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Chromium speciation modifies root exudation in two genotypes of *Silene vulgaris*

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ABSTRACT

The extensive use of Cr compounds in industry has led to the disposal of this element in soil and water, with consequent hazards to the environment and human health. Phytomanagement of Cr contaminated sites could be a sustainable alternative to obtain a profit from otherwise useless land. The objective of this work was to evaluate the influence of Cr speciation on root exudation in two genotypes of the metallophyte *Silene vulgaris*, which have shown different tolerance to Cr(VI). Plant growth has been related to oxidative stress by malondialdehyde (MDA) measures. No toxicity symptoms were observed at the applied dose of Cr(III), whereas biomass was significantly reduced by exposure to Cr(VI). Shoots have been found to be more sensitive to Cr stress than roots. Cr accumulation in plants was correlated to exudate release rates and induced changes in their composition. The genotype SV21 showed less biomass reduction and oxidative stress than the less tolerant SV38. The increment of the exudation rate in the genotype SV21 under Cr(VI) exposure suggested that exudation could be one of the mechanisms implicated in the tolerance of this species.

1. Introduction

Chromium (Cr) is the second most common metal contaminant in ground water, soil, and sediments due to its wide industrial and agricultural applications. Its toxicity and bioavailability depend strongly on its oxidation state, which ranges from 0 to VI. In the environment, Cr occurs primarily in two valence states, trivalent chromium (Cr III) and hexavalent chromium (Cr VI). The speciation is determined by the biogeochemistry of Cr in soil and water. In general, small concentrations of Cr(VI) can be the result of the natural oxidation of Cr(III), but larger concentrations are usually the result either of pollution with Cr(VI) or of the oxidation of Cr(III) by manganese oxides in ultramafic soils (Dhal et al., 2013). Cr(VI) is water-soluble and hence bioavailable, and the chromate and dichromate ions are fairly strong oxidising agents. Those characteristics make Cr(VI) between 10 and 100 times more toxic than Cr(III) (Deflora et al., 1990), and it is classified as a Group A carcinogen by the EPA (USEPA, 1998).

Phytomanagement could be considered a good alternative for the remediation of Cr-polluted soils because the most mobile forms of Cr in soils can be taken up by plants. This technology uses vegetation, alone or in combination with other technologies, to deliver the most cost-effective means of mitigating any environmental risks associated with the site (Robinson et al., 2009). To develop this technology, a deep knowledge of the relationships between pollutant and plant is required.

The study of plant-Cr has received little attention from researchers due to the lack of Cr functions in plants and of accurate analytical methods for determining the oxidation state of Cr (Shanker et al., 2009). For the last few years, interest in studying Cr as a pollutant has increased because of the development and implementation of phytotechnologies for soil remediation (Dhal et al., 2013; Singh et al., 2013; Choppala et al., 2013). One of the most useful methods for decontaminating Cr-polluted soils is the capacity of certain plants to reduce Cr toxicity by converting highly toxic and readily mobile Cr(VI) into less toxic and less mobile Cr(III) (Chaney et al., 1997). One related process concerns the role of root exudates. In response to Cr(VI) toxicity, plants can release organic compounds that maintain nonessential metals below their toxicity threshold and enhance the accumulation of Cr in the root.

The microbial activity and bacterial communities in soil are greatly influenced by root exudates due to the loss of carbon-containing metabolites from roots into the soil matrix as a result of rhizodeposition (Doornbos et al., 2012). In this way, the border cells

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and their exudates act as a defence barrier for the roots (Baetz and Martinoia, 2014). In the case of Cr, the organic compounds can form complexes with Cr, thereby making then available for uptake by the root (Hayat et al., 2012); also, microorganisms can use the organic compounds as substrates, resulting in increased microbial activity around the roots, which could affect the remediation effectiveness.

While the rates of exudation vary with species and environmental conditions (Lambers et al., 2009), the composition of root exudates depends on the plant species and cultivar, developmental stage, plant growth substrate and stress factor (Uren, 2000). Under Cr stress, changes in organic acid exudation and rhizosphere pH have been found in different genotypes of rice plants (Zeng et al., 2008). The metal speciation of Cr should affect the exudation rate and composition of the root exudates due to the differences in toxicity exhibited by the Cr species.

Silene vulgaris, the bladder campion, is a perennial dicotyledonous facultative metallophyte widely distributed throughout Europe, North America, Asia and North Africa. The tolerance of this species to a diversity of metals (Ernst and Nelissen, 2000; Jack et al., 2005; Paliouris and Hutchinson, 1991) makes it highly useful in the initial stages of revegetation and soil remediation. Previous studies (Pradas-del-Real et al., 2013) showed that Cr uptake in *S. vulgaris* was higher in the presence of Cr(VI) than of Cr(III). *S. vulgaris* presented high diversity at the genotypic level because treatment with hexavalent Cr increased the differences among genotypes. Thus, the use of cuttings from a homogeneous genotype seems to be an adequate method for the study of this species.

The objective of this work was to study whether root exudation in two genotypes of *S. vulgaris*, which show different tolerances to Cr, could change as a result of exposure to Cr(III) or Cr(VI) to use this species during soil remediation processes.

2. Material and methods

2.1. Plant material and growth condition

Two genotypes of *S. vulgaris* were chosen from two different populations of Madrid (Spain) according to their different response to Cr(VI) (Pradas-del-Real et al., 2013): (i) genotype SV21 (Rozas de Puerto Real; 200 μM < $EC_{100\,Cr(VI)}$ < 1200 μM) and (ii) genotype SV38 (Valdemaqueda; 200 μM < $EC_{100\,Cr(VI)}$ < 1000 μM). Clones from each genotype were vegetatively propagated in the field (Alcalá de Henares, Madrid, Spain) in a permanent 10 m × 10 m plot (divided into 1 m^2 quadrats). Cuttings of each genotype were collected and rooted in a mixture of peat for three months in a controlled research greenhouse. They were then thoroughly washed with MilliQ water and transferred into a hydroponic system (four cuttings of each genotype per tray with a total of 4 trays per treatment and genotype) with vermiculite and 1 L of half-strength modified Hoagland nutrient solution: (3 mM KNO_3; 2 mM Ca $(NO_3)_2{\cdot}4H_2O$; 1 mM $NH_4H_2PO_4$; 0.5 mM $MgSO_4{\cdot}7H_2O$; 50 μM NaCl; 25 μM H_3BO_3; 2 μM $ZnSO_4{\cdot}7H_2O$; 2 μM $MnSO_4{\cdot}H_2O$; 0.1 μM $CuSO_4{\cdot}5H_2O$; 0.5 μM $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$; 20 μM FeEDTA) into a phytotron chamber (photoperiod 16 h/8 h, temperature 20 °C/16 °C, 164.527 μmol photons/m^2 s^1). The pH of the solutions was buffered with 2 mM of MES and adjusted to 5.5 with KOH. Plants were acclimated for 2 weeks by a progressive increase of the nutrient solution concentration. Afterwards, plants were randomly selected for treatment as follows: (a) Control, no Cr addition; (b) 60 μM Cr(VI), (c) 300 μM Cr(VI) and (d) 300 μM Cr(III). Hexavalent chromium was provided as $K_2Cr_2O_7$ (ACS grade Aldrich) and trivalent chromium as $Cr(NO_3)_3{\cdot}9H_2O$ (RT grade Aldrich). The nutrient solution was replenished daily and completely changed every 3 days. Aliquots (20 mL) of the nutrient solution were collected before and after each change to check the pH and the oxidation state of Cr. The plants were treated for 12 days. The concentration of Cr(VI) in the nutrient solution was determined after each change by a UV–vis light spectrophotometer at 540 nm (Thermo Spectronic Helios Alpha) (EPA method 7196A) (USEPA, 1992). The total Cr concentration was measured in previously acidified samples of nutrient solution by atomic absorption spectrophotometry (VARIAN fast sequential, model AA240FS). The total concentration of Cr(III) was calculated by subtracting the concentration of Cr(VI) from the total Cr concentration.

2.2. Estimation of chemical speciation in nutrient solution

In silico estimations of the concentrations of Cr(III) and Cr(VI) ionic species in the different nutrient solutions were obtained using MINTEQA2 for Windows (version 3.0 visual basic.NET 2005 compiled by Jon Petter Gustafsson).

2.3. Collection and analysis of root exudates

After two weeks of Cr treatment, plants were dipped in 20 mM Na_2EDTA and thoroughly washed in autoclaved MilliQ H_2O. Then, each plant was placed in one sterile Falcon tube with 40 mL of autoclaved MilliQ water to collect root exudates (Tu et al., 2004; Quartaccia et al., 2009). After 24 h, the extracts of root exudates were filtered through sterile 0.20 μm PVC filters and immediately frozen in liquid N_2. The process was performed inside a laminar flow cabinet using standard axenic techniques. Four independent replicates were made per cutting, genotype and Cr dose (n = 4). The extracts were kept at −80 °C to be lyophilised in a Heto Drywinner 3. All freeze-dried root exudates were characterised by elemental analysis (Perkin-Elmer CHNS Analyser 240-III).

2.4. Plant analysis

After collection of the root exudates, the roots and shoots were separated and washed thoroughly with MilliQ water. The fresh weight was recorded, and subsamples of the shoots (2 g) and roots (3 g) were frozen in liquid nitrogen and stored at −80 °C. The rest of the plant tissues were dried in a forced air oven for 48 h at 70 °C. The dry weights were determined, and the samples (∼30 mg) were digested by adding 0.5 mL of HNO_3 (65% Suprapur®) and 0.5 mL $HClO_4$ (70%, Suprapur®) (Zhao et al., 1994). The samples were left to stand overnight and heated at 130 °C for 2 h. After cooling, the digest was diluted in 15 mL of MilliQ water. Total Cr concentrations were determined using an Atomic Absorption Spectrometer (Zeman AA2407) equipped with a graphite tube atomiser GTA 120. Tobacco leaves were used as certified reference materials (CTA-VL2, tobacco leaves). The recovery percentages were 109% in Cr.

2.5. Estimation of lipid peroxidation: malondialdehyde (MDA).

Lipid peroxidation was evaluated based on malondialdehyde (MDA) by the method of Reilly and Aust (2001) modified by Catala et al. (2010). Fresh samples (0.1 g) of shoots and roots were homogenised on ice with 1 mL of deionised water and centrifuged at 16,000 g for 10 min. The supernatants were removed, and the pellets were re-suspended in 500 μL of 0.01% butylated hydroxytoluene (BHT) in 80% ethanol. Then, 900 μL of TBA (2.57×10^{-2} M), TCA (9.18×10^{-1} M) and HCl (3.20 M) were added to each sample. Samples were vortexed, incubated in a water bath at 70 °C for 30 min, cooled on ice and then centrifuged at 16,000 g for 10 min. The absorbance of supernatants was measured at 532 nm. Absorbance at 600 nm was subtracted from this measure to eliminate the interference of soluble sugars in the samples. Absorbances