Characterization of Chromium Toxicity in Food Crops and their Role in Phytoremediation

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Abstract

This study evaluates and compares the remediation potential of rapeseed, soybean and wheat plants for Cr contamination. Seedlings of rapeseed (Brassica campestris cv Pusa Gold), soybean (Glycine max cv Pusa 20) and wheat (Triticum aestivum cv HD 2402) were grown hydroponically and subjected to four levels of Cr treatment (T0: Control, T1: 50 µM, T2: 100 µM and T3: 200 µM). The treated plants exhibited extreme variations in their defence behaviour against Cr stress. The extent of oxidative stress caused by Cr was measured by the rate of lipid peroxidation. Compared with the control, the level of thiobarbituric acid reactive substances (TBARS) was enhanced at all Cr treatments in all the crops studied, being the lowest in rapeseed. There was a multifold increase in the activities of antioxidant enzymes like superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and glutathione reductase (GR). Enzymatic activity, coupled with significantly high Cr accumulation, was maximum in rapeseed. Wheat and soybean on the contrary, did not show much modulation of their antioxidant defense system. Glutathione content showed significant increase with increase in Cr doses. The rapeseed was able to combat the metal-induced oxidative injury involving activation of enzymatic and non-enzymatic antioxidants, coupled with a high Cr accumulation, in both roots and shoots, thereby exhibiting superior Cr-remediating potential among the selected food crops.

Keywords: Antioxidant enzymes; Chromium; Food crops; Phytoremediation

Introduction

Chromium (Cr) is an environmental pollutant that ranks seventh in abundance in the earth crust. The major contributors of Cr contamination are the leather tanning, electroplating, and stainless steel industries. India is one of the largest producers of leather, and nearly 80% of the tanneries are engaged in the chrome-tanning process. Tanning industries in India release 2000 to 3200 MT of elemental Cr into the environment annually, with Cr concentration varying from 2000 to 5000 mg L⁻¹ of the effluent [1]. Heavy metals (HMs) pose a critical concern to human health due to their increasing usage as industrial inputs [2-3]. These metals can also influence the quality of atmosphere and surface water, and threaten the health of animals and humans upon entering the food chain [4]. Many different remediation methods have been tried to address the rising number of HM contaminated sites. Most of the traditional methods like excavation, solidification and land filling are extremely costly and disruptive in nature, cause adverse effect on biological activity and soil fertility [5]. Clean up of contaminated soils needs special attention because these soils support agricultural crops and so the consumption of crop plants grown on these soils may create serious health hazards.

Plants have been shown to sequester heavy metals in roots and/or shoots and, therefore, significantly contribute to metal removal from the environment through the mechanism of phytoremediation, a process that effects in situ risk reduction of contaminated soil, sludge, sediments and groundwater through removal, degradation or containment of the contaminant with the help of green plants [6-7]. For phytoextraction to be effective, plants must take up HM from the soil, tolerate high levels of plant or soil HM, and produce sufficient harvestable biomass [8-10]. Plants may show enhanced accumulation of metals in the wake of their ability to tolerate considerably high metal concentrations. Hypertolerance of plants to heavy metals is the key plant characteristic required for hyperaccumulation, and the hypertolerant capacity depends on an interrelated network of physiological and molecular mechanisms. One of the mechanisms that make a plant species tolerant to HM stress is the presence of strong antioxidant defense system [11-14].

As Cr is one of the most abundant HM pollutants in both aquatic and terrestrial environments, and rapeseed, wheat, and soybean serve as staple food for the majority of world population, it is desirable to study the effect of Cr toxicity in reference to the defence mechanism operational in these plants and evaluate their potential for Cr remediation. Moreover, in a country like India, faced with land crisis on one hand and burgeoning population on the other, cultivation of non-food crops merely for land remediation ends up as a futile practice. So, preference was given to food crops in choosing plants for phytoremediation and preparing them to counteract metal toxicities. The present study examines the Cr accumulation potential of rapeseed, soybean and wheat, and determines the Cr-induced induction of oxidative stress the consequent alterations in the behaviour of enzymes of antioxidant defense system in order to assess the suitability of these plant species with high annual biomass yield for phytoremediation of Cr-contaminated nutrient solution. The results thereby should clarify the potential of these food crops for phytoremediation and elucidation of varying degrees of defense mechanisms in these plants.

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Materials and Methods

Plant material

Seeds of rapeseed (Brassica campestris cv Pusa Gold), soybean (Glycine max cv Pusa 20) and wheat (Triticum aestivum cv HD 2402) procured from Indian Agricultural Research Institute, New Delhi, were sown in soilrite and kept for germination for four days. Four day old seedlings were transferred to 2.5 L plastic pots with nutrient solution with the following composition: 2 mMol/L Ca(NO₃)₂, 4H₂O, 1 mMol/L MgSO₄·7H₂O, 0.9 mMol/L K₂SO₄, 0.2 mMol/L KH₂PO₄, 10⁻⁵ mol/L H₂BO₃, 2x10⁻⁷ mol/L MnSO₄·H₂O, 10⁻⁶ mol/L ZnSO₄·7H₂O, 2x10⁻⁷ mol/L CaSO₄·SH₂O, 2x10⁻⁸ mol/L (NH₄)₂MoO₄·4H₂O and 10⁻⁶ mol/L C₆H₃FeN₂NaO₈ and allowed to grow in growth chambers under controlled environmental conditions (with 20°C in the light and 15°C in the dark, 16-h light/8-h dark photoperiod with a photon flux density of 380 μmol m⁻²s⁻¹ and 60% humidity). After growing in hydroponic culture for fourteen days, the seedlings were subjected to four levels of Cr treatments (T₀: Control, T₁: 50 μM, T₂: 100 μM and T₃: 200 μM) on the fifteenth day and analyzed for various biochemical parameters.

Size and fresh weight

Seedlings were collected and cut at the root-shoot junction and the lengths of their root and shoot were measured with a metric scale and expressed in centimeters. The fresh weight of seedling samples was recorded on an electronic top pan balance (Sartorius BL-210S, Germany) and expressed in g per plant.

Chromium content

The harvested plant material was rinsed thoroughly in Milli Q water, dried at 65°C ± 2°C for 72 h, and then ground to fine powder. About 0.25 g of dry material (root and shoot) from each treatment was added to a 3 mL of concentrated HNO₃ in a 50 mL digestion tube and mixed gently by swirling. The digestion tubes were placed in a heating block set at 150°C for 1 h. Two mL of a 30% H₂O₂ was added to each digestion tube after cooling. They were heated further for 3 hours at 150°C and then cooled to room temperature. Upon complete digestion of the plant tissue, the solution was diluted to 50 mL and the upper clear part was separated from the lower sand-grit portion. The upper portion was sampled for determination of Cr content with the help of atomic absorption spectrometer (Model ZEEnit 600/650, Analytik Jena, Germany).

Estimation of lipid peroxidation

The level of lipid peroxidation in the leaves was determined through estimation of malodealdehyde (MDA), a major thiobarbituric acid reactive substance (TBARS), by the method of [15]. MDA level was expressed in nmol g⁻¹ fresh weight.

Assay of antioxidant enzyme activities

Plant shoot (0.1g) was ground with a mortar and pestle under chilled conditions in a homogenization buffer (2ml) consisting of phosphate buffer (0.1 M, pH 7.5), and ethylene diamine tetraacetic acid (EDTA, 0.5 mM). The homogenate, filtered through four layers of muslin cloth, was centrifuged at 12,000 g for 10 min at 4°C. The resulting supernatant was used for the assay of different enzymes. Protein estimation was carried out using bovine serum albumin as the standard [16].

For estimation of SOD (EC 1.15.1.1) activity, the method of [17] was followed. Measurement of inhibition of photoreduction of nitroblue tetrazolium (NBT) was carried out at 560 nm, using UV-Vis spectrophotometer (Model λ-Bio-20, Perkin-Elmer, Germany). One unit of SOD was defined as the amount of SOD present in the volume of extract required to cause inhibition of photo-reduction of nitroblue tetrazolium (NBT) by 50%, and expressed in mg⁻¹ protein h⁻¹.

Activity of APX (EC 1.11.1.11) was measured on the basis of the rate of H₂O₂-dependent oxidation of ascorbate in a reaction mixture that contained 0.5 M phosphate buffer (pH 7.0), 0.5 mM ascorbic acid and enzyme extract [18]. The reaction was initiated by addition of 10 μL of 10% (v/v) H₂O₂ and the rate of ascorbic acid oxidation was estimated by following the decrease in absorbance at 290 nm for 3 min on a UV-Vis spectrophotometer (Model λ-Bio-20, Perkin-Elmer, Germany). APX activity was calculated by using the extinction coefficient (2.8 mM⁻¹ cm⁻¹) and expressed as enzyme units (mg protein)⁻¹. One unit of enzyme is the amount necessary to decompose one μmol H₂O₂ per min at 25°C.

Catalase (EC 1.11.1.6) activity was determined by the method of [19] based on the monitoring of disappearance of H₂O₂, carried out by measuring the decrease in absorbance at 240 nm. The reaction was carried in a reaction mixture containing 1.0 mL of 0.5M (pH 7.2) phosphate buffer, 3mM EDTA, 0.1 mL of the enzyme extract and 0.3% H₂O₂, and allowed to run for 3 min. The enzyme activity was calculated using the extinction coefficient 0.036 mM⁻¹ cm⁻¹. One enzyme unit (EU) denotes the amount of enzyme necessary to decompose one μmol of H₂O₂ per mg protein per min at 25°C and is expressed as EU mg⁻¹ protein.

Activity of GR (EC 1.6.4.2) was determined by the method of [20], modified by [21]. Fresh supernatant was used to assay the GR activity through glutathione-dependent oxidation of NADPH at 340 nm. One mL reaction mixture, containing 0.2 mM NADPH, 0.5 mM GSSG and 50 μL of enzyme extract, was run on a UV-Vis spectrophotometer (Model λ-Bio-20, Perkin-Elmer, Germany) for 5 min at 25°C. Corrections were made for any GSSG oxidation in the absence of NADPH. The activity was calculated by using the extinction coefficient of 6.2 mM⁻¹ cm⁻¹ and expressed in enzyme unit (mg protein)⁻¹. One unit of enzyme is the amount necessary to decompose one μmol of NADPH per min at 25°C.

Estimation of non-enzymatic antioxidant

Reduced glutathione (GSH) content was determined by the recycling method of [22]. Fresh leaf material (0.5 g) was homogenized in 3.0 ml of a 5% (w/v) sulfosalicylic acid under cold conditions and centrifuged at 10,000 rpm for 10 min. Half mL aliquot was taken in a microfuge tube, to which were added 0.5 ml reaction buffer [0.1 M phosphate buffer (pH 7.0), 3 mM ethylenediaminetetraacetic acid (Na₂EDTA) and 50 μL of 5’ dithio-bis-(2-nitrobenzoic acid). After 5 min, absorbance for determination of GSH was read at 412 nm using a UV-Vis spectrophotometer (Model λ Bio-20, Perkin-Elmer, Germany). The level of GSH was expressed in nmol g⁻¹ fresh weight.

Statistical Analysis

Each treatment was analyzed with three replicates (n=3). Statistical analysis of the data was done by two-way classification of ANOVA, using Instat software [23], to determine whether the means were significantly different (p<0.05).

Results

Chromium accumulation

Chromium accumulation in roots and shoot as well as the extent...
of plant-growth decline increased in all the three plant species with increase in Cr concentrations in the nutrient solution. The roots of all the three plant species accumulated more Cr than the shoots. The amount of Cr in roots of rapeseed and soybean (Table 1) at 200 μM at 7 DAT was 667 μg g⁻¹ DW and 266 μg g⁻¹ DW, which was much higher than in the shoots (Table 1). Rapeseed was more tolerant to Cr and had a higher Cr-accumulating ability than soybean and wheat. In the case of wheat, since the plant could not survive till 7 DAT (200 μM), no data on Cr accumulation were available.

**Lipid peroxidation**

The level of lipid peroxides, measured in terms of TBARS, increased in the seedlings of all the three species at 1-7 DAT with increase in the concentration of K₂Cr₂O₇ in the growth medium. The 200 μM treatment in rapeseed caused about 185% increase in MDA at 7 DAT, as compared with the control. A time-dependent increase was the major highlight in the APX activity of wheat over the control at 5 and 7 DAT with all the treatments (Table 3). On the contrary, there was a decline of 41-53% and 27-35% in the APX activity of wheat from 7-77% in soybean, increase was significant at 1, 3 and 5 DAT, but a significant decline of 26% and 48% occurred at 7 DAT with T2 and T3, respectively. In wheat, a significant enhancement (113-236%) was observed up to 5 DAT, followed by a decline at 7 DAT (77%) (Table 3). The APX activity increased with increasing doses of treatment in the seedlings of the rapeseed (Table 3). The percent increase was in the range of 42-82% and 28-55% at 1 and 3 DAT respectively, which was significant with respect to the control. At 5 and 7 DAT, the effect of Cr treatments was non-significant. In soybean also, activation of APX was observed, which became more pronounced with the passage of time (Table 3). On the contrary, there was a decline of 41-53% and 27-35% in the APX activity of wheat over the control at 5 and 7 DAT with all the treatments (Table 3). Catalase activity declined with time in all the species, with the effect being most evident in wheat (Table 4). In case of GR, rapid activation of defence response was observed in rapeseed, as an increase of 1-54, 9-51 and 15-50% was observed (Table 4). By way of contrast, a non-significant decline over the control was witnessed in treated samples at 7 DAT. Similarly, enhancement of GR activity was observed in soybean and wheat, though not at par with that in rapeseed (Table 4).

**Glutathione content**

With increasing doses of Cr, the GSH content in the rapeseed seedlings increased by 21-145%, 123-254%, 132-225% and 106-191% as monitored at 1, 3, 5 and 7 DAT, respectively. The percent increase was significant with respect to the control and within the treatments. The maximum increase figured with T3, followed by T2, as shown in (Table 5). The GSH content in the soybean seedlings also increased with the increase in Cr doses. It was found to be statistically significant when compared with the control and among the treatments (Table 5). A dose-dependent increase in the GSH content of wheat seedlings varied from 20-27% and...
Effect of chromium treatments on the SOD and APX activity (EU (mg protein)^{-1}) of the rapeseed (cv Pusa Gold), soybean (cv Pusa 20) and wheat (cv HD 2402) seedlings at various days after treatment (DAT).

Table 3: Data are mean ± SE, n = 3. Values denoted by similar letters are not significant. ND = Non-significant

Table 4: Data are mean ± SE, n = 3. Values denoted by similar letters are not significant. ND = Non-significant

Table 5: Data are mean ± SE, n = 3. Values denoted by similar letters are not significant. ND = Non-significant

Table 6: Root length increased in all the plants with age, however, reduced by 10-48%, at 1, 3, 5 DAT respectively, relative to the control at any DAT (Table 6). Similarly, fresh weight of wheat seedlings was also reduced by 3-53%, as compared with the control at 1 DAT. Chromium dose of 50 µM (T1) did not reduce fresh weight significantly at any DAT (Table 6). Similarly, fresh weight of wheat seedlings was also reduced by 10-48%, at 1, 3, 5 DAT respectively, relative to the control (Table 6). Root length increased in the plants with age, however, with increase in treatment doses, a gradual decline was evident. Cr treatments decreased the root-elongation rate by 2-34%, 2-58% and 2-71% in rapeseed, soybean and wheat respectively (Table 6). The shoot length of the rapeseed seedlings also declined, with reference to the control, by 22-29%, 10-21%, 8-27% and 20-32% as observed at 1, 3, 5 and 7 DAT, respectively (Table 6). In soybean seedlings, shoot length increased with age of the seedlings. At 7 DAT, a decline of 8-47% was evident, showing the maximum decline with 200 µM Cr dose (Table 6). Similarly, Cr doses resulted in shortening of shoot length in wheat, with the maximum drop at highest Cr treatment in wheat.

Discussion

With the perspective of assessing differential remediation potential of three food crops and their differential levels of tolerance for Cr, it was revealed that rapeseed responded as a more Cr tolerant crop.
Table 6: Effect of chromium treatments on the fresh weight, root length and shoot length of the rapeseed (cv Pusa Gold), soybean (cv Pusa 20) and wheat (cv HD 2402) seedlings at various days after treatment (DAT).

<table>
<thead>
<tr>
<th>Days After Treatment (DAT)</th>
<th>Rapeseed</th>
<th>Soybean</th>
<th>Wheat</th>
<th>Rapeseed</th>
<th>Soybean</th>
<th>Wheat</th>
<th>Rapeseed</th>
<th>Soybean</th>
<th>Wheat</th>
<th>Rapeseed</th>
<th>Soybean</th>
<th>Wheat</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 DAT</td>
<td>0.97±0.12a</td>
<td>13.18±0.13a</td>
<td>9.49±0.019a</td>
<td>9.20±0.019a</td>
<td>9.31±0.019a</td>
<td>9.00±0.019a</td>
<td>15.31±0.037a</td>
<td>13.97±0.033a</td>
<td>20.24±0.026a</td>
<td>20.21±0.026a</td>
<td>20.37±0.026a</td>
<td>21.47±0.026a</td>
</tr>
<tr>
<td>3 DAT</td>
<td>0.97±0.12a</td>
<td>13.18±0.13a</td>
<td>9.49±0.019a</td>
<td>9.20±0.019a</td>
<td>9.31±0.019a</td>
<td>9.00±0.019a</td>
<td>15.31±0.037a</td>
<td>13.97±0.033a</td>
<td>20.24±0.026a</td>
<td>20.21±0.026a</td>
<td>20.37±0.026a</td>
<td>21.47±0.026a</td>
</tr>
<tr>
<td>5 DAT</td>
<td>0.97±0.12a</td>
<td>13.18±0.13a</td>
<td>9.49±0.019a</td>
<td>9.20±0.019a</td>
<td>9.31±0.019a</td>
<td>9.00±0.019a</td>
<td>15.31±0.037a</td>
<td>13.97±0.033a</td>
<td>20.24±0.026a</td>
<td>20.21±0.026a</td>
<td>20.37±0.026a</td>
<td>21.47±0.026a</td>
</tr>
<tr>
<td>7 DAT</td>
<td>0.97±0.12a</td>
<td>13.18±0.13a</td>
<td>9.49±0.019a</td>
<td>9.20±0.019a</td>
<td>9.31±0.019a</td>
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<td>20.37±0.026a</td>
<td>21.47±0.026a</td>
</tr>
</tbody>
</table>

Data are mean ± SE, n = 3. Values denoted by similar letters are not significant. ND = Non-significant.
organelles to cytosol may have an adverse effect due to formation of hydroxyl radicals through the metal-catalysed Haber–Weiss reaction. Increased activities of APX and GR in Cr-treated plants show that they were functioning concurrently to remove $H_2O_2$. The decline in catalase activity observed by us may be due to inhibition of enzyme synthesis or a change in assembly of enzyme subunits.

Glutathione (GSH) is a major non-protein thiol in plants, which plays a pivotal role in protecting plants from environmental stress. As an antioxidant, GSH together with ascorbate and antioxidative enzymes (SOD, APX and CAT) controls the cellular concentration of $H_2O_2$. It showed a concentration- and time-dependent increase in its level in the Cr-exposed plants. The elevated GSH concentration is correlated with the ability of plants to withstand a metal-induced oxidative stress [32]. Active participation of GSH in detoxification of oxygen species and free radicals has been elucidated by [13,32,33], among others.

The toxic effect of Cr was also evident from the reduced plant growth and fresh weight. We have found variability among rapeseed, soybean and wheat in their response to the exposure to various levels and durations of Cr treatment. In rapeseed and wheat, reduction in the fresh weight (FW) of plants was significant. Soybean showed tolerance to 50 µM Cr (T1) at all the sampling days. In similar earlier studies, very little or no effect of Cr has been reported on biomass accumulation in plants like Oryza sativa [34], Raphanus sativa, Zea mays [35] and Phaseolus vulgaris [36]. It has been argued that the decline in the wheat weight might be due to increased tissue permeability and tissue loss [37]. It was also observed that as the cellular concentration of Cr metal increased, plant growth was affected despite increase in the activity of the enzymes because Cr is known to interact with certain micronutrients essential for plant growth and limit their availability by the decreased uptake or immobilization in the roots. Cr in the soil affects root growth adversely, depending on its concentration and the nature of plant species. In our experiment, the reduction in the root length depended on metal concentration and plant species. In rapeseed, soybean and wheat, 100 µM and 200 µM of Cr reduced the root length significantly. The reduction in root length could be due to the accumulation of high HM concentrations in roots, and/or a non-existence of any defined HM-translocation mechanism, thereby enhancing the HM sequestration in the tissue and, thus, inhibiting the root development [38]. Shoot development is affected by chromium differentially. We noticed that Cr concentrations and exposure durations caused reduction of shoot length in all the species studied. The absence of any significant effect on shoot development with low Cr concentrations suggests that these concentrations served as the threshold of plant tolerance, up to which the plant effectively defended itself from the metal toxicity. However, beyond the threshold level, Cr became deleterious for plant growth [39]. The reduction at higher Cr applications could also be due to impaired root growth leading to a reduced uptake of essential nutrients and water and the consequent impact on the cellular metabolism of shoot. It was also observed that as the cellular concentration of Cr increased, plant growth was affected because Cr is known to interact with certain micronutrients essential for plant growth and limit their availability through decreased uptake or immobilization in roots. Remediation potential of rapeseed has an edge over that of soybean and wheat, as highlighted by its defence behaviour based on modulation of its enzymatic activities and Cr-accumulating tendency in response to Cr toxicity. Post-phytoremediation, the plants can be incinerated and the heavy metal can be retrieved from the ash, thereby leading to phytomining and metal recycling.

**Conclusion**

Reclamation of soils contaminated with high concentrations of heavy metals requires induction of environment-friendly remediation technologies such as phytoremediation. This exercise requires plant species with good accumulation capacity and remediation capability. A comparison of three widely grown food crops with different Cr-accumulation ability has established superior hyperaccumulation and hypertolerating capacities of rapeseed over soybean and wheat on the basis of its antioxidant defence system characterized by high enzymatic activities and high Cr-accumulating and tolerating potential. These results suggest a new role of food crops in modern technological context for phytoremediation of metal-contaminated soil.

**References**