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Characteristics of chromium(III) uptake in hyperaccumulator *Leersia hexandra* Swartz

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ABSTRACT

Characteristics of Cr(III) uptake by roots of hyperaccumulator *Leersia hexandra* Swartz were studied using pot-culture experiment. The results showed that the uncoupler, 2,4-dinitrophenol (DNP), and low temperature significantly limited the Cr(III) uptake ($p < 0.05$), which indicated an active uptake of Cr(III) in the roots. The calcium channel blocker "$LaCl_3$" and the kalium channel blocker "tetraethyl ammonium chloride (TEA)" did not inhibit the Cr(III) uptake, i.e., the Cr(III) uptake was not related to the K^+ or Ca^{2+} ion channels. However, an inhibiting effect of Fe(III) on the Cr(III) uptake was observed in the roots of *L. hexandra*. The Cr(III) uptake followed Michaelis–Menten kinetics with a Michaelis constant (K_m) of 123.9 μmol/L, which was increased by 2-fold in the presence of 300 μmol/L Fe(III). The Fe-deficiency increased the maximum uptake velocity (V_{max}) by 33.5% and decreased the Michaelis constant by 23.2%. The antagonistic effect of Fe(III) on Cr(III) uptake suggest that the Cr(III) uptake by the roots of *L. hexandra* may be mediated partly via transporters for the Fe(III)–phytosiderophore complex.

1. Introduction

Phytoremediation is an emerging green technology that can be considered for remediation of contaminated sites because of its low cost, aesthetic advantages, and long term applicability (Zhao and McGrath, 2009). A major factor behind the interest in phytoremediation of metal-polluted soils has been the growing awareness of the existence of a number of metal-accumulating plant species. These plant species, called hyperaccumulators, are capable not only of tolerating high levels of heavy metals, but also of accumulating high metal concentrations in shoots (Sarma, 2011). Root uptake is the first step of metal accumulation in the plant tissue. Some evidence indicates there are specific physiological characteristics of metal uptake in the roots of hyperaccumulators. Root Zn^{2+} uptake followed Michaelis–Menten kinetics, with similar Michaelis constant values for Zn hyperaccumulator (*Thlaspi caerulescens*) and nonaccumulator (*Thlaspi arvense*). However, the maximum initial velocity for Zn^{2+} influx in *T. caerulescens* root cells was 4.5-fold higher than that in *T. arvense* (Lasat et al., 1996). Zhao et al. (2002) found that the metabolically dependent uptake of Cd was 4.5-fold higher in the high Cd-accumulating ecotype of *T. caerulescens* than in the low Cd-accumulating ecotype. Lu et al. (2009) reported that low temperature treatment significantly inhibited Cd uptake and reduced upward translocation of Cd to shoots by 9 times in hyperaccumulating ecotype of *Sedum alfredii*, whereas no such effect was observed in non-hyperaccumulating ecotype.

To date, the uptake mechanisms of some univalent and divalent cations were understood to a certain extent. Several cation transporters in the ZIP (ZRT, IRT-like protein) family and the NRAMP (natural resistance associated macrophage proteins) family have been found to be involved in the uptake of univalent and divalent micronutrients with the use of molecular techniques (Wu et al., 2009; Wei et al., 2009). However, little is known about the uptake mechanism of trivalent cation such as Cr(III). Zayed and Terry (2003) reported that Cr(III) enters root cells by a passive mechanism, while Cr(VI) uptake is an active process. On the other hand, an active uptake of both Cr species has been reported for several crop plants (Ramachandran et al., 1980). *Leersia hexandra* Swartz, a graminaceous plant species, has been identified as a new Cr hyperaccumulator plant species (Zhang et al., 2007). Under nutrient solution culture, the highest bioaccumulation coefficients of *L. hexandra* root for Cr(III) were 1026. It is hypothesized that this species would have an active uptake of Cr(III) resulting in high concentration of Cr in plant tissue.

Cr is a toxic, non-essential element to plants; hence, they do not possess specific mechanisms for its uptake. Therefore, the uptake of this heavy metal is through carriers used for the uptake of essential metals for plant metabolism (Shanker et al., 2005). However, the carriers involved in Cr(III) uptake remain unclear. Cr(III) uptake showed similar features as Fe(III) uptake, which gave rise to investi-

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gate the interaction of both elements in plants (Bülent et al., 2007). It was reported that leafy vegetables that tend to accumulate Fe (e.g., spinach and turnip leaves) appeared to be the most effective in accumulating Cr (Cary et al., 1977). Low Cr supply to Fe-deficient plants could slightly alleviate plants from Fe deficiency-induced chlorosis (Bonet et al., 1991). Our recent works found that the cellular distribution patterns of Cr and Fe in the root cells of *L. hexandra* were similar, indicating a possible relationship between the uptake of Cr and Fe (Liu et al., 2009). Since previous reports suggest a possible interaction of Cr with Fe, it is essential to investigate the relationship between the uptake of Fe and Cr.

In the present work, the effect of Fe on Cr uptake of *L. hexandra* was studied by Michaelis–Menten kinetics. To investigate whether an active uptake occurred in *L. hexandra*, the uptake of Cr(III) was compared between the plants with and without metabolic inhibitor treatment. Furthermore, we also explored the relationship of Cr(III) uptake with Ca and K ion channels using channel blockers.

2. Materials and methods

2.1. Plant culture

Seedlings of *L. hexandra* were collected from the riverside of Taohua River in Guilin where Cr concentration in soil is 8–14 mg/kg. Chromium concentrations in seedling of *L. hexandra* grown in this site were not detectable. The seedlings were washed thoroughly (at least tree times) with redistilled water and placed in 15 cm diameter round plastic pots filled with 1.5 L 20% Hoagland's nutrient solution. The nutrient solution renewed every 3 days with the volume being restored to its original level. Plants were grown in the pots for 20 days in a controlled environment growth cabinet (14 h photoperiod, 25 °C day/18 °C night, relative humidity 70–75%, and a light intensity of 300 μmol/(m^2 s)).

2.2. Metabolic inhibitor on uptake of Cr(III)

Twelve hours before the uptake experiment, the nutrient solution was buffered at pH 5.5 with 2 mmol/L Mes–Tris (Lasat et al., 1996). The plants exposed in 300 μmol/L $CrCl_3$ were treated with different concentrations (0, 25 and 50 μmol/L) of 2,4-dinitrophenol (DNP). The plants were harvested after 1, 2, 4, 8, 12, 24, and 48 h treatment duration. Plant roots were cut, and washed tree times with 10 mmol/L EDTA in an ultrasonic equipment to completely remove adsorbed Cr as described (Leita et al., 1991). Samples were blotted dry and kept in oven at 70 °C. Dried plant samples were digested in a mixture of HNO_3 and $HClO_4$ (5:3, v/v) that was heated on an oven. After cooling, the extracts were diluted up to 50 mL with 0.2% HNO_3. Chromium concentrations of the extracts were determined by flame atomic absorption spectrophotometer (AAS).

2.3. Low temperature on uptake of Cr(III)

After the plants were cultured for 12 h in the nutrient solution which was adjusted to pH 5.5 by 2 mmol/L Mes–Tris, they were exposed in 300 μmol/L $CrCl_3$ and placed in a growth cabinet at 2 °C. After 1, 2, 4, 8, 12, 24, and 48 h, the plants were harvested and Cr concentrations in roots were determined as described above. The control of this experiment was the same as the metabolic inhibitor experiment.

2.4. Ion channel blocker on uptake of Cr(III)

The experimental procedure was similar to that described above. There were three treatments for plant: control (300 μmol/L $CrCl_3$), Ca^{2+} channel blocker treatment (300 μmol/L $CrCl_3$ + 1 mmol/L $LaCl_3$), and K^+ channel blocker treatment (300 μmol/L $CrCl_3$ + 5 mmol/L TEA). The plants were harvested after 4 and 8 h treatment duration, respectively. Cr, Ca and K concentrations in roots were also determined by AAS.

2.5. Fe on uptake of Cr(III)

The plants were divided into three groups. One group was precultured for 48 h without Fe supply, and two groups were cultured with normal nutrient solution. Then the $CrCl_3$ was added to the all groups in six levels: 50, 150, 300, 600, and 1000 μmol/L. Meanwhile, 300 μmol/L $FeCl_3$ was added into one group of plant with normal nutrient solution. Four hours after treatment, the plants were harvested and washed tree times with 10 mmol/L EDTA for 30 min to remove adsorbed Cr. Samples were dried and analyzed for Cr as described above.

2.6. Statistical analysis

The data presented in this paper are the average of three independent replicates ± standard error of means (SD). Each experiment was repeated at least twice. Analysis of variance (ANOVA) was performed on all data sets, and least significant difference (LSD) was used to compare treatments.

3. Results

3.1. The effect of metabolic inhibitor on Cr(III) uptake

Time course of Cr(III) uptake at 300 μmol/L external Cr^{3+} level with or without DNP (Fig. 1) have shown that uptake of Cr(III) by *L. hexandra* was significantly inhibited in the presence of DNP ($p < 0.05$). Moreover, the inhibition effect of 50 μmol/L DNP on Cr(III) uptake was more pronounced than that of 25 μmol/L DNP (Fig. 1). However, DNP did not substantially reduce uptake of Cr(III) before 2 h. The probable reason was that apoplastic binding in roots was predominant in Cr(III) uptake before 2 h or DNP inhibited metabolic with a time delay.

3.2. The effect of low temperature on Cr(III) uptake

The metabolic level of plant could also be inhibited by low temperature. To exclude the possible interference of metabolic inhibitor with Cr(III) uptake, the effect of low temperature on Cr(III) uptake was tested. As shown in Fig. 2, the uptake of Cr(III) by roots of *L. hexandra* was strikingly depressed at 2 °C. After the plants were exposed in 2 °C for 4 h, Cr concentrations in roots were significantly lower than those in control

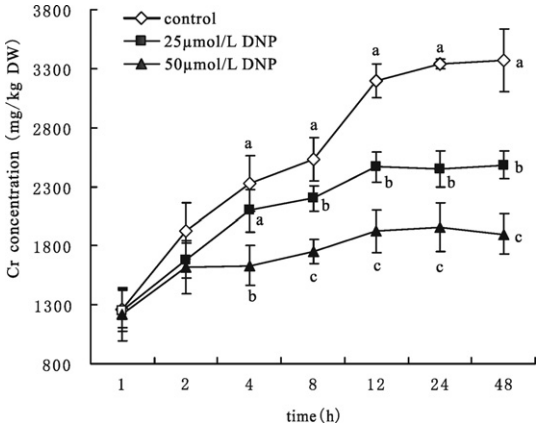

Fig. 1. The effect of DNP on uptake of Cr(III) by *L. hexandra*. Different letters denote that differences are statistically significant (LSD, $p < 0.05$).