100 r/min at 4 °C. Zhou et al. (2007) reported that this washing procedure desorbed up to 90% of Cu previously adsorbed to the root apoplast. We also tested this method in a preliminary trial. Nickel remaining in EDTA-washed roots was considered to be symplastic Ni, whereas Ni desorbed from roots was considered to be apoplastic Ni, which was estimated by the difference between Ni concentrations in unwashed and EDTA-washed roots.

Ni accumulation in shoots was also calculated, and the sum of symplastic Ni in roots and Ni accumulation in shoots was taken as the true Ni uptake in the whole plant. The relative translocation of Ni from root to shoot was calculated as the percentage of Ni accumulation in the shoot to that in the root symplast. It must be noted that the biomass, Ni accumulation or uptake of wheat seedlings