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Implication of citrate, malate and histidine in the accumulation and transport of nickel in *Mesembryanthemum crystallinum* and *Brassica juncea*

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ABSTRACT

Citrate, malate and histidine have been involved in many processes including metal tolerance and accumulation in plants. These molecules have been frequently reported to be the potential nickel chelators, which most likely facilitate metal transport through xylem. In this context, we assess here, the relationship between organics acids and histidine content and nickel accumulation in *Mesembryanthemum crystallinum* and *Brassica juncea* grown in hydroponic media added with 25, 50 and 100 μM $NiCl_2$. Results showed that *M. crystallinum* is relatively more tolerant to Ni toxicity than *B. juncea*. For both species, xylem transport rate of Ni increased with increasing Ni supply. A positive correlation was established between nickel and citrate concentrations in the xylem sap. In the shoot of *B. juncea*, citric and malic acids concentrations were significantly higher than in the shoot of *M. crystallinum*. Also, the shoots and roots of *B. juncea* accumulated much more histidine. In contrast, a higher root citrate concentration was observed in *M. crystallinum*. These findings suggest a specific involvement of malic and citric acid in Ni translocation and accumulation in *M. crystallinum* and *B. juncea*. The high citrate and histidine accumulation especially at 100 μM $NiCl_2$, in the roots of *M. crystallinum* might be among the important factors associated with the tolerance of this halophyte to toxic Ni levels.

1. Introduction

Among environmental pollutants, heavy metals (HMs), have received a particular attention as potent hazards to human health in relation to increasing industrialization and disturbance of natural biogeochemical cycles. Unlike organic substances, HMs cannot be degraded by chemical or biological processes and therefore accumulate in the environment. Nowadays, soil contamination with HMs is becoming a serious environmental problem which stimulates the efforts to propose new approaches for cleaning up HMs-contaminated soils (Ghnaya et al., 2013). Phytoremediation consists in the use of green plants to reduce the toxicity of these persistent contaminants in the environment. It has recently emerged as a new promising alternative to expensive physico-chemical techniques. It is a cost-effective and efficient remediation strategy that can be applied to both organic and inorganic pollutants present in the soil, water or air (Vithanage et al., 2012).

Nickel (Ni) is one of the most important environmental HM pollutants. Nickel soil contamination may originate from natural sources such as weathering of rocks, forest fire and volcanic eruptions, but may also result from anthropogenic activities including industrial effluents, surgical instruments, kitchen appliances, steel alloys and automobile batteries (Knox et al., 1999). Nickel is an essential micronutrient for plant but its concentration in the vast majority of plant species is rather low (0.05–10 μg/g dry weight) (Nieminen et al., 2007). However, unlike Cd, Pb, Hg, Ag, and several other metals that do not assume any biological role in plants and are highly toxic, even at low concentration, Ni acts as a cofactor for enzyme activities including urease, superoxide dismutases and hydrogenases (Krämer et al., 1996; Sirko and Brodzik, 2000; Küpper and Kroneck, 2007). Nickel homeostasis is therefore crucial for cell metabolism (Yusuf et al., 2011). Nevertheless, Ni excess drastically affects vital processes in plants and leads to visible symptoms of toxicity (Yusuf et al., 2011). It induces a wide range of toxic effects at morphological, physiological, and biochemical levels. Those Ni-induced deleterious effects on plants

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may result from direct (toxicity of metal accumulated in tissues) and/or indirect processes including alteration of the plant water status, impairment of photosynthesis, disturbance or imbalance of mineral nutrition (Amari et al., 2014), inhibition of enzymatic activities and oxidative stress resulting from the overproduction of reactive oxygen species (ROS) (Gajewska and Sklodowska, 2007).

Despite Ni high phytotoxicity, some plant species are able to tolerate and accumulate high levels of this element in their aboveground organs and thus behave as "Ni-phytoextractors" (Baker et al., 1994; Kerkeb and Krämer, 2003; Montargès-Pelletier et al., 2008). Most of these plants are salt sensitive species, and can therefore not be used to extract metals from salt-affected soils while the phytoremediation of HMs contaminated salty soils constitutes an important challenge for scientists. Phytoremediation of salt-affected areas requires the use of plants which are able to tolerate salt and to accumulate metals in their tissues. Recently, several studies demonstrated the superiority of halophyte, *i.e.* native salt tolerant species, to cope with HMs stress (Zaier et al., 2010; Eid, 2011; Taamalli et al., 2014). Some of these plants were also reported to accumulate high amounts of Cd (Ghnaya et al., 2007), Pb (Zaier et al., 2010), Zn (Eisa and Eid, 2011) and Ni (Amari et al., 2014) within their shoots.

Metal detoxification mechanisms in plants mainly rely on the distribution of metals in the apoplasm on the one hand, and on the chelation of metals by cytosolic ligands followed by the sequestration of the metal–ligand complex into the vacuole on the other hand. Organic acids, amino acids, phytochelatins (PCs) and metallothioneins (MTs) are natural ligands synthesized by plants and able to chelate free bivalent cations (DalCorso et al., 2008). Removing heavy metal complexes with organic acids from the cytoplasm through their vacuolar sequestration is considered as an efficient mechanism of pollutants detoxification (Wei et al., 2009).

Organic acids are commonly present in various plant organs and it was suggested that they are involved in root-to-shoot translocation of several metal ions in the form of bound complexes (Tatár et al., 1999). For instance, citric acid is the main ligand of Ni in leaves of *Thlaspi goesingense* (Krämer et al., 2000), and in roots of *Thlaspi caerulescens* (Richau et al., 2009). In *Alyssum murale,* X-ray experiments demonstrated that citric acid is the main ligand responsible for long distance transport of nickel (Montargès-Pelletier et al., 2008). Similarly, the higher Pb accumulation potential of *Sesuvium portulacastrum* is associated with a higher concentration of citrate in shoots and xylem sap (Ghnaya et al., 2013). Amino acids, especially histidine, were also shown to play a key role as Ni chelators (Kerkeb and Krämer, 2003). Histidine is also considered as an efficient ligand involved in plant Ni-tolerance (Krämer, 2010). Thus, considering the putative role of organic acids and histidine in conferring plants tolerance to HMs and their aptitude to contribute to root-to-shoot translocation, we hypothesize that monitoring the level of organic acids and histidine in the plant tissue after exposure to Ni could be used as an indicator to identify Ni tolerant species. In this work, we aimed to analyze the putative implication of organic acids and histidine in Ni transport and accumulation in the halophyte *Mesembryanthemum crystallinum* as compared to the reference species *Brassica juncea*. We therefore determined acids concentrations in the shoots, xylem sap and the roots of the two species submitted to increasing doses of Ni. Data obtained were analyzed in relation to the mean level of Ni accumulation in the considered plant species, as previously reported (Amari et al., 2014).

2. Materials and methods

2.1. Plant materials and treatments

M. crystallinum seeds were collected near Thina (Sfax, 300 km south of Tunis) whereas *B. juncea* seeds (Acc PI 173874) were kindly provided by the North Central Regional Plant Introduction Station (NCRPIS-USDA-USA). Seeds of both species were sterilized by dipping into a 10% H_2O_2 solution during 20 min. They were then washed and sown on perlite imbibed with distilled water and remained for 4 days in the dark at 24 ± 1 °C and 70% relative humidity. Twelve days after germination, plants were grown hydroponically for 3 weeks in 5L continuously aerated pots (8 plants per pot) using the Hoagland's nutrient solution (Arnon and Hoagland, 1940). Pots were placed in a growth chamber at $25 \pm 1/18 \pm 1$ °C, 55/75% relative humidity (RH) and 16 h/8 h photoperiod day/night regime. The nutrient solution of control plants was renewed every 3 days. Four Ni treatments (3 pots per treatment) were applied as follows: 0, 25, 50 and 100 μM $NiCl_2$. The hydroponic medium of Ni-treated plants was daily changed to avoid Ni depletion in the nutrient solution. Plants were harvested after 21 days of treatment and used for physiological analyses.

2.2. Reagents

All solutions were prepared in MilliQ purified water (Millipore, Molsheim, France). The Ni standard solution was prepared by appropriate dilution of a Nickel standard solution (Merck, Darmstadt, Germany). All reagents used were analytical-reagent-grade. Organic acids were obtained from Sigma (St. Louis, MO, USA), and the other reagents were purchased from Merck (Darmstadt, Germany). Stock solutions were prepared by dissolving malic and citric acids in bidistilled water and were stored at 4 °C. Analytical standard solutions were prepared from these stock solutions by serial dilutions.

2.3. Xylem sap collection

At harvest, shoots were separated from roots, rinsed three times with cold water and blotted between two layers of filter-paper. Roots were immediately dipped in a cold solution of HCl (0.01 M) during 5 min to eliminate elements adsorbed at the root surface, then washed three times with cold distilled water and blotted dry with filter paper. The xylem sap collection was performed at the end of the 3rd week of treatment. The shoots of both the species were excised 2 cm above the root and the solution exuded from the cut surface, after discharging the first drop, was considered as the xylem sap according to Schurr (1999) and thus collected by means of trapping into a 1.5 mL plastic vial filled with a small piece of cotton for 2 h after cutting. After determination of their volumes the xylem sap samples were stored at −20 °C.

2.4. Nickel accumulation

Dried samples were ground to a fine powder using a stainless mill and digested by concentrated HNO_3 (10/100 v/w) in a microwave digester (ETHOS D, milestone, Italy) at 100 °C (Ghnaya et al., 2005). Ni and nutrients concentrations were measured by inductively coupled plasma mass spectrometry (ICP-MS; Perkin Elmer, Sciex-Elan 5000).

2.5. Determination of organic acids

Organic acids were extracted according to Rabotti et al. (1995). For each treatment, frozen tissues (about 2 g FW) were pulverized in a cold mortar with a pestle and then homogenized with ice-cold