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Full Length Research Paper

Low molecular weight organic acids in root exudates and cadmium accumulation in cadmium hyperaccumulator *Solanum nigrum* L. and non-hyperaccumulator *Solanum lycopersicum* L.

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Changes in the roots exudation of low molecular weight organic acids (LMWOA), cadmium (Cd) accumulation and translocation and soil solution pH in *Solanum nigrum* L., the Cd-hyperaccumulator were examined and compared with a non-hyperaccumulator *Solanum lycopersicum* L. The differences were significant ($P < 0.05$ or < 0.01) between *S. nigrum* and *S. lycopersicum* for all the five LMWOA at all soil levels. The total amount of LMWOA exuded by *S. nigrum* was significantly higher than that of *S. lycopersicum* ($P < 0.05$ or < 0.01). The *S. nigrum* accumulated and translocated more Cd than *S. lycopersicum*. The soil solution pH of *S. nigrum* was significantly lower than *S. lycopersicum*. The *S. nigrum* with higher concentrations of LMWOA in roots exudation accumulated more Cd in the plants. The results indicated tha LMWOA secretion by *S. nigrum* root, especially in Cd-contaminated soils, was likely to be an important role in Cd hyperaccumulation.

Key words: Cadmium, Solanum nigrum *L.*, low molecular weight organic acids, root exudates.

INTRODUCTION

With the development of modern industry and agriculture, cadmium (Cd) has become one of the most harmful and widespread pollutants in agricultural soils and soil-plant-environment system mainly due to anthropogenic activities, such as industrial emission, the application of Cd-containing sewage sludge and phosphate fertilizers and municipal waste disposal (Wu et al., 2005; Lima et al., 2006). Therefore, it is important and urgent to develop methods to cleanup Cd-contaminated soils. Phytoremediation which has been increasingly given more attention and considered as a promising, cost-effective and aesthetically pleasing technology is defined as the use of hyperaccumulator plants in removing pollutants from the environment or in rendering them harmless (Salt et al., 1998; Garbisu et al., 2002). Hyperaccumulators are plants that can exceptionally accumulate high quantities of heavy metals. At present some progresses have been made in our understanding of the ability of hyper-accumulators to tolerate and accumulate metals within their tissues (Wei et al., 2006). However, it is still necessary to fully understand some related mechanisms involved in metal hyperaccumulation, especially the role of low molecular weight organic acids (LMWOA) in the root exudation.

LMWOA are important components of root exudates and are typically negatively charged anions capable of reacting strongly with metal ions in both the soil solution and solid phases (Jones et al., 1996). Simple organic acids have the potential to enhance metal mobility in soil profiles by reducing soil pH and forming complexes with heavy metals (Renella et al., 2004). The organic acids exuded by plant roots can influence heavy metal in soil by reducing sorption, increasing solubility, enhancing bioavailability and mobilizing heavy metals in soils (Krishnamurti et al., 1997; Liao and Xie, 2004). The interactions of LMWOA with metals in the soil-plant

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system may affect solubility and phytoavailability of metals in soil and their transport in plant. The bioavailability of Cd in the soil-plant system is considered to be a key factor controlling plant uptake. Some LMWOA, such as citric, malic and oxalic have been reported to be potential metal chelators (Naidu and Harter, 1998). The more Cd complexed with organic acids, the greater the enhanced Cd uptake (Han et al., 2006). The rice cultivar with higher concentrations of LMWOA in soil accumulated more Cd in the plants (Liu et al., 2007). The total amount of LMWOA in the rhizosphere soil of the high Cd-accumulating wheat cultivar was significantly higher than that of low Cd-accumulating cultivar and Cd accumulation by the high and low accumulating cultivars was proportional to the levels of LMWOA found in the rhizosphere soil of each cultivar (Cieslinski et al., 1998). In pot experiments, organic acids triggered uranium accumulation in Indian mustard and Chinese cabbage (Huang et al., 1998) and increased chromium accumulation in tomato (Srivastava et al., 1999). In addition, organic acids can increase desorption of heavy metals from soil and consequently increase metal concentrations in the soil solution (Qin et al., 2004).

The Solanum nigrum L. are worldwide weeds of arable land, but in many developing countries they constitute a minor food crop, with the shoots and berries not only being used as vegetables and fruits, but also for various medical and local uses. A cadmium-hyperaccumulator *S. nigrum* was first discovered by the pot-culture method arranged in outdoor and sample-analyzing experiments carried out in heavy metal contaminated areas (Wei et al., 2006).

The aim of this study was to investigate the mechanisms of enhanced Cd uptake by the hyperaccumulator, *S. nigrum* as influenced by LMWOA in root exudation compared with non-hyperaccumulator, *S. lycopersicum* L. Five LMWOA exudation, Cd bioaccumulation and translocation and pH alterations of soil solution are reported, in order to fully understand the processes involved in Cd hyperaccumulation by *S. nigrum* L. in the presence of LMWOA.

MATERIALS AND METHODS

Soil samples were collected from an agricultural field in the Shenyang Station (123°41'N and 41°31'E) of Experimental Ecology, Chinese Academy of Sciences. This soil is meadow burozem with 32.1% clay, 46.5% silt and 21.4% sand, and organic matter, total nitrogen, pH and Cd concentration were 1.55%, 0.11%, 6.50 and 0.20 mg kg^{-1}, respectively. The fresh soil samples were air dried, passed through a sieve of 4.0 mm and thoroughly mixed with basal fertilizers. Basal fertilizers applied were 150 mg N kg^{-1} dry soil as urea, 60 mg P kg^{-1} and 80 mg K kg^{-1} as KH_2PO_4. Cd was applied as $CdCl_2{\cdot}2.5H_2O$ and mixed thoroughly with the soil samples, and equilibrated for 14 days before the pot culture. There were five Cd-level treatments corresponding to each plant species: Cd 0 (control), 1, 5, 10 and 20 mg kg^{-1}.

Seeds of *S. nigrum* were collected from a non-contaminated field in the Shenyang Station of Experimental Ecology, Chinese Academy of Sciences. Seeds of *S. nigrum* and *S. lycopersicum* were sown into the pots with a diameter of 9.0 cm and a depth of 12.0 cm, each filled with 1.0 kg of soil samples. Plants were grown under a controlled-environment growth chamber with a 16 h light period (light intensity of 350 µmol m^{-2} s^{-1}), a 25/15°C light/dark temperature regime and 60% relative humidity. Five weeks after germination, samples of both plants were collected and analyzed, respectively.

During harvest, the roots and aerials were rinsed with distilled water and shoot and root were separated. The plant samples were oven dried at 70°C for 48 h to a constant weight, after which dry weight of shoots and roots were determined by electronic balance. After milling, 200 ± 5 mg dried plant tissue were weighed into a 30 ml porcelain crucible. The plant tissues were ashed at 500°C for 5 h in a muffle furnace and cooled down. A mixture of 5 ml HNO_3 (65%), 2 ml H_2O_2 (30%) and 2 ml purified water (Milli-Q reagent grade water) were added at room temperature. Subsequently, the sample volume was adjusted to 20 ml with deionized water and analyzed for Cd by flame atomic absorption spectroscopy (Spectra AA220, Varian). Soil solution samples were collected from the pots, and soil solution pH was measured using a pH meter. One soil moisture suction sampler was installed in the centre of each pot to permit sampling of the soil solution.

The translocation factor (TF) indicated the ability of plants to translocate heavy metals from the roots to the shoots (Mattina et al., 2003). It was calculated as: TF = the metal concentration in shoots/the metal concentration in roots.

The whole root systems of intact plants were washed carefully and transferred to Erlenmeyer flasks (1 plant in 200 ml). The flasks were filled with deionized water. The pH of the collection solution was 6.0 to 6.2. The flasks were covered with black plastic cloth and the collection experiment was carried out in the same controlled climate conditions as where plants were grown. The solutions were permanently aerated. The roots were stored for 1 h in solution to remove exudates from possibly injured cells caused by washing the roots. Solutions obtained during the first hour were not considered and analyzed. After the first hour, solutions were renewed and the plants were allowed to exude into the collection solution for 4 h. Sterility checks were conducted in collection of exudates simultaneously. The root exudation was poured onto anion-exchange (DEAE) column (1.2 cm × 8 cm, 9 ml DEAE32). The column was washed with 25 ml of deionized water and the organic acids were eluted with 15 ml of 1 M HCl. The exudate was dried with rotary evaporator (40°C). The residue was dissolved with 1 to 2 ml of the HPLC (high-performance liquid chromatography) mobile phase solution of 0.5% KH_2PO_4. The mixture was filtered through 0.45 µm filter to remove suspended material prior to injection into the HPLC (Agilent 1100). Separation was conducted on a 150 × 4.6 mm reverse phase column (Extend Zorbax). Sample solutions (20 µl) were injected into the column with a flow rate of 0.6 ml·min^{-1} at 50°C and UV detection at 210 nm. 0.5% KH_2PO_4 solution was used for isocratic elution. Identification of organic acids was performed by comparing retention times and absorption spectra with those of known standards of five different organic acids. External standard method was the quantitative analysis method.

Controls and treatments were performed in triplicates. Data were tested for statistical significance using one-way ANOVA by SPSS13.0 software package, followed by the least significant difference (LSD) test for comparison of individual means. The difference was considered significant at the $P < 0.05$ and $P < 0.01$ levels.

RESULTS AND DISCUSSION

The LMWOA concentrations in root exudates of *S. nigrum* were higher than those of the root exudates of *S.*

Table 1. Differences between *S. nigrum* L. and *S. lycopersicum* L. in low molecular weight organic acid concentrations of root exudates (µmol g^{-1}dry wt root, n = 3).

Organic acid	Control		Cd1		Cd5		Cd10		Cd20	
	S. nigrum	*S. lycopersicum*	*S. nigrum*	*S. lycopersicum*	*S. nigrum*	*S. lycopersicum*	*S. nigrum*	*S. lycopersicum*	*S. nigrum*	*S. lycopersicum*
Acetic acid	36.56	28.23**	43.13	39.78*	55.13	43.24**	78.39	64.56**	59.46	48.49**
Citric acid	7.56	5.58**	8.52	6.54**	10.26	8.23**	12.45	11.34*	1 9.56	14.56**
Malic acid	4.35	2.35**	9.56	7.56**	10.28	8.24**	14.26	12.36**	10.56	7.26**
Oxalic acid	1.26	0.78**	0.88	0.69*	1.58	1.12**	3.26	2.48**	2.59	1.89**
Tartaric acid	280.12	265.41**	310.26	287.26**	335.85	316.25**	385.26	348.26**	3 40.25	305.26**
Total content	329.85	302.35**	372.35	341.83**	413.10	377.08**	493.62	439.00**	432.42	377.46**

* Significant difference at P = 0.01 level, ** significant difference at the P = 0.05 level.

lycopersicum at all soil Cd levels ($P < 0.05$ or < 0.01) (Table 1). However, the magnitudes of the differences between the two plants varied with the kind of LMWOA and depended on soil Cd concentrations. The differences were significant ($P < 0.05$ or < 0.01) between *S. nigrum* and *S. lycopersicum* for all the five LMWOA at all soil levels. *S. nigrum* exuded 8.42 to 29.51% more acetic acid, 9.79 to 35.48% more citric acid, 15.37 to 85.11% more malic acid, 27.54 to 61.54% more oxalic acid and 5.54 to 10.62% more tartaric acid than *S. lycopersicum* across all the Cd treatments.

With regard to the differences in the total amount of the five kinds of LMWOA between the two plants, *S. nigrum* was higher than *S. lycopersicum* ($P < 0.01$) across all the Cd treatments. The largest difference was in soil Cd of 20 mg kg^{-1} treatment (*S. nigrum* was 14.56% higher than *S. lycopersicum*). The smallest difference in soil Cd was 1 mg kg^{-1} (S. nigrum was 8.93% higher than *S. lycopersicum*). With increasing levels of Cd, root exudation of *S. nigrum* and *S. lycopersicum* both increased from the control to 10 mg kg^{-1} Cd treatment and then decreased. The largest amount of root exudation for each LMWOA in *S. nigrum* and *S. lycopersicum* both appeared at Cd 10 mg kg^{-1}. With regard to the composition of the LMWOA in the present experiments, tartaric acid was dominant. They occupied more than 78.05 to 84.92% and 79.33 to 87.78% of the total concentration of the five kinds of LMWOA for *S. nigrum* and *S. lycopersicum* at all soil Cd levels. The five organic acids in roots exudation of *S. nigrum* and *S. lycopersicum* were in the same sequence; tartaric > acetic > malic ≈ citric > oxalic under all the Cd treatments. As presented in Figure 1, the soil solution pH of *S. nigrum* and *S. lycopersicum* nearly had no change with increasing Cd concentrations in the soil. However, the pH in soil solution of *S. nigrum* was significantly lower than those of *S. lycopersicum* at all soil levels ($P < 0.05$).

The Cd accumulation and translocation in the *S. nigrum* and *S. lycopersicum* observed in this experiment are presented in Figures 2 and 3. Cd accumulation in shoots and roots of the two plants both increased with rising Cd concentrations in the soils. In all the treatments, the concentration of Cd in the shoots of *S. nigrum* was always higher than that in the roots, with the ratio of shoot Cd/root Cd concentrations varying between 1.33 and 1.89, and furthermore, the Cd concentrations in shoots of Cd treated *S. nigrum* greatly exceeded the threshold value of 100 µg Cd g^{-1} DW in shoots of a plant, which is used to define a Cd-hyperaccumulator (Baker et al., 1994). On the contrary, *S. lycopersicum* had higher Cd concentrations in the roots than those in the shoots, which is unexpected for a hyperaccumulating species. The translocation factor of *S. lycopersicum* was from 0.45 to 0.84, which indicated that Cd accumulation was mainly at the roots. The concentration of Cd in the shoots of *S. nigrum* was 2.08 to 3.45 folds as much as that of *S. lycopersicum*. The maximum concentration of Cd accumulated from the soil by the *S. nigrum* shoots was 127.5 µgCd g^{-1}, but only 61.4 µgCd g^{-1} was accumulated by the *S. lycopersicum* shoots.

Hyperaccumulation of heavy metal by higher plants is a complex phenomenon. Heavy metal bioavailability for uptake into roots is a major factor limiting the effects of phytoremediation. Thus, increasing metal bioavailability in the soil is an important prerequisite to enhance the potential for phytoextraction. Root exudates, especially LMWOA released by plant root, have the ability to form complexes with heavy metals, so they are likely to increase the solubility and phytoavailability of metals in soil (Jones et al., 1996). In

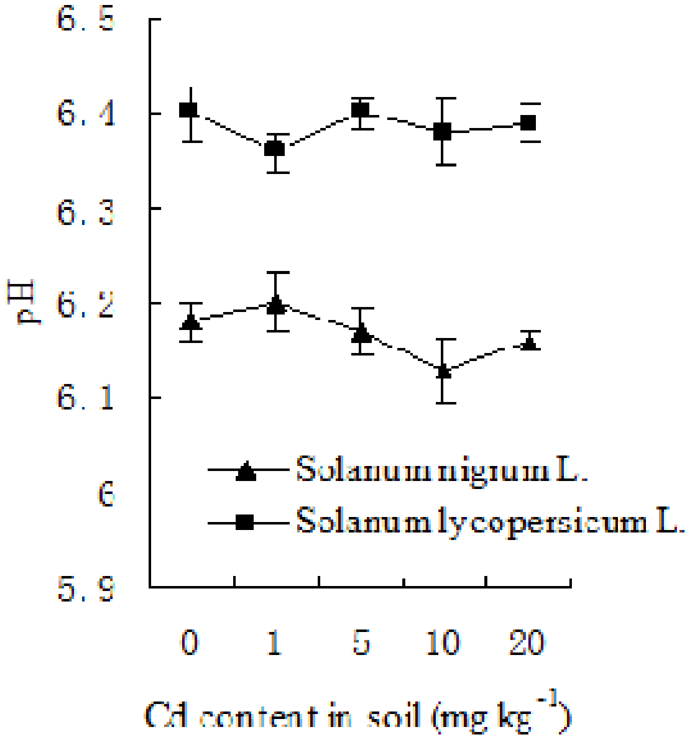

Figure 1. Soil solution pH change of *Solanum nigrum* L. and *Solanum lycopersicum* L. grown at different soil Cd concentrations. Vertical bars represent ± SE of triplicates (n = 3).

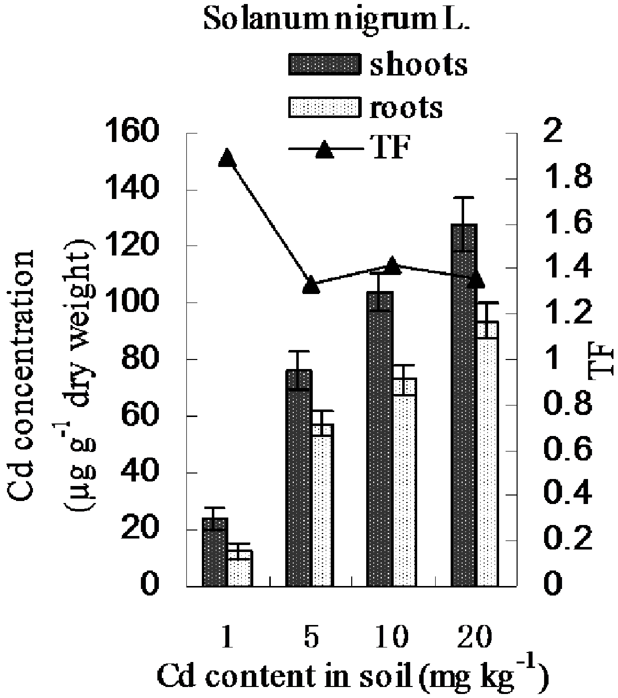

Figure 2 .TF and Cd accumulations in the shoot and root tissue of *S. nigrum* L. grown at different soil Cd concentrations. Vertical bars represent ± SE of triplicates (n = 3).

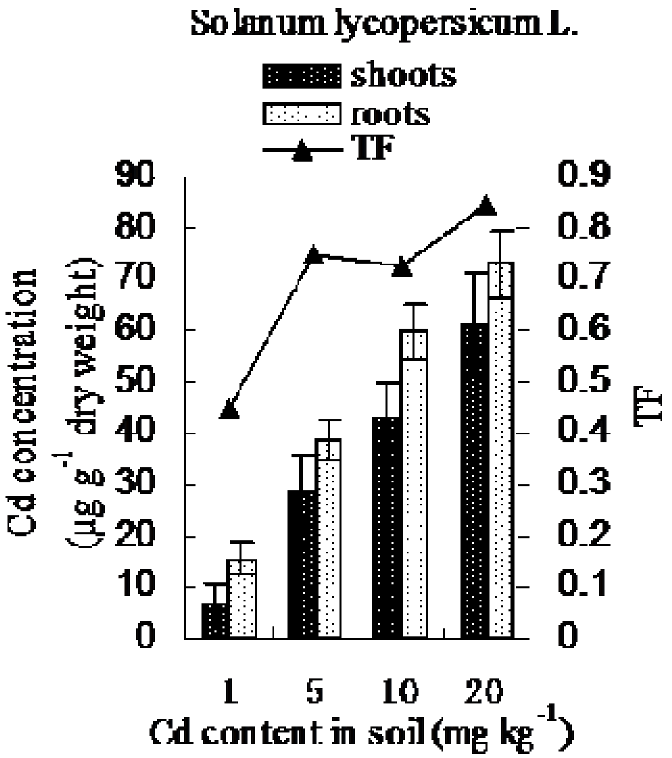

Figure 3. TF and Cd accumulations in the shoot and root tissue of *S. lycopersicum* L. grown at different soil Cd concentrations. Vertical bars represent ± SE of triplicates (n = 3).

this work, with increasing Cd concentration in the soils, the *S. nigrum* accumulated and translocated more Cd than *S. lycopersicum*. Simultaneously, *S. nigrum* significantly exuded more LMWOA *than S. lycopersicum* at each Cd treatment. These results indicate that the more LMWOA exuded by roots possibly are involved in the process of hyperaccumulation Cd by *S. nigrum*. An increase in Cr uptake from the Cr^{3+}-treated soils with increasing supplementation of organic acids may be ascribed to the interaction of Cr^{3+} with organic ligands leading to the formation of mobile organically-bound Cr^{3+} and highlights the interactions between Cr^{3+} and organic acid as a major contributor for Cr uptake by plants (Srivastava et al., 1999). Han et al. (2006) showed that the more Cd complexed with organic acids, the greater the enhanced Cd uptake. The rice cultivar with higher concentrations of LMWOA in soil accumulated more Cd in the plants (Liu et al., 2007). Cd accumulation by the high and low accumulating cultivars was proportional to the levels of LMWOA found in the rhizosphere soil of each cultivar (Cieslinski et al., 1998). In pot experiments, organic acids triggered uranium accumulation in Indian mustard and Chinese cabbage (Huang et al., 1998). Under hydroponic conditions, lanthanum uptake by barley, maize and wheat roots was enhanced in the presence of organic acids (Han et al., 2005). Organic acids can increase desorption of heavy metals from soil and consequently increase metal concentrations in the soil solution (Qin et al., 2004). Various LMWOA were able to influence the rate of Cd release from different soils and increase the solubility of Cd in bulk soil through the formation of soluble Cd–LMWOA complexes (Krishnamurti et al., 1997).

The bioavailability of heavy metals in soils is influenced by many factors, such as the cation exchange capacity, the organic matter content and especially the pH which has been regarded as a master variable regulating the mobility of metals (Lim et al., 2002). Root exudates can change the pH of the rhizosphere, provide ligands for metal complexation and facilitate microbial activity, which in turn enhance the concentrations of soluble metals in soil (Tao et al., 2004). In this study, the pH of soil solution of *S. nigrum* was significantly lower than *S. lycopersicum*, because large amount of LMWOAs were exudated in soil by *S. nigrum*. For example, organic ligands could induce changes in soil pH and solubilization of solid bound Cd may take place as pH decreases (Collins et al., 2003). Chen et al. (2000) indicated that the decrease of pH could increase the content of exchangeable Cd in soil, that would make Cd more bioavailable and easy to be phytoextracted by plants.

It can be concluded from our research that the differences between the hyperaccumulator *S. nigrum* and non-hyperaccumulator *S. lycopersicum* with regard to Cd uptake abilities may be related to their differences in the properties of root LMWOA secretion, especially in a soil Cd stress environment. The hyperaccumulator *S. nigrum* could secrete more LMWOA than *S. lycopersicum*, and therefore moved more Cd from the soil for root uptake. Moreover, the decrease in the soil solution pH of *S. nigrum* made Cd more bioavailable and easy to be phytoextracted.

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