Responses of the maize plant to chromium stress with reference to antioxidation activity

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ABSTRACT

Two maize varieties, viz. Sartaj and Deccan, have been evaluated according to their responses to chromium (Cr) in terms of specific physiological and biochemical attributes. During the exposure to Cr concentration (0, 50, 100, 200 and 300 µM) of potassium dichromate ($K_2Cr_2O_7$) for seven days, significant changes of oxidative stress in terms of lipid peroxidation and protein oxidation were recorded along with other antioxidative pathways. It is revealed that irrespective of varieties, Sartaj was more prone to oxidative stress since the amount of superoxide due to Cr exposure was found to be significant, unlike the second variety, Deccan. In addition, for Sartaj the activity of NAD(P)H oxidase recorded a significant linear increase in a dose response manner. The changes of antioxidative enzymes recorded significant variation in both varieties under stress compared to control. However, Deccan maintains a consistent up regulation in SOD and GPX activities. Contrarily, APX activity was less sensitive to both varieties. The activation of antioxidative mechanisms due to the impact of Cr-induced oxidative stress has been described subsequently. The results deriving from this investigation might be effective for the selection of tolerant plant types under chromium contamination.

Keywords: antioxidative enzymes, MDA, protein oxidation, ROS.

Abbreviations: APX: Ascorbate peroxidase, AsA: Ascorbic acid, CAT: Catalase, DTT: Dithiothreitol, DNPH: Dinitrophenylhydrazine, GPX: Guaiacol peroxidase, MDA: Malondialdehyde, PMSF: Phenylmercuric sulfonyl fluoride, PVP: Polyvinylpyrrolidone, PVPP: Polyvinylpolypyrrolidone, ROS: Reactive Oxygen Species, SOD: Superoxide dismutase.

INTRODUCTION

Chromium (Cr) is the 21st most abundant element in the Earth's crust with an average concentration of 100 ppm (Emsley, 2001). It is a transition metal located in group VI B of the periodic table. Mainly due to tanning and chrome-plating industrial activities, large quantities of Cr compounds are discharged as liquid, solid and gas waste into the environment, and ultimately have significant adverse biological and ecologic effects (Kotas and Stasicka, 2000). Cr occurs in the environment especially in two valence states: trivalent Cr [Cr (III)] and hexavalent Cr [Cr (VI)]. These two forms display quite different chemical properties in terms of mobility, bioavailability and toxicity (Panda and Choudhury, 2005). Of these two forms, Cr (VI) shows more phytotoxicity when absorbed by plants (Panda, 2007). Cr interferes with several metabolic processes and its toxicity to plants is exhibited by reducing seed germination, stunted seedling and inadequate accumulation of phytomass. Subsequently, this aggravates the further degradation of pigments, stunting and, finally, plant death (Vajpayee et

al., 2000; Gikas and Romanos, 2006; Zou et al., 2006). Being a heavy metal, Cr is a potential agent of oxidative stress, inducing oxidative damage to lipids, proteins and DNA-like biomolecules (Shanker et al., 2004). Cr changes in activity of antioxidant enzymes as well as of other enzymes in order to disrupt the nutrients and water balance (Pandey and Sharma, 2003). It is also reported that it could stimulate the formation of free radicals and reactive oxygen species (ROS) such as superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($\cdot OH$). Furthermore, under natural growth conditions, plants are adapted to minimize the damages induced by ROS (Jinhua et al., 2009). In response to such abiotic stress, plants are also tuned to mitigate the oxidative stress by number of ways. Non-enzymatic and enzymatic anti-oxidation cascades are common defense mechanisms as upregulated under such metal induced oxidative stress. This defense system is composed of antioxidant enzymes: superoxide dismutase (SOD), peroxidase with different variants (e.g., ascorbate peroxidase, guaiacol peroxidase and glutathione reductase), catalase (CAT). Some other non-enzymatic antioxidant moieties (e.g., ascorbic acid, glutathione, carotenoids, flavonoids, α-tocopherol etc.) are also important to quench the ROS developed under such condition (Gratão et al., 2005).

Maize (*Zea mays* L.), a typical cereal crop of temperate and subtropical zones is displayed as a variable stress tolerant plant under environmental extremities. By dint of the C_4 advantages, maize is able to thrive well under the condition of soil moisture deficit coupled with high illumination inducing oxidative stress. Besides, maize plants have been reported to hyperaccumulate a number of heavy metals and Cr is one of them (Shanker et al., 2005).

Maize varieties have been reported with the illustration of wider adaptability to various abiotic stresses; however, in the present experiment the varieties Sartaj and Deccan have earlier been clarified with its potential for salinity stress (Uma et al., 1990). Since salinity stress is most integrally associated with oxidative damage, the way maize plants are to sensitize under heavy metal stress might be justified to observe. With this background, Deccan and Sartaj were investigated under the condition of heavy metals, e.g. Cr, as in the present case. Cr has already been mentioned to cause oxidative induction to change the cellular responses in many crop species. Therefore, maize being a potential practiser of C_4 photosynthetic pathway, having to overcome the induction of oxidative stress under heavy metal contamination and how these two varieties could be discriminated to accomplish the antioxidation pathway are our focus. The effects of Cr and subsequent antioxidative responses to different concentrations (0, 50, 100, 200 and 300 µM) applied to two maize varieties (viz, Sartaj and Deccan) are investigated. We describe the effects of different concentrations of Cr on growth attributes, generation of O_2^- and H_2O_2, membrane lipid peroxidation, protein carbamylation along with the changes in activities of enzymatic antioxidation pathway, including SOD, guaiacol peroxidase (GPX) and ascorbate peroxidase (APX).

MATERIAL AND METHODS

Plant culture and chromium treatments: In order to fulfill the aforementioned objectives, an experiment was conducted in the laboratory of Plant Physiology and Plant Molecular Biology, Department of Botany, University of Kalyani, West Bengal, India. Seeds of two maize varieties (namely, Sartaj and Deccan) were collected from Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, West Bengal, India. Seeds were soaked in 0.1% mercuric chloride ($HgCl_2$) for 15 minutes, rinsed twice with distilled water, transferred to Petri dishes and kept for germination at 30ºC for 5 days. Germinated seedlings (their ages being recorded from the day of radical emergence) of uniform appearance were selected and grown in plastic pots, supplemented with Hoagland's nutrient medium (Hoagland and Arnon, 1950). Afterwards, the seedlings were able to grow for 10 days in the open air in order to attain their full growth under natural conditions at around 30/25°C during day/night (photo period = 13/11h light/dark) and 70–75% relative humidity (RH) in the month of March. It is already reported that Cr (VI) is hexavalent Cr (in which oxidation state +6 could easily be obtained from any salts of chromate and dichromate). Moreover, chromate of any alkali metal, like Na or K, when undergoing aqueous solution, releases Cr (VI) (Zou et al., 2009). Therefore, in the present experiment we used potassium dichromate ($K_2Cr_2O_7$, analytical reagent, E-MARK) as a source of Cr (VI). From the stock solution of $K_2Cr_2O_7$, a series of dilution (0, 50, 100, 200 and 300 µM) was made in deionized water and supplemented with full strength Hoagland's nutrition solution. Plants were kept at each concentration with ten seedlings for seven days, and thereafter destructive sampling was done. For each experiment, equal amounts of fresh tissues with three replications were taken both from control and treated plants and following parameters were measured under identical laboratory conditions.

Measurement of growth and biomass: Growth was measured in terms of shoot and root length along with their dry biomass. Shoot and root length were measured following the harvest in fresh samples. For dry biomass, 2 g of shoot and root tissue for each sample were dried at 80ºC for 48h and made complete dry until constant weights achieved.

Estimation of reactive oxygen species: superoxide and hydrogen peroxide: To detect the generation of $O_2^{.-}$, the method was adopted from Elstner and Heupel (1976), with some modification. One gram of leaf sample was thoroughly homogenized with 65 mM phosphate buffer (pH 7.8) and supernatant was saved following centrifugation at 5,000 g_n, for 15 min, at 4°C. The assay mixture (2 mL) consisting of 0.9 mL of 65 mM phosphate buffer (pH 7.8), 0.1 mL of 10 mM of hydroxylamine hydrochloride and 1.0 mL of supernatant was incubated at 25°C for 30 minutes. 1 mL of 17 mM sulphanilamide and 1.0 mL of 7.0 mM α-naphthylamine were added and incubated at 25°C for 20 minutes. The changes in absorbance against a reagent blank were recorded spectrophotometrically at 530 nm and generated $O_2^{.-}$ was calculated from a standard curve of nitrite salt.

H_2O_2 content was determined (Sun et al., 2006). Approximately 1.0 g leaf sample was crushed with liquid nitrogen followed by extraction of 0.1% (w/v) TCA. The supernatant was centrifuged at 12,000 g_n for 20 minutes at 4°C. The assay mixture (consisting of 0.5 mL supernatant, 1 mL of 1.0 M KI and 0.5 mL of 10 mM potassium phosphate buffer, pH 7.0) was prepared. The absorbance of supernatant was read at 390 nm. The content of H_2O_2 was calculated from a standard curve.

Lipid peroxidation: For the lipid peroxidation, malondialdehyde (MDA) content was determined. Leaf homogenate was extracted with 20% (w/v) trichloroacetic acid (TCA) containing 0.5% (w/v) thiobarbituric acid (TBA). MDA content was determined by reading the absorbance at 532 nm and corrected for nonspecific absorbance at 600 nm (Heath and Packer, 1968).

Protein oxidation assay: For protein oxidation, protein carbonyl content was determined. It was measured by the reaction with di-nitro-phenyl-hydrazine (DNPH) (Verbeke et al., 2000). The leaf homogenate was extracted with 6% (w/v) SDS followed by 25 minutes incubation at room temperature (4°C). Afterwards, 10 mM DNPH in 10% TCA, being incubated with gentle shaking for 40 minutes. Precipitation of protein was done by 15% (w/v) TCA on ice for 15 min. The samples were centrifuged at 10,000 g_n and

pellets were washed twice with 20% (w/v) TCA. Finally, the protein pellets were resuspended in 0.2 M sodium phosphate buffer (pH 7.0) and the absorbance was measured at 360 nm.

Determination of the NAD(P)H oxidase activity: Leaf homogenate was extracted by 50 mM potassium phosphate buffer (pH 7.0) and centrifuged at 10,000 g_n at 4°C for 20 minutes (Smiri et al., 2010). Finally, NAD(P) H oxidase activity was determined in an assay mixture containing 100 mM sodium acetate (pH 6.5), 1 mM $MnCl_2$, 0.5 mM p-coumaric acid and 50 μg of protein (enzyme extract). Protein content was determined with BSA as standard (Bradford, 1976). The control set was done, and p-coumaric acid was withdrawn in the same assay mixture. The reaction was initiated by adding 0.2 mM NAD(P)H in the same assay mixture and incubated at 37ºC for 5 minutes. The activity of NAD(P)H oxidase was recorded at A_{340nm}. The enzyme activity was calculated from change of OD per g of protein with extinction coefficient of 0.622 $mM^{-1} mm^{-1}$.

Assay of guaiacol peroxidase (EC 1.11.1.7): The enzyme extract was prepared by thoroughly homogenizing the leaf tissues (1.0 g) in liquid nitrogen followed by 0.1 M potassium phosphate buffer (pH 7.0) under cold condition. The homogenate was centrifuged at 12,000 g_n at 4°C for 15 min. The protein content of the supernatant was concentrated using 80% ammonium sulphate $[(NH_4)_2SO_4]$ precipitation followed by dialysis and lyophilization (Sambrook and Russell, 2001). The protein was measured according to Bradford (1976). The concentrated protein samples were used as sources of enzyme and incubated in an assay mixture containing 0.1 M phosphate buffer (pH 6.5), 1.5 mM O-dianicidine, 0.2 M H_2O_2, 50 μg of protein at 37°C. The absorbance was recorded at 430 nm using extinction coefficient of O-dianicidine (2.62 $mM^{-1}mm^{-1}$). One unit of enzyme activity was determined as the amount of enzyme required changing the absorbance by 0.1 per unit time (Hu et al., 2009).

Assay of ascorbate peroxidase (EC 1.11.1.11): APX activity was determined as described by De Leonardis et al. (2000), by measuring the decrease in absorbance at 290 nm in a reaction mixture containing 50 mM phosphate buffer pH 7.0, 500 mM AsA, 1 mM H_2O_2 and 100 μg of enzyme extract. The extinction coefficient of AsA (2.62 $mM^{-1}mm^{-1}$) was used to calculate the APX activity.

Assay of superoxide dismutase (EC 1.15.1.1): SOD activity was assayed spectrophotometrically

according to Alonso et al. (2001). Plant samples were homogenized with 50 mM potassium-phosphate buffer (pH 7.5) and then centrifuged at 20,000 g_n for 30 minutes, at 4°C. Supernatant was added to a reaction mixture containing 50 mM phosphate buffer (pH 7.0), 13 mM methionine, 75 mM NBT, 100 mM EDTA and 2 mM riboflavin. The total mixture was kept for 10 min under 15-W fluorescent tubes of 40 µmol m^{-2} s^{-1} photon flux density. The control set of reaction mixture was kept in the dark. The activity was calculated from the absorbance at 560 nm.

Statistical analysis: All the observations were recorded with three replications (n=3) and data were expressed as mean±SE. The statistical analysis was performed with SPSS 10 statistical package (SPSS Inc., Chicago) by one-way ANOVA, taking p≤0.05 as significant followed by least significance difference (LSD) test taking p≤0.05 levels of significance (Gomez and Gomez, 1984).

RESULTS

Plant growth: The oxidative exposure of Cr and its impact were revealed in the present study for both the varieties. Plants were found to be morphologically weaker in relation to growth and vigor. However, to complete the experiment plants were collected for measurement of length and dry biomass of shoot and root. Cr exposure significantly inhibited (p≤0.05) plant length and biomass (Figure 1). A linear decrease of shoot length (Figure 1A) and dry biomass (Figure 1B) were recorded at different concentrations of Cr. Shoot length was reduced by 37.60 and 19.57% for varieties Sartaj and Deccan, respectively, at maximum concentration of Cr, i.e. 300 µM. Shoot biomass also decreased by 59 and 53% for Sartaj and Deccan at the same maximum concentration. Root length (Figure 1C) and its dry weight (Figure 1D) followed the similar trends of decreasing growth as that recorded in shoot. Root length was decreased by 36% and 21% in Sartaj and Deccan. Likewise, the loss of root biomass
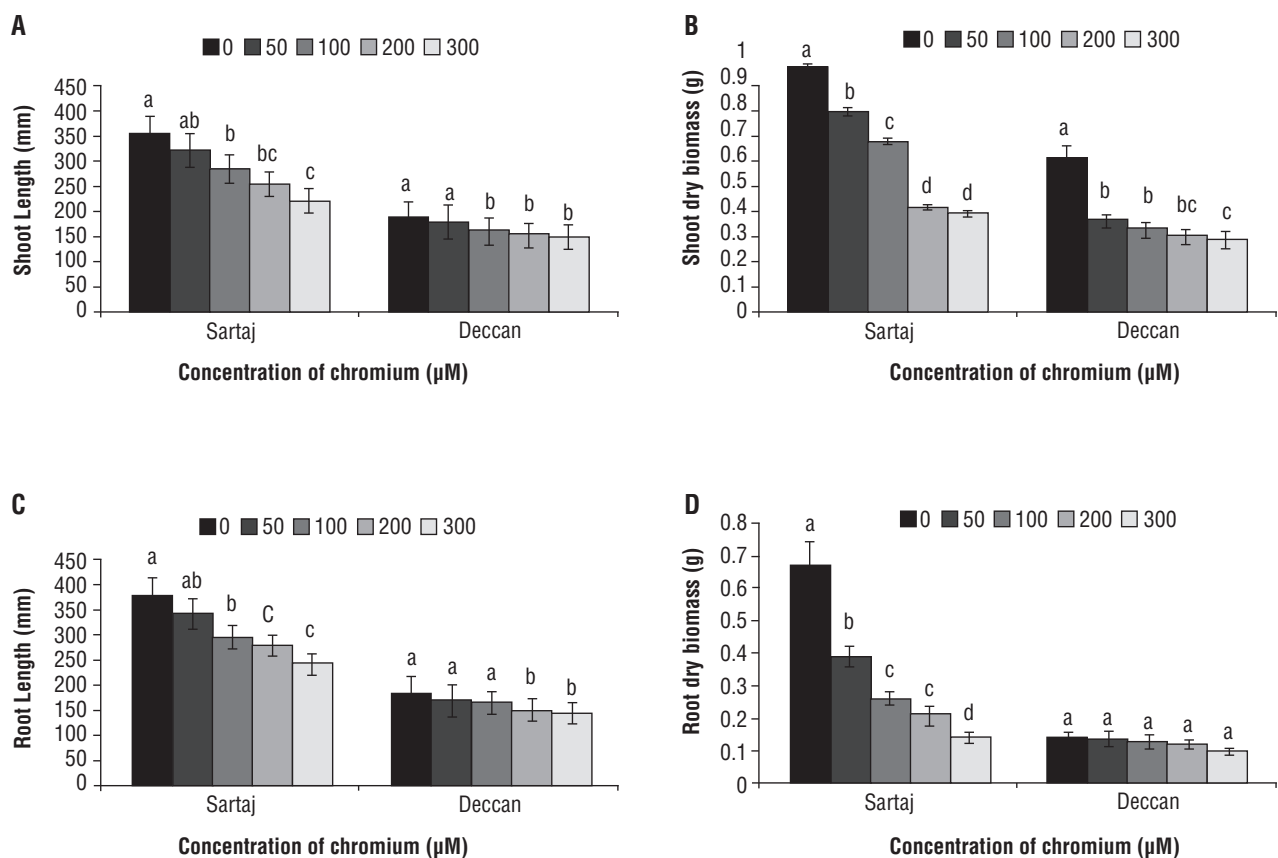
Figure 1. Changes in (A) shoot length, (B) root length, (C) shoot dry biomass, and (D) root dry biomass under different concentrations of Cr (VI) (0, 50, 100, 200 and 300 µM). Data are mean of three replicates ± SE, compared to control (0 µM) at p≤0.05. Different letters indicate significant differences among mean values within each variety (Student t-test, p≤0.05).

was 79% for Sartaj and 32% for Deccan at the highest concentration of Cr (300 µM).

Hydrogen peroxide and superoxide radical content: The oxidative stress in plants may be illustrated by showing the accumulation pattern of $O_2^{.-}$ (Figure 2) and H_2O_2 in the tissue (Figure 3). Under each Cr concentration, the $O_2^{.-}$ and H_2O_2 generation increased significantly ($p \leq 0.05$) as compared to those under control condition irrespective of varieties. After seven days of incubation, the variety Sartaj recorded the increase of $O_2^{.-}$ by 1.17 fold, 1.33 fold, 1.47 fold, 1.58 fold over control under 50, 100, 200 and 300 µM of Cr salt respectively. Likewise for Deccan, the increase of $O_2^{.-}$ under the same concentrations of Cr was 1.04, 1.29, 1.33 and 1.48 fold respectively. Therefore, Sartaj recorded
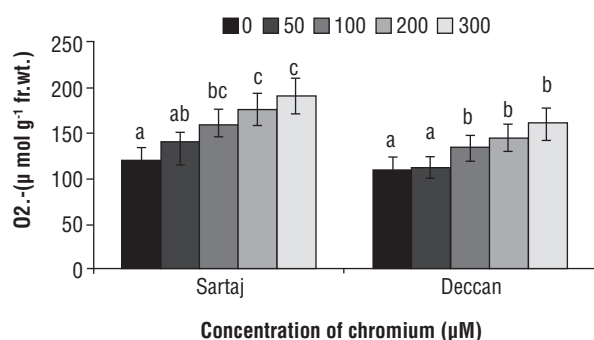

Figure 2. Generation of superoxide in Sartaj and Deccan under different concentrations of Cr (0, 50, 100, 200 and 300 µM). Data are mean of three replicates ± SE, compared to the control (0 µM) at $p \leq 0.05$. Different letters indicate significant differences among mean values within each variety (Student *t*-test, $p \leq 0.05$).
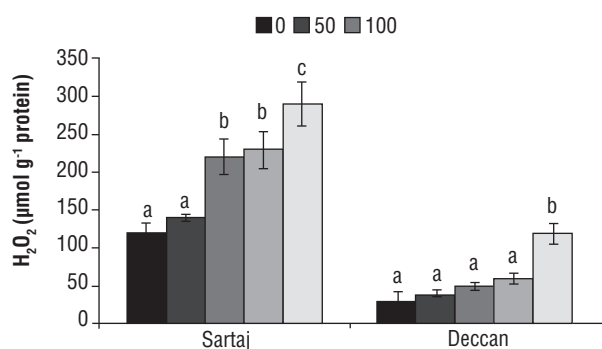

Figure 3. Generation of hydrogen peroxide in Sartaj and Deccan under different concentrations of Cr (0, 50, 100, 200 and 300 µM). Data are mean of three replicates ± SE. Data are mean of three replicates ± SE, compared to the control (0 µM) at $p \leq 0.05$. Different letters indicate significant differences among mean values within each variety (Student *t*-test, $p \leq 0.05$).

more accumulation of $O_2^{.-}$ than variety Deccan uniformly over any concentration of Cr.

Similar findings for H_2O_2 accumulation strengthen the evidence for oxidative stress in plants irrespective of varieties. Thus a linear increase in H_2O_2 concentration was the feature for both varieties. The H_2O_2 content in Sartaj becomes almost 2 and 3 fold for 100 and 300 µM of Cr concentrations, respectively, compared to the control. However, for Deccan accumulation of H_2O_2 it was 2 fold at 300 µM concentration of Cr over control.

Malondialdehyde content and protein oxidation: Malondialdehyde and carbonyl contents, measures of lipid peroxidation and protein oxidation respectively, were increased ($p < 0.05$) in both varieties under Cr stress. For MDA the variety Sartaj proved to be more sensitive in terms of lipid peroxidation by 1.86, 2.15, 2.39 and 3.42 fold over control at different concentration of Cr. Deccan recorded 1.4, 1.5, 1.66 and 2.43 fold MDA content over control under those concentration of Cr (Figure 4).

Similarly, the carbonyl formation was found maximum in Sartaj (1.06, 1.73, 3.03 and 3.34 fold over control respectively for 50, 100, 200 and 300 µM of Cr salt), while comparatively lower in Deccan (1.6, 2.15, 2.58 and 3.66 fold over control respectively for the same concentration of Cr salt) (Figure 5).

Estimation of NAD(P)H oxidase: One of the source of ROS at cellular level is furnished by the oxidation of NAD(P)H in intercellular spaces by membrane bound NAD(P)H oxidase activity, because NAD(P)H oxidase
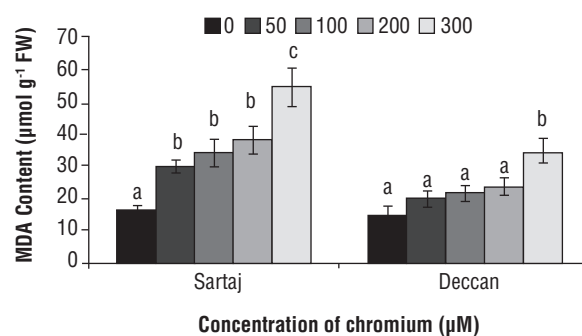

Figure 4. Malondialdehyde (MDA) content of Sartaj and Deccan under different concentrations of Cr (0, 50, 100, 200 and 300 µM). Data are mean of three replicates ± SE, compared to the control (0 µM) at $p \leq 0.05$. Different letters indicate significant differences among mean values within each variety (Student *t*-test, $p \leq 0.05$).

transfers electrons from NAD(P)H to O_2 to form superoxide radical (O_2^-). Thus, in the present studies plants were found to upregulate the NAD(P)H oxidase activity by varying concentration of Cr (Figure 6). The activity increased by 1.02, 1.18, 1.26 and 1.3 fold in variety Sartaj, whereas those were 1.04, 1.05, 1.14 and 1.17 fold in the case of Deccan. On comparative basis, Sartaj was recorded 1.35 fold higher than Deccan at the highest concentration of Cr.

Estimation of antioxidative enzymes: The antioxidative responses of those varieties in terms of enzymes like SOD, guaiacol peroxidase (GPX), and

APX showed significant ($p \leq 0.05$) variation to Cr. The GPX activity was at maximum in Sartaj, with records of 1.08, 1.23, 1.88 and 1.96 fold over control (Figure 7). Deccan showed similar trends of GPX activity, i.e., 1.05, 1.25, 1.75 and 1.96 fold over control at different concentrations of Cr. The APX activity was also increased in both varieties under treatment. In comparison, the level of APX was higher in Sartaj (1.04, 1.08, 1.12 and 1.16 fold over control) than Deccan (1.01, 1.04, 1.08 and 1.16 fold over control) at every concentration of Cr (Figure 8). On the other hand, the SOD activity was stimulated in response to Cr. The trend of SOD activity was almost similar in two varieties, differing at most at 300 µM of Cr (Figure 9). It recorded 1.38, 2.0, 3.6 and 4.85 fold higher than the control in Sartaj, whereas 1.54, 2.18, 3.82 and 5.36 fold over control in Deccan.
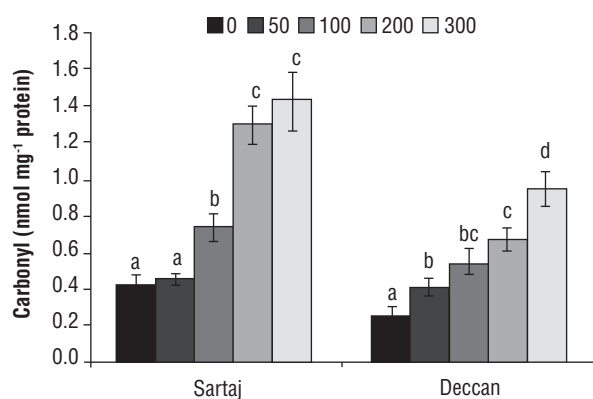

Figure 5. Protein carbonyl content of Sartaj and Deccan under different concentrations of Cr (0, 50, 100, 200 and 300 µM). Data are mean of three replicates ± SE, compared to the control (0 µM) at $p \leq 0.05$. Different letters indicate significant differences among mean values within each variety (Student t-test, $p \leq 0.05$).

Figure 7. Assay of guaiacol peroxidase in Sartaj and Deccan under different concentrations of chromium (0, 50, 100, 200 and 300 µM). Data are mean of three replicates ± SE, compared to the control (0 µM) at $p \leq 0.05$. Different letters indicate significant differences among mean values within each variety (Student t-test, $p \leq 0.05$).
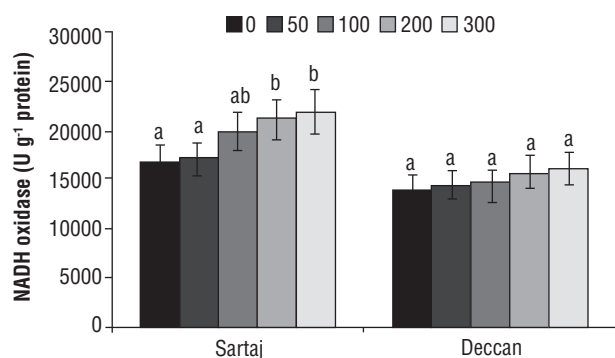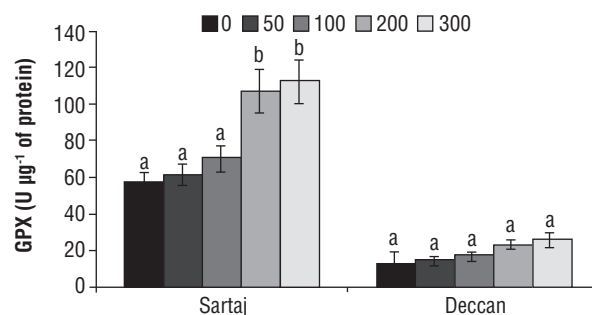

Figure 6. Changes in NAD(P)H oxidase content of Sartaj and Deccan under different concentrations of chromium (0, 50, 100, 200 and 300 µM). Data are mean of three replicates ± SE, compared to the control (0 µM) at $p \leq 0.05$. Different letters indicate significant differences among mean values within each variety (Student t-test, $p \leq 0.05$).
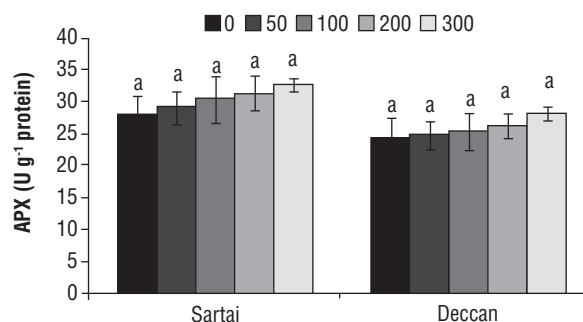

Figure 8. Assay of ascorbate peroxidase in Sartaj and Deccan under different concentrations of chromium (0, 50, 100, 200 and 300 µM). Data are mean of three replicates ± SE, compared to the control (0 µM) at $p \leq 0.05$. Different letters indicate significant differences among mean values within each variety (Student t-test, $p \leq 0.05$).
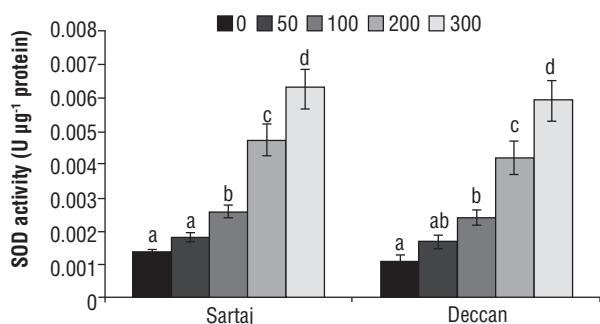
Figure 9. Assay of superoxide dismutase in Sartaj and Deccan under different concentrations of Cr (0, 50, 100, 200 and 300 μM). Data are mean of three replicates ± SE, compared to the control (0 μM) at p≤0.05. Different letters indicate significant differences among mean values within each variety (Student t-test, p≤0.05).

DISCUSSION

The present investigation aimed at evaluating the physiological and cellular performances of two maize varieties under different concentrations of Cr. Maize plants have been found to significantly suffer under Cr treatments, especially as to root growth and biomass. A distinct reduction in root growth as well as in allied attributes has been documented in the results section. The changes of root growth extrapolate in succession to the other phytotoxic symptoms on shoot under Cr contamination (Liu et al., 2008). In general, Cr-induced alteration of physiological activities in all plants starts with disease development and malfunctioning of root physiology (Dhir et al., 2009), which is manifested, to impair photosynthetic activities, water relation, translocation of organic solutes and so on as reported in cereal crop (Panda, 2007). Oxidative stress caused by Cr or other heavy metals as a severe phytotoxicity in every possible way limit successful plants growth and development. Moreover, treatments with Cr salt had recorded in dose response the inhibition of seedling growth leaf development, proper pigment and its functioning, etc. (Panda and Choudhury, 2005). At cellular level, the uptake of Cr in excess has been more inductive to oxidative stress by generation of ROS (Wang et al., 2010). Therefore, the uptake of Cr being mainly facilitated by specific membrane transporter, generation of ROS and its impact on the membrane should have more attention. Plants under normal physiological conditions can accommodate ROS produced in different cellular compartments

harbouring electron transport chain. Those include cell wall, apoplastic space, plasma membrane, chloroplast, mitochondria etc. But the production of ROS beyond the capacity of cells may create the oxidative damage (Dhir et al., 2009).

In the present experiment, a significant rise of O_2^- and H_2O_2, regardless of varieties, is interesting to notice. It could be considered as a preliminary outcome of oxidative induction. H_2O_2, by nature being not a typical radical, still, very often is regarded to be safe within the threshold level of tissue. Thus, with lower concentration of Cr both the varieties tended to minimize the H_2O_2 level, but peaked at 300 μM of Cr. Hydrogen peroxide may act as a secondary messenger to trigger many physiological processes, as recorded under various biotic and abiotic stress. Inhibition of root cell by application of Cr had been reported by Arora et al. (2006) with moderate increase in H_2O_2 content. On the other hand, since O_2^- is more reactive, unstable and impermeable to biological membrane, it reacts with H_2O_2 to generate hydroxyl radicals. The latter, being the most potent ROS, plays a major role in membrane lipid peroxidation. The exposure exposure of Cr undoubtedly accelerates the oxidative damage in crop plants in natural environmental conditions by inducing lipid peroxidation (Dhindsa et al., 1981). Regarding the source of O_2^- and other ROS generation, there is some oxidase located in apoplastic spaces and ionically bound cell wall polymers, or even cell membrane bound becoming over activated. In the present experiment, NAD(P)H oxidase mediates the oxidation of NADH with simultaneous reduction of O_2 to O_2^- and H_2O_2. This oxidase activity has a significant sharing in generation of oxidative bust with other membrane bound peroxidises and oxidases (Achary et al., 2007). Cr and other heavy metals are mostly observed with a significant expression of NADH oxidases activity in many plant species. Moreover, the variation in activities between the two varieties (Sartaj and Decan) is one more confirmation of oxidative stress under Cr induction. The variation in NAD(P)H oxidase activity could be regarded as discriminatory sensitivity of oxidative stress in terms of the same enzyme activity, as reported earlier (Sharma et al., 2012), under heavy metal stress. Meanwhile, the exposure to redox metals (in this case, the Cr) could be able to induce the ROS by Fenton type and other redox types of reaction. On the other hand, the impact of oxidative stress on plants could easily be justified through its lipid peroxidation and protein oxidation level.

This is considered as a consequence of ROS imposed lipid peroxidation of cell membrane and other organelles (Ghosh et al., 2011). The level of MDA content recorded to induce differences between the two varieties is suggestive to their differential sensitivity to oxidative stress. Lipid peroxidation happens to be a crucial effect of ROS, where a chain of reactions are operated with different intermediates at successive levels of peroxidation. The physical structure of plant cell membrane is placing the fatty acid side chains in close proximity. Thereby, it enables the autocatalytic process in consecutive stress. Initially, lipids in the membrane are converted into alkaline radicals. In the propagation steps, it is converted into hydroperoxides, epoxide. Finally, fatty acids are produced and complete lipid peroxidation (Bhattacharjee, 2005). This is followed by an irreversible oxidation of protein moieties, and that turns over some carbonylated derivatives. Metal ions with strong redox potential (Cr being one of those) accelerate the the membrane peroxidation more rapidly than other non-redox metals (Dhir et al., 2009).

The accumulation of Cr in plant tissues happens in order to induce the alteration of metabolic reaction of plants with up or down regulation of enzymatic cascade. The antioxidative enzymes tolerant to Cr induced oxidative stress recorded a similar response. With the illustration of peroxidase activity taken both guaiacol and ascorbate as phenolic substances, plants were induced according to concentrations of Cr. Peroxidases in plants are commonly referred as monomeric heme containing enzymes which are able to catalize the reactions with phenolic moieties as electron donor to reduce H_2O_2 into H_2O. The hyperactivity of peroxidase under Cr contaminated waste water in many aquatic plants has been implicated as constant detoxification of H_2O_2. Our results are in conformity with earlier studies on upregulation of antioxidative enzymes under heavy metal (Shanker et al., 2005). Peroxidase is attributed to lignifications by the cross linking of phenolic residues in the cell wall matrix. This is probably forwarded for the rigidity of cell wall or shielding the membrane from peroxidation under the condition of metal stress (Bhattacharjee, 2005).

Likewise, APX enzyme is displayed with its variable isozymic forms for antioxidation processes of H_2O_2. In most cases, APX is responsible for such activities with cytosolic, chloroplastic, mitochondrial and other organelle under various metal stresses (Hu et al., 2007). Notably, in the present experiment the variety Deccan recorded the highest activity only at 300 µM Cr concentration, whereas Sartaj recorded a steady linear increase against the increasing Cr concentrations. Two varieties showed discriminatory responses of APX to H_2O_2. To be more specific, APX is supposed to be less involved in H_2O_2 detoxification rather than the regulation of H_2O_2 as a signalling molecule (Cuypers et al., 2011). SOD is furnished with first line of defense, where $O_2^{.-}$ is dismuted in H_2O_2 and O_2. The accumulation of $O_2^{.-}$ or other free radicals in general could be detoxified by SOD. Cr, like other heavy metals, is quite efficient to induce SOD to detoxify the effect of $O_2^{.-}$. However, a discriminatory pattern is also reported in many studies. Those findings are coordinated with variation of metal concentrations, duration of exposure, tissue types and even the valence of metals (Tanaka et al., 1996). Whatever the cases might be, the elevation of SOD activity in general is a clear indication to detoxify the O_2^{-} and other ROS in downstream pathways in different cell organelle under metal stress. The two varieties in the present experiment established their differential antioxidation pathway under the same concentration of Cr. However, Sartaj proved to be more efficient in this process.

Conclusively, the present experiment showed that the bioaccumulation of Cr had significantly affected the maize with reference to oxidative stress. Cr, for being a hazardous redox metal, has significantly curtailed root growth and biomass accumulation along with oxidative damage of cell membrane. Out of the two varieties, Deccan was more potential to minimize the lipid peroxidation and protein oxidation than Sartaj. On that basis, the former variety is likely to be more tolerant than the latter. So, the accumulation of ROS could induce the antioxidation pathway with regards to those enzymes, like SOD, GPX and APX. From the overall responses of varieties, the sensitivity of the oxidative stress is likely to be correlated with the antioxidation capacity directly. Admittedly, a short term effect of metal stress under *in vitro* condition may not necessarily be a reflection of behaviour of plants as compared to prolonged metal stress in field. Still, the findings could help to assess the survival of the plants at seedling stage. Maize is a C_4 plant and is well adaptive to water stress through its modified carbon metabolism, which may lead to improved antioxdation pathways. So, future scope still remains to study other aspects of antioxidation pathways, which could be reliable indexes in selection for C_4 plants under Cr stress.

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