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Full Length Research Paper

Comparison of antioxidant enzyme activities in leaves stems and roots of Sorghum (*Sorghum bicolor* L.) exposed to Chromium (VI)

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This study was conducted to determine the response between different organs to assess which one was more severely affected. Thus, we exposed sorghum (*Sorghum bicolor* L.) to five level 0, 30, 60, 100 and 130 mg/L concentration of chromium (Cr) VI during two weeks and measuring the activity of glutathione-s-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPX), catalase (CAT), total glutathione (GSG) and lipid peroxidation (LPO) in upper, middle and lower leaves, stems and roots of sorghum plants. Reduction in dry matter of roots, stem and leaves was noticed in Cr (VI) stress. GST and Cr (VI) activities were induced in all organs of the exposed plant, but was inhibited in 130 mgL^{-1} Cr (VI). GPX and CAT activities were induced in all the organs at all studied concentrations, whereas inhibited was observed in the roots and higher leaves above 100 and 130 mgL^{-1}. Significant increases in lipid peroxidation in plants exposed to 100 and 130 mgL^{-1}.These results suggest that in roots and upper leaf of sorghum the tolerance to Cr (VI) toxicity is more dependent on the activities of GST and GR than other enzyme activities. Hyperactivity of the GR and GST indicated that these enzymes played an important role in protecting the sorghum from Cr (VI) toxicity. However, GPX took a little part in detoxification of Cr (VI). Furthermore, the conjugation of GSH with Cr (VI) ions by GST helped them to sequester into vacuole. GR participates in the GSG biosynthesis and might build up a reduced form GSG against poisoning of the Cr (VI). This might be a part of defense strategy adapted by *S. bicolor* against chromium toxicity to protect themselves. We find that the roots and upper leaves, often considered as a first biomarker for Cr (VI) treated plants organ compared to the other organs. A higher level of LPO was correlated with lower CAT and GPX activities. Therefore, hyperactivities of GST and GR in roots and upper leaves might be attributed to the strategies adopted by *S. bicolor* to overcome the toxicity of the Cr (VI).

Key words: Hyperactivity, GST, CAT, GR. activities and *Sorghum bicolor*, LPO.

INTRODUCTION

A high level of chromium (Cr) accumulation in soils and waters poses a threat to normal functions of plants and human health (Kimbrough et al., 1999). Cr is a hazardous heavy metal which interferes with several metabolic processes, causing toxicity to the plants as exhibited by reduced growth and chlorosis, impaired photosynthesis, stunting and finally plant death (Vajpayee et al., 2000). However, the plants have developed a very potential mechanism to combat with such adverse environmental heavy metal toxicity problems (Sinha et al., 2002). Yadav et al. (2009) showed that Cr could be translocated from the roots to the leaves.

The increased chlorophyll and carotenoid concentrations caused by exposure to small concentrations of Cr and have been reported iron deficiency in *Ocimum tenuiflorum* (Vartika et al., 2004). Due to Cr application the activity of ironporphyrin enzyme catalase was improved in bean plants (Samantary et al., 2002).

The Cr exposure decreased the activity of catalase which coincides with the findings of Panda et al. (2003), who reported depression in catalase activity due to borderline elements. Panda et al. (2003) found improved activity of catalase due to Cr in leaves of wheat.

However, the presence of Cr at higher concentrations retarded growth and development of plants, by interfering with certain important metabolic processes. Whether Cr has any role in plant metabolism is still ambiguous (Gardea-Torresdey et al., 2004).

In plants, glutathione (GSH) is involved in cellular processes, including defense against reactive oxygen species (ROS). Glutathione exists in two form reduced glutathione (GSH) and oxidized glutathione (GSSG) . The reduction potential of glutathione depends on the intracellular GSH/GSSG ratio (Pekker et al., 2002). Change in the redox ratio of glutathione mainly depends on the pH, total GSH concentration, GSH biosynthesis and GSH catabolism. Ammonium ion, derived either from nitrogen assimilation or from photorespiration, is incorporated into glutamine by a reaction catalyzed by glutamine synthase (GS), and glutamine is further converted into glutamate catalyzed by glutamate synthase (GOGAT). The conjugation of GSH with such molecules is governed by glutathione S-transferase (GST). GST catalyze the conjugation of GSH with metal ions and help them to sequester into vacuole (Shanker et al., 2004; Hossein et al., 2006).

The enzymatic mechanism of detoxification involves GPX, GR and other enzymes. The oxidized glutathione (GSSG) formed is converted back to GSH by NAD(P)H-dependent glutathione reductase (GR) glutathione peroxidation (GPX) and its regenerating cycle (Shanker et al., 2004). Moreover, Sies (1999) provided evidence that GR is the rate limiting enzyme in the ascorbate dependent H_2O_2 destruction. One of the most deleterious effects induced by heavy metal exposure in plants is lipid peroxidation, which can directly cause biomembrane deterioration. Malondialdehyde (MDA), one of the decomposition products of polyunsaturated fatty acids of membrane, is regarded as a reliable indicator of oxidative stress (Dey et al., 2007).

Although the oxidative response of leaves has been well studied, organ response is quite unexplored in the plants (Scandalios et al., 2002). The coordination among enzymatic activities in organs might be different from that of leaves, even though these organs share almost the different enzymatic machinery (Panda et al., 2005). Thus, little is known about the integration and functioning of organs and the antioxidative apparatus, especially under oxidative stress conditions induced by Cr (VI).

Sorghum (*Sorghum bicolor* L.) serves as staple food for the majority of world population and its productivity is drastically limited under metal stress. In this work, we have studied different parts (examle, leaves, stems and roots) of the plant enzymatic antioxidant system (GST, CAT, GR and GPX activities) associated with GSG and lipid peroxidation from sorghum organs induced by different Cr level. The possible mechanisms of effectiveness and protection against the Cr toxicity by enzymatic action in each organ are different and extending our knowledge about the antioxidant process especially in different organs could provide information regarding the regulation of this process.

MATERIALS AND METHODS

Plant material

The experiments were carried out between 22 May and 13 July, 2009 outdoor under natural daylight in the University of Bu Ali Sina Hamadan. During growth season the temperature ranged between 20±5°C. Sorghum (S*. bicolor* L.) seed cultivar Munch (132) was obtained from seed research center of Isfahan. The seeds were sterilized for 20 min in a 10% sodium hypochlorite solution. Seeds of sorghum were cultivated in cycle pots with surface of 1015 cm^2 and depth of 60 cm.

The medium culture was river sand and peat in 3:1 ratio, respectively. Various concentrations (0, 30, 60, 100 and 130 (mg/L) of Cr (VI), were prepared by diluting 1000**/**xm stock solution of $K_2Cr_2O_7$ - $7H_2O$ using 5% Hoagland solution. The Hoagland's nutrient solution was added to each pot once in two week.

Sample preparation

The seedlings were cultivated for the next 72 h under five concentrations of Cr (VI). After 3 days the medium was replaced by a normal condition and the plant samples were harvested 50 days after sowing. The plant material was frozen in liquid nitrogen and stored at -80**°**C or directly used in determination. Extraction for enzyme activities was carried out as following, the 500 mg, different parts such as leaves, stem and roots were homogenized in 1 ml of 100 mmol/L chilled potassium phosphate buffer (pH 7.0) containing 0.1 mmol/L EDTA and 1% polyvinyl pyrrolidone (PVP, w/v) at 4°C. The homogenate was centrifuged at 15.000×g for 15 min. at 4°C. The supernatant was used to measure the activities of GST, CAT, GPX and GR were assayed at 25°C in a final reaction volume of 0 to 6 ml. In all assays the blank consisted of the components of the reaction mixture except for the enzyme extract, which was replaced by an equal volume of the assay buffer. In the remaining cases the enzyme blanks were subtracted from the assay measurements.

MDA content and antioxidants

The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) content by the thio-barbituric acid (TBA) reaction (Buege and Aust, 1978), 500 mg of material was homogenized in 5 ml of 0.1% TCA. The homogenate was centrifuged at 10,000×g for 5 min. For every 1 ml of aliquot, 4 ml of 20% TCA containing 0.5% thiobarbituric acid was added. Mixture was heated at 95°C for 30 min. and then cooled quickly on ice bath. The resulting mixture was centrifuged at 10.000 ×g for 15 min and the absorbance of the supernatant was taken at 532 and 600 nm. The non-specific absorbance at 600 nm was subtracted from the absorbance at 532 nm. The concentration of MDA was calculated by using the extinction coefficient of 155 mM^{-1} cm^{-1}.

Glutathione reductase GR (EC 1.6.4.2)

Estimation of GR was done according to Smith et al. (1988), following the decrease in absorbance at 340 nm due to NADPH oxidation *(*E = 6.2 mM^{-1} cm^{-1}). The reaction mixture consisted of 25 mM phosphate buffer (pH 7.8), 0.5 mM oxidized glutathione (GSSG), 0.12 mM NADPH and enzyme extracts. The enzyme activ-ity was measured in terms of µmol of NADPH, H^+ oxidized min^{-1} g^{-1}

fresh weight at 25+2°C. In the case of the GR assay, an additional blank without oxidized glutathione was included in order to account for the presence in the extracts of other enzyme activities able to oxidize NADPH.

Catalase CAT (EC 1.11.1.6)

The CAT activity was measured by the method of Chance and Maehly (1955) with minor modifications. The assay system comprised of 50 mM sodium phosphate buffer (pH 7.0), 20 mM H_2O_2 and a suitable aliquot of enzyme in the final volume of 3 ml. Decrease in the absorbance was taken at 240 nm. The molar extinction coefficient of H_2O_2 at 240 nm was taken as 0.04 cm^2/ mol. Enzyme activity was expressed as moles of H_2O_2 degraded min^{-1} g^{-1} fw.

Glutathione peroxidase GPX (EC 1.11.1.9)

The GPX activity was also determined spectrophotometrically according to the method of Aebi and Bergmeyer (1983). Prepare a reaction by pipetting (in ml) the 9.20 Sodium Azide Solution, 0.1 GPX Solution and glutathione into ß- NADPH vial. Mix by inversion and adjust to pH 7.0 at 25°C with 1 M HCl or 1 M NaOH, if necessary. In a 3.05 ml reaction mixture, final concentrations are 48 mM sodium phosphate, 0.38 mM ethylenediaminetetraacetic acid, 0.12 mM ß-NADPH, 0.95 mM sodium azide, 3.2 units of glutathione peroxidase, 1 mM glutathione, 0.02 mM DL-dithiothreitol, 0.0007% (w/w) hydrogen peroxide and 0.075 to 0.15 unit of glutathione. Monitor the $A340_{nm}$ until constant, for approximately 5 min.. Obtain the AA 340_{nm}/min. using the maximum linear rate for both the Test and Blank.

Glutathione S-transferase GST (EC2.5.1.18)

The GST activity was determined spectrophotometrically according to the method of Aebi and Bergmeyer (1983). One gram plant samples were extracted in 5 ml medium containing 50 mM phosphate buffer, pH 7.5, 1 mM EDTA and 1 mM DTT. The enzyme activity was assayed in a reaction mixture containing 50 mM phosphate buffer, pH 7.5, 1 mM 1-chloro-2, 4-dinitrobenzene (CDNB). The reaction was initiated by the addition of 1 mM GSH and formation of S-(2, 4-dinitrophenyl) glutathione (DNP-GS) was monitored an increase in absorbance at 340 nm to calculate the GST specific activity.

Glutathione concentration (GSG)

The glutathione concentration was measured by the method of Shi and Dalal (1989) . Plant material (500 mg each of leaves and roots) frozen in liquid nitrogen, was homogenized in 0.1 M sodium phosphate buffer (pH 8.0) containing 25% meta-phosphoric acid. The homogenate was centrifuged at 20.000 × g for 20 min. at 4°C and total glutathione (GSSG and GSG) content was determined fluorometrically. In the supernatant after 15 min. incubation with o-phthaldialdehyde (OPT). Fluorescence intensity was recorded at 420 nm after excitation at 350 nm on a Perkin-Elmer LS 55 micro plate reader.

Cr uptake

Harvested plants were washed thoroughly with distilled water for total metal accumulation. The oven dried (80°C) plant organs (leaves, stem and roots) of treated and control plants were digested in HNO3:HClO4 (3:1, v/v) mixture at 80°C. Chromium concentration was determined using a Flame Atomic Absorption Spectrophotometer (Perkin-Elmer 2380).

Statistical analysis

The data of plant biomass, metal accumulation and enzymes activity under different Cr treatments were analyzed using the SPSS 15 software program and by two-way analysis of variance (ANOVA) to compare treatments with control and between means of the different treatments. All values are given the mean of four replicates ± standard deviation.

RESULTS AND DISCUSSION

In the present study, sorghum (S.*bicolor* L.) plants were exposed to five concentration of Cr (VI) on the medium culture. The amount of Cr (VI) accumulated by different plant organs (roots > leaves> stem) varied significantly (ANOVA, $p<0.05$) (Table 2). Roots accumulated maximum amount (463 gg^{-1} DW) of Cr (VI) when exposed to 130 mgL^{-1} Cr (VI) for 72 h. The maximum Cr (VI) (343 gg^{-1} DW) content in lower leaves was also recorded at same concentration. The accumulation of Cr(VI) in the leaves (upper, middle and lower), stem and roots at various concentrations was presented in Table 2, which increased in concentration ($p<0.01$) dependent manner. The analysis of the results revealed that the primary site of Cr(VI) accumulation was the root and the amount of Cr (VI) translocated into the leaves was found less by the order of magnitude (upper leaves <stem<middle leaves<lower leaves<roots) . The lower leaves and roots accumulated more Cr (VI) than the upper leaves and stems in all treatments. Comparatively lower accumulation of Cr (VI) in upper leaves and stem than roots and lower leaves were probably due to reduction of Cr (VI) to Cr (III), which reduces its mobility from roots and lower leaves to upper and middle leaves. It has been reported that Cr (III) readily forms complexes with polyphenols which inhibits the translocation of metal from roots and lower leaves to upper leaves (Yadav et al., 2009). Another important reason for the lack of transport of Cr (VI) from roots to stems could be because the plants lack any specific mechanism of transport of Cr (VI), as it is a toxic and nonessential element to plant growth (Yadav et al., 2009; Zayed et al., 1998). We observed that, In comparison with the control a significant decrease in DW leaves, stem and roots was observed at 60, 100 and 130 mg/L^{-1}, Cr (VI) (Table 1). The decrease in DW of upper, middle leaves and roots was higher than other organs (ANOVA, $P < 0.05$).

Compared to control, concentration of MDA was found increased in all the Cr (VI) concentrations in the roots, stem and leaves of the Cr (VI) treated plants, indicating enhanced lipid peroxidation (Figure 5). Maximum increase of 110% at 130 mgL^{-1} after 72 h in the roots and 72, 64 and 60% after 72 h in the (upper>middle >lower) leaves was observed (Figure 5) . Dey et al. (2007) and Panda et al. (2003) also reported similar findings showing

Table 1. The effects of Cr on leaves, stems and roots dry weight of sorghum grown on different concentrations of Cr. Data are means SD of four replicates. Within the same treatment, mean values followed by different letters (*, ** and ***) are significantly different ($p<0.05$).

Concentration (mgl^{-1})	($g.g^{-1}$ dw)				
	Root	Stem	Upper leaf	Middle leaf	Lower leaf
0	1.5	1.86	0.74	0.83	0.94
30	1.42	1.79	0.78	0.8	0.96
60	1.2***	1.94	0.64*	0.71*	0.91
100	0.92***	1.25***	0.43**	0.58***	0.82*
130	0.75**	1.03**	0.32***	0.45***	0.7***

All values are means of four replicates ±SD. LSD ($p<0.01$): Cr= concentration; roots = 0.08, stem = 0.19, upper leaves = 0.02, middle leaf = 0.03, lower leaf = 0.03 Significant ($p<0.01$) compared to control.

Table 2. Accumulation of Cr ($g.g^{-1}$ dw) in the examined organs of sorghum at different concentrations.

Concentration (mgl^{-1})	($g.g^{-1}$ dw)				
	Root	Stem	Upper leaf	Middle leaf	Lower leaf
0	5	2	2	-	3
30	79*	23	13	25*	42***
60	184***	42*	17	38	79*
100	358***	117***	53**	85***	158***
130	463**	163**	124***	174***	343***

All values are means of four replicates ±SD. LSD ($p < 0.01$): Cr= concentration; roots = 73.20, stem= 36, upper leaves = 25.3, middle leaf= 25, lower leaf=34 Significant ($p < 0.01$) compared to control.

increase in MDA in the metal treated plants.

Amongst various enzymes involved in the antioxidant metabolism of ROS, the GST, CAT and GR activities was found higher at all the Cr (VI) concentrations than their controls (Figures 2, 1 and 3). The order of magnitude is lower leaves, stems, middle leaves, upper leaves and roots, respectively.

The GST and GR activities in all the examined organs of the plant increased with higher Cr (VI) concentrations at 72 h of exposure than its control (Figures 2 and 3). However, it increased up to 100 and 130 mgL^{-1} Cr (VI), respectively, followed by decrease as compared to their respective controls. The maximum decrease of 60% was found in GST activity in the upper leaves. Similar findings were also reported in previous studies (Radotic et al., 2000; Pekker et al., 2002). The GPX activity in the roots was found higher at 30 and 60 mgL^{-1} Cr (VI) at 72 h, compared to their respective controls and negatively correlated with Cr (VI) accumulation (Figure 4) . Therefore, reduced GPX activity in Cr (VI) treated other plants was recorded (Shanker et al., 2004; Vajpayee et al., 2002). This reflects the sensitivity of the enzyme to the Cr(VI). Further, Cr(VI) treated plants yielded lower glutathione (a major component of GR and GPX activities) in comparison to the control (Figure 6). The decomposition of O_2 is always accompanied by production of H_2O_2, which rapidly diffuses across the membrane and is toxic as it acts both as an oxidant as well as a reductant (Vartika et al., 2004; Toppi et al., 2002). H_2O_2 can be scavenged in the cell either by CAT or GPX. CAT is a universally present oxido reductase that decomposes H_2O_2 to water and molecular oxygen and CAT and GPX are the key enzymes involved in the removal of toxic peroxides (Shanker et al., 2004; Samantary et al., 2002). A decline in CAT activity under Cr (VI) toxicity was observed in the present study the enzyme activity was also increased from 66 to 130% in 30 and 60 mgl^{-1} Cr (VI) (Figure 1). However, it increased up to 60 and 100, 130 mgL^{-1} Cr(VI) at 72 h, respectively, followed by decrease as compared to their respective controls. The maximum decrease of 50% was found in CAT activity in the upper leaves at 72 h as compared to their respective 60 mgL^{-1} Cr (VI). The results are in confirmation with the report of excessive levels of H_2O_2 minimized through the activities of CAT and GPX. Similar decrease in CAT activity was also reported in Fe, Cu and Cd toxicity (Rai et al., 2002; Panda et al., 2005; Noctor and Foyer, 1998).

The GPX activity in all the organs was found higher at 30 and 60 mgL^{-1}, the concentrations of Cr (VI) at 72 h, compared to their respective controls and negatively correlated with Cr (VI) accumulation (Figure 4) . Therefore, reduced GPX activity in Cr (VI) treated other plants was recorded (Rai et al., 2002; Panda et al., 2005). Therefore, Cr treated plants yielded lower glutathione (a major

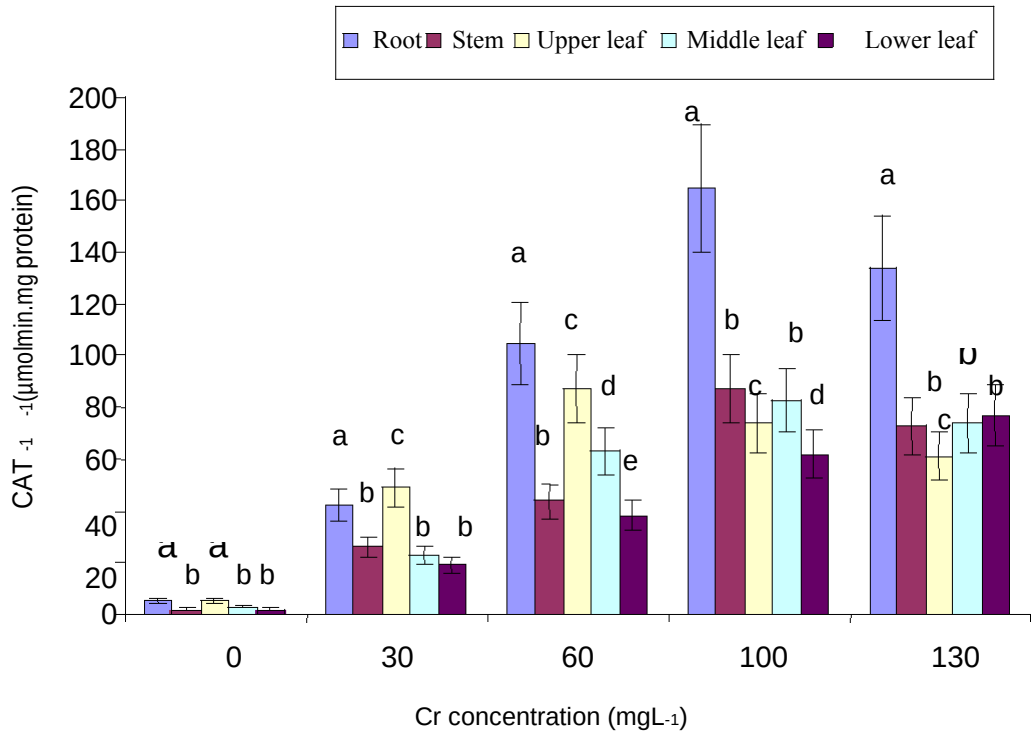

Figure 1. Effects of Cr on activity of CAT (µmol min^{-1}. mg^{-1} protein) in the organs of sorghum plants grown in medium solution containing different concentration Cr. data are means ± SD of four replicates. Within the same treatment concentration, mean values followed by different letters (a, b and c) are significantly different (P< 0.05).

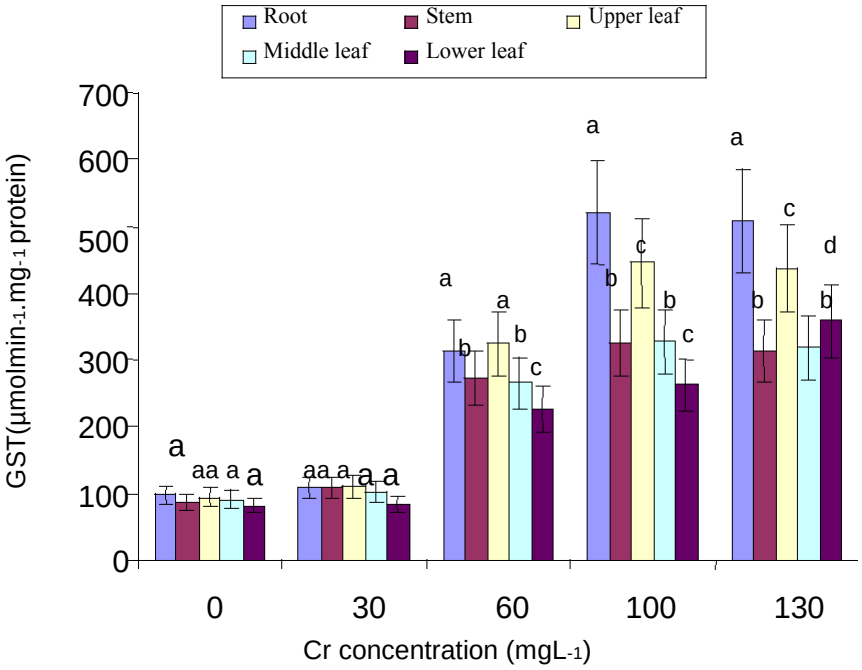

Figure 2. Effects of Cr on activity of GST (µmol $^{-1}$ min^{-1}. mg^{-1}, protein) in different organs of sorghum plants grown in medium solution containing different concentration of Cr. data are means ± SD of four replicates. Within the same treatment concentration, mean values followed by different letters (a, b and c) are significantly different (P< 0.05).

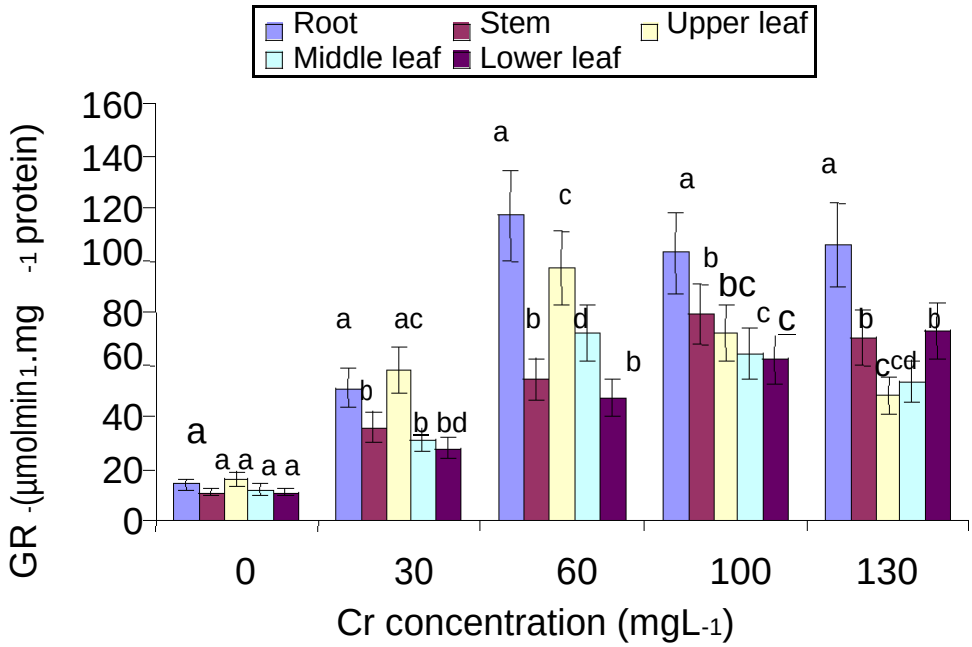

Figure 3. Effects of Cr on activity of GR (µmol^{-1} min^{-1} mg^{-1}, protein) in the organs of sorghum plants grown in medium solution containing different concentration Cr. data are means ± SD of four replicates. Within the same treatment concentration, mean values followed by different letters (a, b and c) are significantly different (P< 0.05).

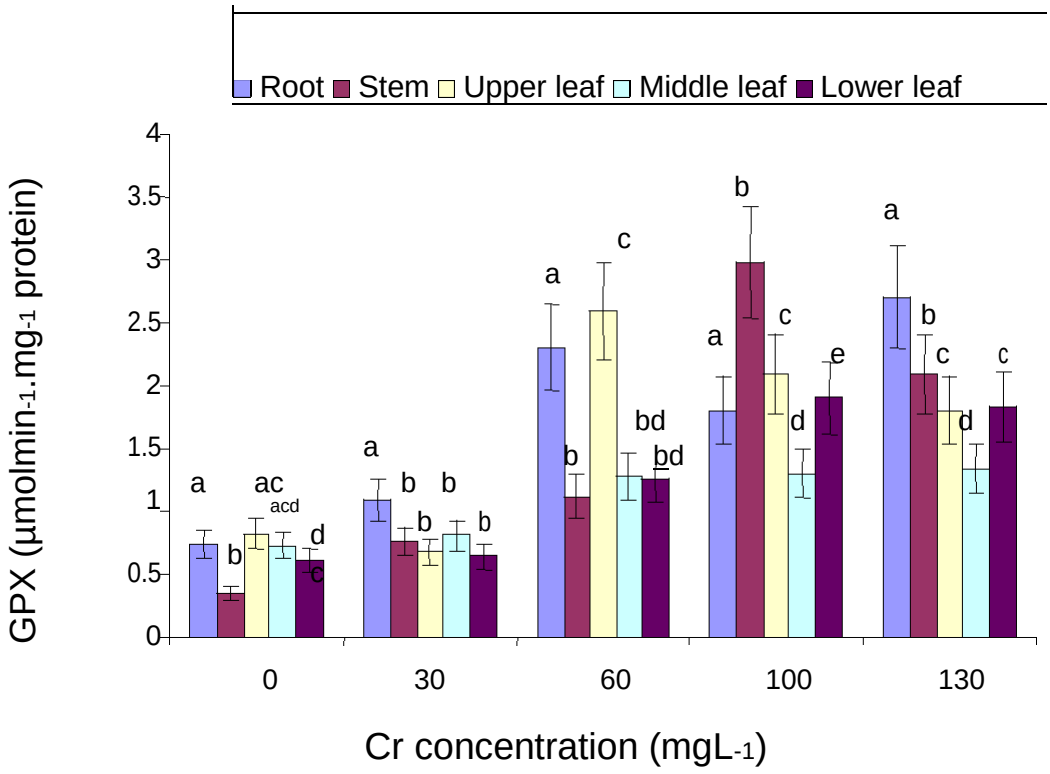

Figure 4. Effects of Cr on activity of GPX (µmol^{-1} min^{-1}mg^{-1}, protein) in the organs of sorghum plants grown in medium solution containing different concentrations of Cr. data are means ± SD of four replicates. Within the same treatment concentration, mean values followed by different letters (a, b and c) are significantly different (P< 0.05).

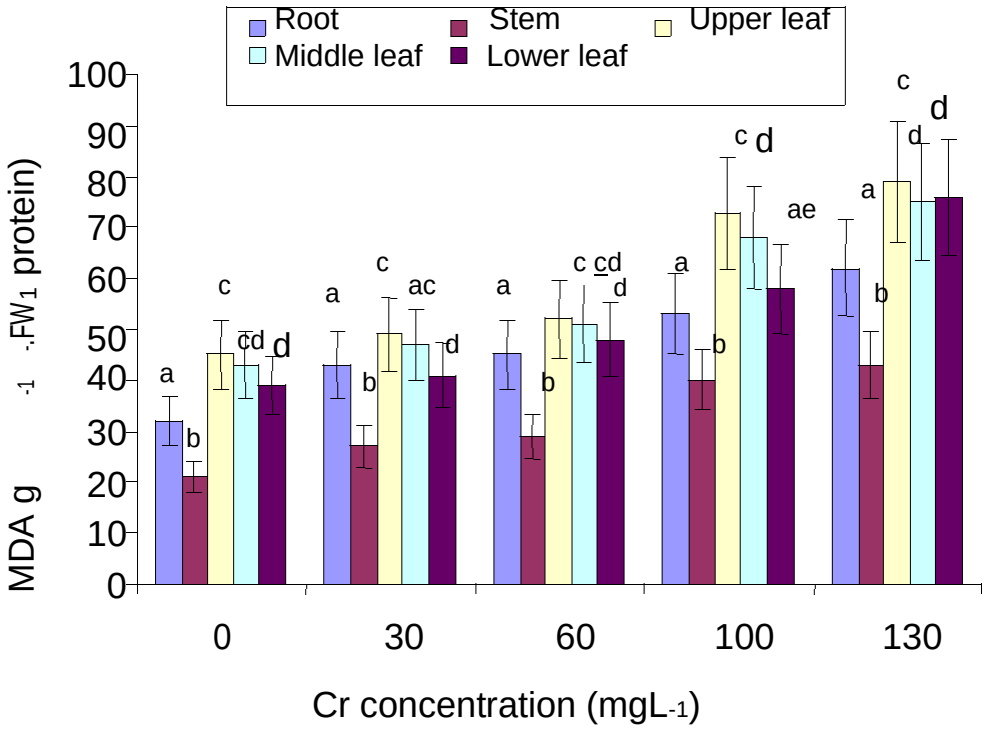

Figure 5. Effects of Cr on concentration of MDA (mol g^{-1} FW^{-1}) in the organs of sorghum plants grown in medium solution containing different concentration of Cr. data are means ± SD of four replicates. Within the same treatment concentration, mean values followed by different letters (a, b and c) are significantly different (P< 0.05).

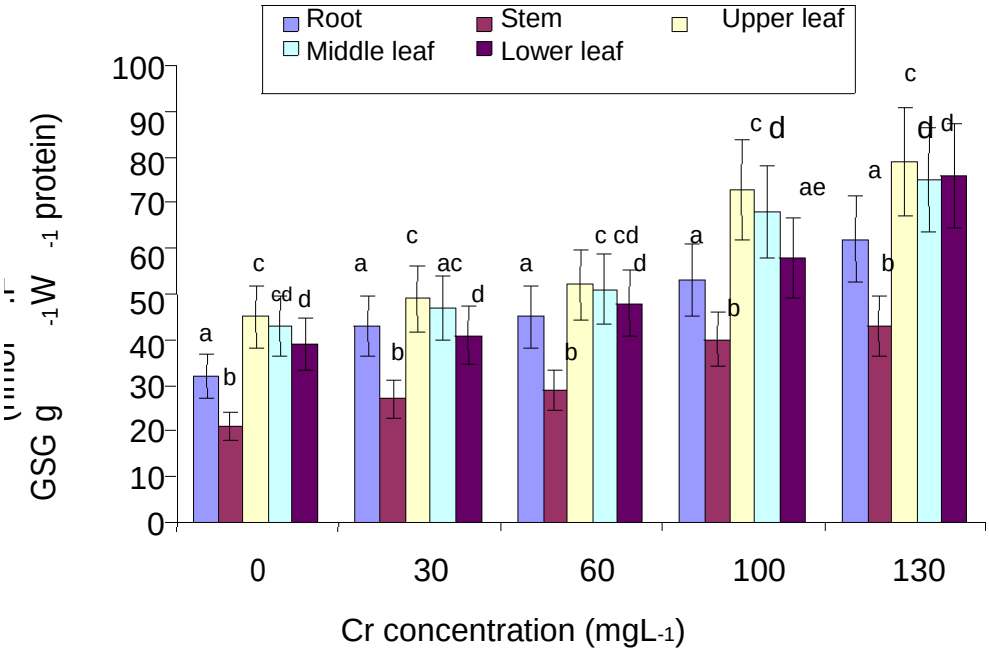

Figure 6. Effects of Cr on concentration of GSG (µmol g^{-1}. FW^{-1}) in the organs of sorghum plants grown in medium solution containing different concentration of Cr. data are means ± SD of four replicates. Within the same treatment concentration, mean values followed by different letters (a, b and c) are significantly different (P< 0.05).

component of GR and GPX activities) in comparison to the control (Pekker et al., 2002). In plants, GPX plays an important role in scavenging H_2O_2; however under excess Cr (VI) stress, its function becomes more complex. In previous research we found a close relationship between the oxidation of Al^{3+} in the presence of GPX and phenols (Malmir et al., 2009). The GPX catalyzed formation of polyphenols, which are responsible for the browning of the leaves, is accompanied by the formation of phenoxy radicals (Vartika et al., 2004; Hossain et al., 2006). In the present study, excess Cr (VI) caused a significant elevation in GPX activity (Figure 4), than the Cr (VI) supply significantly decreased GPX activity. This reduction of GPX activity may be related to the increased of formation MDA and therefore the toxicity were inhibited (Rock et al., 2001). The GST and GR activities in all the organs of the plant increased with higher Cr concentrations at 72 h of exposure than its control. However, it increased up to 100 mgl^{-1} and 130 mgl^{-1} Cr (VI), respectively, followed by decrease as compared to their respective controls. The order of magnitude is lower leaves, stems, middle leaves, upper leaves and roots, respectively (Figures 2 and 3).

In the process of Ascorbate-glutathione cycle, GPX plays a most important role in removing H_2O_2, and GR can provide substrate for GPX by catalyzing reaction (Shanker et al., 2004). In the present study, activities of GST and GR were increased in sorghum plants when exposed to excess Cr (VI) (Figures 2 and 3), suggesting the important role of GST in the detoxification of Cr(VI). GR could keep higher substrate for GST by increasing activity, as reported in Cr-stressed *Pisum sativum* (Dixit et al., 2002; De Vos et al., 1992). In the present study, GSH concentrations decreased greatly in sorghum organs treated with excess Cr(VI) (Figure 6), and similar results have been obtained from Cu-stressed *Convolvulus arvensis* (Gardea-Torresdey et al., 2004). GSG is oxidized to GSSG during the conversion of dehydrate ascorbic acid to ascorbic acid, which depends on GR to reform GSH by oxidizing NAD(P)H to NAD(P) (Foyer et al., 1991; Gardea-Torresdey et al., 2004). These results suggest that in roots and upper leaves of sorghum plants the tolerance to Cr (VI) toxicity is more dependent on the activities of GST and GR than other enzyme activities. Hyperactivity of the GR and GST indicated that these enzymes played an important role in protecting the sorghum from Cr (VI) toxicity. Exposure to high concentration of Cr (VI) caused GPX and CAT inhibition and increase of GST and GR activities in upper and middle leaves and roots (Shanker et al., 2004). However, GPG took a little part in detoxification of Cr (VI). Furthermore, the conjugation of GSH with Cr (VI) ions by GST helps them to sequester into vacuole. GR participates in the GSG biosynthesis and might build up a reduced form GSG against poisoning of the Cr (VI). This might be a part of defense strategy adapted by sorghum against chromium toxicity to protect themselves. A higher level of LPO was correlated with lower CAT and GPX activities. Vartika et al. (2004) and Hossain et al. (2006) also reported similar findings. Therefore, hyperactivities of GST and GR in roots and upper leaves might be attributed to the strategies adopted by sorghum to overcome the toxicity of the chromium.

Conclusions

The present study concluded that sorghum (*S.bicolor* L.) could grow in Cr (VI) contaminated soil and accumulate Cr (VI) in roots followed by other organs. Cr (VI)-induced oxidative stress was tolerated by this plant through the hyperactivity of GST and Cr (VI) system. The antioxidant system in roots and higher leaves was the least altered compared with the other organs. This effect could be related to the route of exposure of this organ to Cr (VI). The upper and middle leaves were the most sensitive organ to oxidative damage; particularly LPO levels were high in upper leaves. We suggest that GPG took a little part in detoxification of Cr (VI). Furthermore, the conjugation of GSH with Cr (VI) ions by GST helps them to sequester into vacuole. GR participates in the GSG biosynthesis and might build up a reduced form GSG against poisoning of the Cr (VI). The upper leaves in sorghum could be effectively used as bioindicator to evaluate the pollution with Cr (VI).

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