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Chromium toxicity tolerance of *Solanum nigrum* L. and *Parthenium hysterophorus* L. plants with reference to ion pattern, antioxidation activity and root exudation

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ABSTRACT

Chromium (Cr), being a highly toxic metal, adversely affects the mineral uptake and metabolic processes in plants when present in excess. The current study was aimed at investigating the Cr accumulation in various plant tissues and its relation to the antioxidation activity and root exudation. Plants were grown in soil spiked with different concentrations of Cr for three weeks in pots and analysed for different growth, antioxidants and ion attributes. Furthermore, plants treated with different concentrations of Cr in pots were shifted to rhizobox-like system for 48 h and organic acids were monitored in the mucilage dissolved from the plant root surface, mirroring rhizospheric solution. The results revealed that the Cr application at 1 mM increased the shoot fresh and dry weight and root dry weight of *Solanum nigrum*, whereas the opposite was observed for *Parthenium hysterophorus* when compared with lower levels of Cr (0.5 mM) or control treatment. In both plant species, Cr and Cl concentrations were increased while Ca, Mg and K concentrations in root, shoot and root exudates were decreased with increasing levels of Cr. Higher levels of Cr treatments enhanced the activities of SOD, POD and proline content in leaves of *S. nigrum*, whereas lower levels of Cr treatment were found to have stimulatory effects in *P. hysterophorus. P. hysterophorus* exhibited highest exudation of organic acid contents. With increasing levels of Cr treatments, citric acid concentration in root exudates increased by 35% and 44% in *S. nigrum*, whereas 20% and 76% in *P. hysterophorus*. Cr toxicity was responsible for the shoot growth reduction of S. nigrum and P. hysterophorus, however, shoot growth response was different at different levels of applied Cr. Consequently, Cr stress negatively altered the plant physiology and biochemistry. However, the enhanced antioxidant production, Cl uptake and root exudation are the physiological and biochemical indicators for the plant adaptations in biotic systems polluted with Cr.

1. Introduction

Environmental contamination with Cr due to industrial (Dixit et al., 2002) and anthropogenic activities (Zayed and Terry, 2003) is a serious concern in recent years, because global discharge of Cr exceeds than those of lead (Pb), mercury (Hg), and cadmium (Cd) (Kabata-Pendias and Mukherjee, 2007). Naturally, Cr exists in two oxidation states; the trivalent form [Cr (III)] and hexavalent form [Cr (VI)] that differs in terms of mobility, bioavailability and toxicity (Panda and Choudhury, 2005). Cr (III) is passively transported in plants (Zayed and Terry, 2003) either because of Cr (VI) that is reduced to Cr (III) as a result of redox/pH dependent reactions in the rhizospheric environment (Zeng et al., 2008) or due to its higher affinity to form complexes with naturally occurring organic compounds, i.e. organic anions (Srivastava et al., 1999).

Under Cr stress, plant roots excrete different organic acids as reported in *Oryza sativa* L. (Zeng et al., 2008). The organic acids may either stimulate the solubility or the immobility of heavy metals depending upon the type and concentration of organic acids, soil properties and other environmental factors (Ding et al., 2014). On the other hand, the released organic acids may protect the plant roots by limiting metal transport across the biological membranes due to metal ion complexes with organic anions (Kochian et al., 2004). Generally, the organic acids are released as anions and their release is balanced by the release of cations. Further, the metal stress may be coupled by the efflux of protons after impairing the H^+-ATPase pumps activities as shown in some plants, e.g. *Cucumis sativus* (Janicka-Russak et al., 2008). In this context, it is likely that metal exposure results in a defence mechanism of releasing organic anions from the plant roots. The anions ultimately consume

Abbreviations: AAS, atomic absorption spectrometer; HPLC, high performance liquid chromatography; EC, electrical conductivity; POD, peroxidase; SOD, superoxide dismutase

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protons, particularly, when the substrate pH is low. Consequently, Cr uptake by plants is modulated by the enhanced production of organic acids upon Cr exposure. However, the mechanism of Cr detoxification of these organic acids has not yet been fully elucidated in plants.

Numerous reports are available describing the effects of Cr toxicity on the nutritional ion imbalance in plants. For example, a reduction of Fe accumulation in *Brassica oleracea* (Pandey and Sharma, 2003), nutrient imbalance in *Spinacia oleracea* (Chatterjee and Chatterjee, 2000) and *Zea mays* (Mallick et al., 2010) has been reported under Cr stress. Additionally, Cr interferes with several plant metabolic processes, therefore, the mechanism of Cr toxicity in plants has been reported as the degradation of essential pigments, stunting and ultimately plant death (Gikas and Romanos, 2006). As well known that high Cr concentrations induce the formation of free radicals and reactive oxygen species (ROS) such as superoxide radicals ($O^{2-}\cdot$), hydrogen peroxide (H_2O_2.) and the hydroxyl radical ($OH^{-}\cdot$), however, the production of certain antioxidants minimised the damage caused by ROS (Maiti et al., 2012). Pandey and Sharma (2003) also reported that antioxidative enzyme system, nutrient and water balance was altered when plants were exposed to Cr stress.

Solanum nigrum L. and *Parthenium hysterophorus* L. are widely distributed wild plants in northern patches of Pakistan, particularly, in areas polluted with industrial effluents. Wild plants have been considered to withstand the heavy metal stresses, including Cr toxicity (Pradas-del-Real et al., 2013) and can be used for restoration of polluted soils through the root exudation. However, the physiological and biochemical modulations that confer adaptation to these wild plants in Cr contaminated environments have been poorly documented. Therefore, the current studies were designed to evaluate (a) the interference of Cr stress with the uptake of Cr and ion homoeostasis of these plants, and (b) to assess if the Cr stress is alleviated by the production of antioxidants in leaves and organic acids in root exudates of these plants. Our hypothesis was that Cr mainly sequestrated in the roots of *S. nigrum* and *P. hysterophorus*, leading to enhanced exudation of organic acids and better plant survival. A better understanding of the physiological adaptation and its dependence on plant root exudates may assist in appropriate utilisation of plant species for soil decontamination.

2. Materials and methods

2.1. Plant cultivation

A pot experiment was conducted in the wire house, botanical research area of Quaid-i-Azam University, Islamabad located between longitude 73.13°E and latitude 33.14°N. Seedlings of *S. nigrum L.* and *P. hysterophorus* L. were uprooted with soil substrate and transplanted into earthen pots (30×45 cm^2) containing 12 kg soil in each. The soil was silty loam having pH 7.5 and EC 3.2 dS/m. The experimental soil was spiked well with chromium as $CrCl_3$ to bring the final concentrations 0, 0.5 mM, and 1 mM and NPK fertilisers (75 kg N+50 kg P_2O_5+50 kg K_2O ha^{-1}) as urea, di-ammonium phosphate and muriate of potash, before filling the pots. Seedlings were exposed to the different Cr treatments using three replicates for each treatment and four seedlings for each replicate. Seedlings were maintained in pots for three weeks and watered to 60% field capacity, which was determined at the onset of the experiment. After three weeks, plants were harvested for physiological and biochemical attributes, whereas two plants were uprooted and shifted into rhizobox like systems for the collection of root exudates.

2.2. Root exudates collection

Step by step procedure for the collection of root exudates from *S. nigrum* and *P. hysterophorus* roots has been demonstrated in Fig. 1. In short, plants treated with Cr in pots were thoroughly

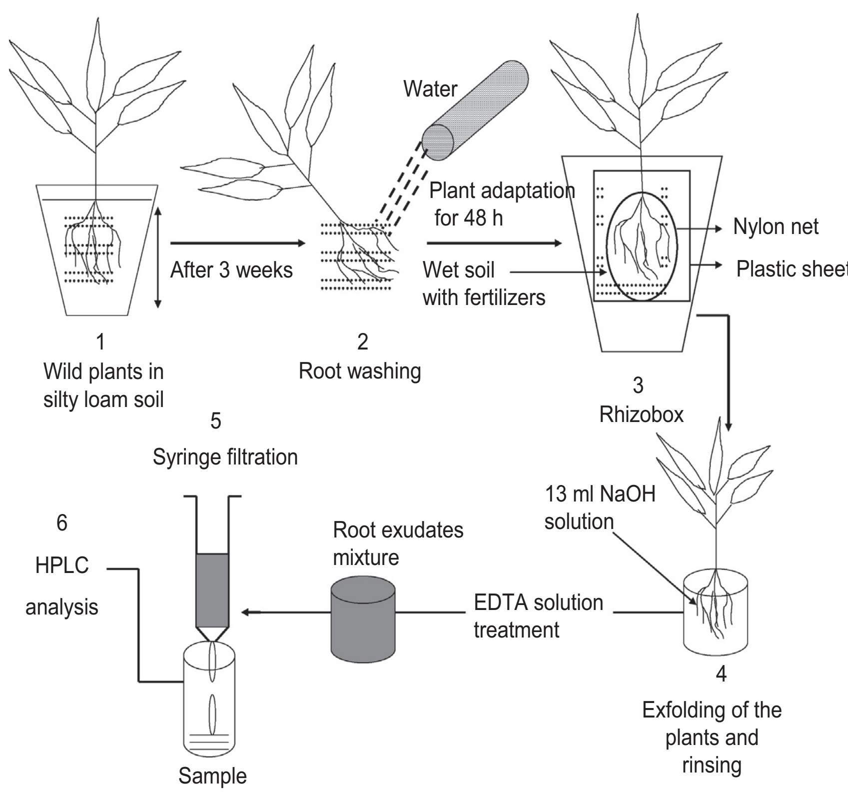

Fig. 1. Flow chart representation of steps used for root exudates collection and preparation of root exudates samples for HPLC analysis.