Research Article

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Antioxidant enzyme response of sorghum plant upon exposure to Aluminum, Chromium and Lead heavy metals

Alüminyum, Krom ve Kurşun ağır metallerine maruz bırakılan sorgum bitkisinin antioksidan enzim tepkisi

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Abstract

Objective: Sorghum has received great interest for resistance to heavy metals. Therefore, effects of Aluminum (Al), Chromium (Cr) and Lead (Pb) concentrations (2, 4, 8, 16, 32 and 64 ppm) on antioxidant enzyme systems of Sorghum in root and leaf tissues were investigated.

Methods: Seeds were cultivated in hydroponic Hoagland solution containing heavy metal concentrations in a growth chamber. Malondialdehyde (MDA), proline levels, catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR) and glutathione S-transferase (GST) activities were evaluated after treatment.

Results: Al doses decreased chlorophyll a (Chl a) at 4 ppm and subsequent doses, and total Chl at 32 and 64 ppm doses, however, it did not cause any change at Chl b except at 2 ppm. Although 64 ppm of Al, Cr, and Pb decreased total Chl, it increased proline level (nearly 5, 21 and 40 times higher compared to control, respectively) in leaf. Common observation is that positive correlation was apparent between proline, MDA, APX and GST activity for Al and Cr treatments and GST activity for three elements in root. Also, the only positive correlation was evident between proline and MDA for Al and Cr applications in leaf.

Conclusion: Although sorghum is resistant to heavy metals, induction of antioxidant enzymes seem to be not enough at higher concentrations to protect cells from heavy metal toxicity; however, it has great importance for further studies to find out whether phenolic compounds produced in sorghum have additive role in this regard.

Keywords: Sorghum; Antioxidant enzymes; Proline; Chlorophyll; Heavy metals; MDA.

Özet

Amaç: Sorgum ağır metallerle dayanıklılığı yönüyle büyük ilgi çekmektedir. Bu nedenle, bu çalışmada, Alüminyum (Al), Krom (Cr) ve Kurşun (Pb) konsantrasyonlarının (2, 4, 8, 16, 32 ve 64 ppm) sorgum yaprak ve kök dokularında antioksidan enzim sistemleri üzerindeki etkisinin belirlenmesi amaçlanmıştır.

Metod: Denemeler tohumların iklim kabinde ağır metal konsantrasyonu içeren Hoagland solüsyonu içerisinde yetiştirilmesiyle gerçekleştirilmiştir. Al, Cr ve Pb’ye maruz bırakılan sorğumda malondialdehit (MDA), prolin seviyesi, katalaz (CAT), süperoksid dismutaz (SOD), askorbat peroksidaz (APX), glutatyon redüktaz (GR) ve glutatyon S-transferaz (GST) aktiviteleri değerlendirilmiştir.

Bulgular: 4 ppm ve sonrası Al dozları klorofill a (Chl a) miktarını, 32 ve 64 ppm dozları da toplam Chl miktarını belirgin şekilde artırtırken, Chl b seviyesinde 2 ppm dozdaki keskin artışın dışında bir değişiklik gözlemlenmemiştir. Al,
Cr, ve Pb’nin 64 ppm dozdaki uygulamasında toplam Chl miktarı belirgin şekilde azalmasına rağmen, yaprakta prolin seviyesini (kontrol ile kıyaslandığında yaklaşık olarak sırasıyla 5, 21, ve 40 kat daha fazla) artırılmıştır. KÖkte Al ve Cr uygulamalarında Chl miktarı belirgin pozitif korelasyon ortak bir sonuç olarak ortaya çıkmaktadır. Ayrıca yaprakta Al ve Cr uygulamalarında görülen tek pozitif korelasyon prolin ve MDA arasında gözlenmiştir.

Sonuç: Sorgum ağır metallere karşı dirençli olmasına rağmen, antioksidan enzim sisteminin indüklenmesi yüksek konsantrasyonlarda hücreleri ağır metal toksitesine karşı korumak için yeterli görülmemektedir. Ayrıca Sorgum, Al ve Cr uygunlukta köktedeki Prolin, MDA, APX, ve GST aktiviteleri ile, tüm ağır metal uygulamalarda belirgin pozitif korelasyon ortak bir sonuç olarak ortaya çıkmaktadır. Ayrıca yaprakta Al ve Cr uygulamalarında belirgin pozitif korelasyon prolin ve MDA arasında gözlenmiştir.

Anahtar Kelimeler: Sorgum; Antioksidan enzimler; Prolin; Klorofil; Ağır metaller; MDA.

Introduction

*Sorghum bicolor* is the fifth-most important cereal crop and used for food, animal fodder, the production of alcoholic beverages and biofuels [1]. It is an important food in Africa, Central America, and South Asia, and is an excellent source of bioactive compounds that can promote benefits to human health [2]. In addition, it contains phenolic compounds and vitamin E which contribute to its high antioxidant activity [3].

Agricultural soils in many places are slightly to moderately contaminated by heavy metal accumulation due to sewage sludge application, use of phosphatic fertilizers, industrial wastes and improper watering applications in agricultural lands [4]. Such heavy metals are considered as soil pollutants due to their prevalent existence, and their prominent toxicity on plants grown of such soil [4].

Heavy metals such as mercury (Hg), cadmium (Cd), Al, Pb, and arsenic (As), are not necessary for any process in plants and their toxic levels result in reactive oxygen species (ROS) and interfere with biochemical and physiological processes such as change in chl a and b content and photosynthesis, damage in cell membranes, chloroplast pigments, nucleic acids, inactivate enzymes and growth inhibition [5, 6].

Lipid peroxidation, directly causing membrane disruption, is one of the most detrimental effects induced by heavy metal exposure in plants. MDA is one of the breakdown products of lipid peroxidation and considered as a reliable indicator of oxidative stress [4, 7, 8].

In plants under stress conditions, the concentration of free radical species may increase and activate the detoxifying enzymes [8] such as CAT, SOD, GR, APX, GST, which are able to eliminate ROS [9, 10]. Under normal circumstances, due to the activity of these enzymes concentration of oxygen radicals remains low [11]. It is known that biosynthesis of proline was increased with proline carboxylic acid synthetize and proline carboxylic acid reductase under stress conditions and improves tolerance in plants via O2 quenching activity, ameliorating antioxidant enzyme system, photosynthetic activity and plant growth [12–14].

As for *Sorghum bicolor* species, previous studies have mainly focused on their chemical composition and nutritional value, response to salt stress and water logging, distribution and accumulation of metals, and effect on photosynthesis of a single heavy metal [2, 10]. Little information is available on the physiological and biochemical mechanisms of antioxidant enzyme activity for comparing the effect of more than one heavy metal stress. Therefore, for improving the Sorghum ecosystem, it may be useful to study the correlation between heavy metals and antioxidative enzymes in Sorghum plants.

In the present study, we examined the response of *Sorghum bicolor* to 2, 4, 8, 16, 32, and 64 ppm doses of Al, Cr, and Pb heavy metal exposure considering the physiological and chemical parameters. In this sense, we investigated whether a positive or negative correlation between Chl a and b, and total chl content, proline, lipid peroxidation, and antioxidant enzymes such as CAT, SOD, APX, GR, and GST activities and heavy metals exist in Sorghum.

Materials and methods

Experimental design

Experiments were carried out by cultivating the *Sorghum bicolor* seeds in hydroponic hoagland solution [15] in a growth chamber to investigate the impact of Al, Cr and Pb concentrations (2, 4, 8, 16, 32 and 64 ppm) on some physiological and biochemical processes. Hoagland solution without heavy metal was used as control. Experiments were carried out in three replications independently. The seeds were let to germinate for 7 days in hoagland solution and heavy metal concentrations were applied for subsequent 10 days. The hydroponic solutions of applications and control were renewed daily. Chl (a, b, and total)
content, membrane lipid peroxidation, proline accumulation and antioxidant activities of SOD, CAT, APX, GR and GST of *Sorghum bicolor* under heavy metal stress was investigated in both root and leaf segments.

**Preparation of enzyme extracts**

Fresh tissues (0.5 g) were ground in liquid nitrogen and extracted in 50 mM Tris HCl (pH, 7.2) containing 2% PVP, 1 mM Na,EDTA, and 2 mM ascorbate. Then centrifuged at 15,000×g for 20 min at 4°C and supernatant was used for SOD, CAT, GST, APX, and GR enzyme studies. The concentration of the protein content was determined using the method of Bradford [16]. Absorbance and enzyme activity measurements were performed using UV-1800 UV-Vis Spectrophotometer (Shimadzu Corporation, Japan).

**Lipid peroxidation (MDA)**

Lipid peroxidation was carried out according to the method of Madhava and Srety [17] with slight modification. One g of fresh tissue was ground in 5 mL of 0.1% TCA solution and centrifuged at 10,000×g for 5 min. For every 1 mL supernatant 4 mL of TCA (20%) solution containing 0.5% TBA was added. The mixture was incubated at 95°C for 30 min and immediately cooled under tap water. Subsequently centrifuged at 10,000×g for 15 min and their absorbance were determined under 532 and 600 nm. For eliminating unspecific turbidity, absorbance at 600 nm was subtracted from that at 532. The concentration of thiobarbituric acid reactive substance (TBARS) formed as byproduct of MDA was calculated by using extinction coefficient of 155 mM⁻¹cm⁻¹. The following formula was used for estimating TBARS content (nmol/gr): \[(Abs 532–Abs600)/155\text{M}^{-1}\text{cm}^{-1}\]*[Reaction volume (mL)/Amount of sample (gr)]*1000.

**Determination of free proline level**

Free proline amount of sorghum samples were determined by the method of Karabal et al. [18]. Two hundred and fifty milligrams of fresh tissue was squashed in 5 mL of 3% sulfosalysilic acid and centrifuged at 15,000×g for 10 min at 4°C. A mixture was prepared by adding 2 mL of supernatant to 2 mL of acid ninhydrin (1 g ninhydrin, 25 mL glacial acetic acid, 16 mL of 6 M phosphoric acid) and then 2 mL 96% acetic acid and 1 mL of 3% sulfusaly-silic acid was added. After heating the tubes at 96°C for 1 h and immediately cooling under tap water, 4 mL of toluene was added and vigorously vortexed. Upper red phase was measured at 520 nm. Toluene was used as control. The proline standard was prepared ranging from 0.01 μM to 1.5 mM.

**Chlorophyll extraction**

Extraction of chl from leaves was carried out using the method described by Hiscox and Israelstam [19]. One hundred milligram of fresh tissue was transferred to 50 mL tubes and 7 mL of DMSO was added. Until the removal of color (30–60 min) the sample was heated at 65°C in water bath. Solution was transferred to a new tube and completed to 10 mL with DMSO. Samples were quickly analyzed or stored at 4°C until analysis. If OD >0.7, then the samples were diluted with 50 or 90% DMSO. Chl a (g/L) = 0.0127*Abs663−0.00269*Abs645; Chl b (g/L) = 0.0229*Abs665−0.00468*Abs645, and Total Chl (g/L) = 0.0202*Abs665+0.00802*Abs645 was calculated according to these formula. Leaf chl amount was given as mg/g chl.

**Enzyme activities**

SOD activity was determined by the method of Grannopolitis and Ries [20]. Reaction was performed in 3 mL quartz cuvette containing 20 mM NaHPO₄ (pH 7.5), 0.1 mM EDTA, 10 mM methionine, 0.1 mM p-nitro blue tetrazolium chloride (NBT), 0.005 mM riboflavin, and 50 μg protein. Reaction was started upon addition of enzyme into the cuvette. Fluorescence lamp (300 lumen) was positioned at a distance of 20 cm from the cuvette for 15 min. Absorbance of the samples was measured at 530 nm. SOD standard was prepared as containing 20–200 ng samples. Light untreated reaction mixture was used as the blank. Reaction mixture without enzyme exposed to light was also used as control. Percent inhibition is calculated according to the formula; [(The absorbance of the control–sample absorbance)/control absorbance]*100. One SOD unit is the amount of enzyme for providing 50% inhibition. A graphic was constructed corresponding to percent inhibition of enzyme concentrations and using the equation of the graphic, SOD contents (ng/mL) of the samples were determined.

For determining APX activity the reaction was started by adding 6 mM H₂O₂ on 50 mM KHPO₄ (pH 6), 0.15 mM ascorbate, and 3 μg enzyme mixture in a quartz cuvette (1mL). The absorbance was recorded during 3 min at OD 290, 25°C. The enzyme activity was calculated as the amount
of \( \text{H}_2\text{O}_2 \) consumed using the extinction coefficient of \( \text{H}_2\text{O}_2 \) (2.8 \text{ mM}^{-1} \text{ cm}^{-1} \text{ at 290 nm}) [21].

CAT activity was measured according to the method described by Misra and Gupta [22]. The reaction was started by adding 6 mM \( \text{H}_2\text{O}_2 \) in 20 mM NaHPO\(_4\) (pH 7.5), 3 \( \mu \text{g} \) enzyme mixture in a quartz cuvette (1 mL), and the absorbance was measured during 3 min at \( \text{OD}_{240} \text{ 25}^\circ \text{C} \). The enzyme activity was calculated as the amount of \( \text{H}_2\text{O}_2 \), consumed using the extinction coefficient of \( \text{H}_2\text{O}_2 \) (40 \text{ mM}^{-1} \text{ cm}^{-1} \text{ at 240 nm} \).

GR activity was measured by mixing 100 mM potassium phosphate buffer (pH 7.4), 0.1 mM Na\(_2\)EDTA, 0.1 mM nicotinamide adenine dinucleotide phosphate (NADPH), 1.0 mM oxidized glutathione (GSSG), and 5 \( \mu \text{g} \) enzyme extract in a final volume of 1 mL in a quartz cuvette. The decrease in NADPH concentration was observed during 5 min at 340 nm, 25\°C. The extinction coefficient of NADPH (6.2 \text{ mM}^{-1} \text{ cm}^{-1} \text{ at 340 nm}) was used for calculating the enzyme activity starting from initial rate of the reaction.

GST activity was performed with a mixture containing potassium phosphate buffer (100 mM, pH 7.5), 0.1 mM Na\(_2\)EDTA, 1.0 mM NADPH, 2.0 mM glutathione (GSH), 1.0 mM 1-chloro-2,4-dinitrobenzene (CDNB) and 50 \( \mu \text{g} \) enzyme extract in a final volume of 1 mL. To detect the non-enzymatic activity, reaction was performed through incubating the assay solution for 5 min prior to addition of enzyme extract. Subsequently, following the addition of 50 \( \mu \text{g/mL} \) enzyme extract the absorbance was measured at 340 nm for 5 min at 10 s intervals. The extinction coefficient of NADPH (6.2 \text{ mM}^{-1} \text{ cm}^{-1} \text{ at 340 nm}) was used for calculating the enzyme activity starting from initial rate of the reaction.

### Statistical analysis

Data were subjected to analysis of variance (ANOVA) and correlation using the SAS packet program [23]. The experimental design was completely randomized with three replications. Significant differences between individual means at \( p \leq 0.05 \) were identified using the Duncan multiple range tests. Standard errors of means were calculated from the residual mean square in the analysis of variance.

### Results and discussion

In the current study, six concentrations (2, 4, 8, 16, 32, 64 ppm) of Al, Cr and Pb were applied to sorghum (Sorghum bicolor L.) plants at different pots in controlled conditions for observing their effects on the amount of proline, chl (a, b, and total) content, lipid peroxidation and enzyme activities of CAT, SOD, APX, GR, and GST in both root and leaf segments.

Heavy metal accumulation in plants results in oxidative stress through producing ROS known as singlet oxygen (\( \text{O}_2^\beta \)), hydrogen peroxide (\( \text{H}_2\text{O}_2 \)), superoxide radical (\( \text{O}_2^- \)) and hydroxyl radical (\( \text{HO}^- \)) [24]. They cause irreversible metabolic disorders unless prevented or eliminated. That’s why production of antioxidant enzymes as CAT, SOD, APX, GR, and GST are the crucial events for minimizing the oxidative stress induced by heavy metal accumulation. In the present study variations in the level of proline, MDA, and antioxidant enzymes in root and leaves are regarded as the indication of physiological response of the plant to Al, Cr and Pb stress.

The results indicated that Al doses significantly decreased the amount of Chl a at 4 ppm and subsequent doses, and total Chl at 32 and 64 ppm doses. Similarly, a decrease was observed in total Chl amount at 8 ppm and subsequent doses of Pb (Table 1). Although not many studies were found in literature considering the antioxidative enzyme activities in sorghum species, the findings are in harmony with those of Panda et al. [25] which was

**Table 1:** Chlorophyll content upon exposure to heavy metals.

| Doses (ppm) | Al | | | Cr | | | Pb | |
|-------------|----|---|---|----|---|---|---|---|
|             | Chl a | Chl b | Total Chl | Chl a | Chl b | Total Chl | Chl a | Chl b | Total Chl |
| 0           | 5.418 a' | 2.787 b | 8.205 ab | 5.418 d | 2.787 d | 8.205 de | 5.418 a | 2.787 a | 8.205 a |
| 2           | 5.272 a | 4.352 a | 9.625 a | 7.216 b | 3.220 bcd | 10.435 b | 3.842 b | 3.358 | 7.199 ab |
| 4           | 4.505 ab | 3.322 b | 7.827 b | 8.433 a | 3.796 ab | 12.229 a | 3.799 b | 3.339 | 7.139 ab |
| 8           | 4.024 bc | 3.235 b | 7.259 bc | 8.781 a | 3.895 a | 12.676 a | 3.851 b | 2.828 | 6.679 bc |
| 16          | 3.840 abc | 3.015 b | 6.855 bc | 6.324 c | 3.513 abc | 9.837 bc | 3.621 b | 2.815 | 6.436 bc |
| 32          | 3.123 cd | 2.793 b | 5.915 c | 5.749 cd | 3.230 bcd | 8.979 cd | 3.486 bc | 2.462 | 5.948 bc |
| 64          | 2.951 d | 2.601 b | 5.551 c | 4.988 d | 2.901 cd | 7.889 e | 3.000 c | 2.385 | 5.386 c |

*The same letters in the same colon indicate lack of significance at 95% confidence interval.*
carried out in wheat species with heavy metal ions. Also, findings about total Chl content upon exposure to Pb concentrations was consistent with the study of Zengin and Munzuroglu [26], which was carried out on bean seedlings. Decrease in chlorophyll content in leaves of especially Al and Pb treated seedlings can be attributed to chlorosis as stated by Stobart et al. [27] as well as an increase in its degradation [5]. In the present study, the decrease in total Chl at 64 ppm of Cr, Al, and Pb was around 4, 32 and 34%, respectively. In a similar manner Oncel et al. [28] reported 50% decrease in total Chl content in *Triticum aestivum* upon exposure to Pb and Cd. Interestingly Al applications didn’t cause any change at the level of Chl b except at 2 ppm with a sharp increase (Table 1). Although change in Chl a and b content was positively correlated with total Chl content for both Al ($r^2 = 0.941$ and 0.829, respectively) and Cr ($r^2 = 0.995$ and 0.938, respectively) applications, it was the case for Chl a at Pb applications ($r^2 = 0.916$). On the other hand, Cr doses covering 2–16 ppm caused considerable increase in Chl b and total Chl amount. Such a situation was reported on *Ocimum tenuiflorum* by Vartika et al. [29]. For Chl b and total Chl, Cr caused significant increasing effect at the doses of 4, 8 and 16 ppm. In resistant plants, Cr concentrations till critical levels cause an increase in chl content but higher doses result in decrease as is the case in our study. Although, there is no specific mechanism for Cr metabolism in chl synthesis, researchers reported that low and moderate concentrations of Cr increased pigment content in leaves, but the higher concentrations reduced chl and carotenoid biosynthesis. The positive effect of low and moderate concentrations of Cr on chl biosynthesis may be attributed to the increased transport of Mg [30]. At Pb applications, reduction was observed in the amount of Chl a when compared with the control, but there was no difference between the doses except for 64 ppm.

Lipid peroxidation in roots and leaves of *Sorghum* plant as MDA content was presented in Figure 1. It was seen that a continuous increase was evident at increasing doses of Al, Cr, and Pb. The difference in the activity of MDA between 2–4 and 16–32 ppm for Cr, and 2–4 ppm for Pb was not significant. On the other hand, for root MDA, maximum levels for Al (39.85) and Cr (23.18), and Pb (39.46) applications were reached at 4 and 8 ppm doses, respectively. It was clear that the MDA response of the plant against the heavy metals was more prominent at leaves compared to root, indicating that antioxidant enzymes work more effectively in roots to eliminate ROS at 16 ppm and after for Al and Cr, and 8 ppm and after for Pb treatment compared to that in leaves (Figure 1). These results indicate that sorghum effectively translocate the heavy metals to shoot parts. This feature of sorghum species can efficiently be utilized in detoxification of heavy metal contaminated areas. For Al applications MDA exhibited negative correlation between Chl a ($r^2 = -0.963$), and total Chl ($r^2 = -0.847$) content. The same trend was observed between total Chl and Chl b for Pb applications. The reason for this result is that an increase in MDA level is regarded as the result of free radical accumulation in tissues under oxidative stress conditions, and reduction in $\text{H}_2\text{O}_2$ levels and membrane damage due to increased antioxidative enzyme activity [8, 31, 32]. The results were in accordance with the study of Malmir [33] indicating that lipid peroxidation is enhanced in tissues. Also other researchers reported significant increase in MDA level under Cr, Zn, Cd, and Pb treated wheat seedlings [25, 32].

Gajewska [34] denoted the proline as one of the most widespread metabolites produced in plant tissues under

![Figure 1](image_url)  
**Figure 1:** MDA levels in leaf and root tissues of *Sorghum bicolor* L.  
The same letters on bars of the same colors indicate lack of significance at 95% confidence interval.
stress conditions. Due to its ROS scavenging [13], osmo-
protecting [35] and membrane stabilizing [36] specifica-
tions proline has a protective role in stress conditions. The
increasing dose implementations of Al, Cr, and Pb caused a
gradual numerical increase in proline level, but Al and
Cr at the doses covering 2–16 ppm, and Pb at 2 ppm didn’t
cause significant change between them in leaf (Figure 2).
On the other hand, when compared with control nearly
5, 21, and 40 times higher proline level was estimated at
64 ppm for Al, Cr, and Pb, respectively. It was evident that
continuous increasing trend in leaf proline level in all
doses of Al, Cr, and Pb is a typical response of the plant to
heavy metal stress. Besides, the simultaneous increase of
leaf MDA level indicates an apparent correlation between
MDA and proline levels [37]. The study carried out on bean
seedlings by Zengin and Munzuroglu [26] supports our
findings about proline levels. Also, the highest amount
at root proline level for Al treatment (91.24) was 4.2 times
lower than the maximum level at leaf (248.76). Yet, the leaf
proline level was sharply increased in 64 ppm of all ele-
ments, but a decrease was evident for root proline level for
Al and Cr at the same dose indicating that Al and Cr were
probably translocated to upper parts of the plant. The
increase in proline content explains its role as an antioxi-
dant in detoxification of heavy metal accumulation [38].

Leaf SOD activity was considerably different at all
doses of heavy metals compared to control. However there
were no differences between the doses 32 and 64 ppm for
Cr, and 4 and 8 ppm for Pb. Maximum rates in leaves for
all tree elements at all doses were reached at 8 ppm, and
then a steady reduction was observed in the subsequent
doses. SOD activity at 8 ppm was nearly 9, 11, and 34 times
higher compared to control for Al, Pb, and Cr applications,
respectively (Figure 3). The decreases in SOD activity at
16 ppm and after suggest that its oxygen scavenging func-
tion was gradually weakened. These findings are in agree-
ment with the data reported by Zhang et al. [8]. But, root
SOD activity especially as a response to Al and Cr doses
resulted in a continuous increase unlike that in leaves
indicating that this enzyme has better preventive func-
tion against oxidative damage compared to that in leaves.
It was thought that the case is possibly due to transloca-
tion of heavy metals and their accumulation in leaf tissues
other than roots. As stated by Scandalios [39], increase in
SOD activity is associated with the tolerance of the plant to
oxidative stress in root. As evidence to previous sentences,
maximum rate at root was achieved at 64 ppm as 0.889 for
Al and 1.478 for Cr, whereas; the highest activity at leaves
was reached at 8 ppm as 1.446 for Cr. For Pb applications
the highest SOD rate was observed at 32 ppm for root
(0.387) and 8 ppm for leaf (0.477) (Figure 3). SOD activity
was positively correlated with GR in both Al (r^2 = 0.975)
and Cr (r^2 = 0.775), and GST for Pb (r^2 = 0.755) applica-
tions in root. On the other hand it was directly correlated
with CAT activity in leaf for Al (r^2 = 0.835) and Pb (r^2 = 0.959)
applications. Another important point is that SOD activ-
ity indicated a positive correlation with Chl a (r^2 = 0.892), b
(r^2 = 0.970) and total content (r^2 = 0.926) only for Cr applica-
tions in leaf.

Leaf APX activity was significantly different with a
fluctuation at 2–32 ppm for Al with the highest rate (0.437)
at 16 ppm. Root APX for Al doses indicated a continuous
increase till 16 ppm (0.244) and then a decrease was seen.
The highest APX activity reached at 8 ppm for Cr (0.440)
and at 4 ppm for Pb (0.220) at leaf and 16 ppm for Cr (1.278)
and Pb (0.187) at root. The APX activity response of the

Figure 2: Proline levels in leaf and root tissues upon exposure to heavy metals.
The same letters on bars of the same colors indicate lack of significance at 95% confidence interval.
plant to heavy metals seems to be higher in root compared to leaf (Figures 3 and 4). The positive correlation between APX activity and CAT, MDA, Proline, and GST was clear for both Al ($r^2 = 0.864$, $0.828$, $0.863$, and $0.963$, respectively) and Cr ($r^2 = 0.875$, $0.837$, $0.924$, and $0.847$, respectively) applications in root. The situation suggests that APX, CAT, and GST seem to act together for eliminating the stress created in the root. However, such a condition was not the case between other enzymes for Al application, and Cr and Pb application except for SOD activity in leaf.

Leaf CAT activity was significantly higher at all doses of Al, Cr, and Pb compared with control. Thus, CAT can be considered as primary stress eliminating enzyme in sorghum plant. The increasing trend was observed at the doses covering 2–16 ppm for Al and Cr, and 2–8 ppm for Pb. At subsequent doses decreasing trend was observed.
At the maximum level of CAT activity, the effect of Al and Pb, and Cr was more than 20 and 9% higher, respectively. On the other hand, root CAT activity for Al indicated an increase only at 4, 8, and 16 ppm doses. Also unlike the value in leaf, no difference was seen in root CAT activity between 32–64 ppm and the control. This is also an indication of heavy metals translocation from roots to leaf without accumulation at high levels. The leaf CAT activity as a response to Al at maximum level was nearly 2.5 times higher than that of root (Figures 3 and 4). Root Cr doses covering 2–16 ppm resulted in a continuous increase, but at 32 and 64 ppm a decrease was observed. The root CAT activity at 64 ppm was not different from that of control. On the other hand the effect of Pb on root CAT activity was continuously increased unlike that in leaf. These findings are in agreement with the results of Malmir et al. [33]
carried out on sorghum plant with Cr ion. They reported that GST, CAT and GR activities were higher at concentrations of Cr treatments. CAT was positively related with proline, GR \( (r^2 = 0.852) \) and GST \( (r^2 = 0.917) \) in root and SOD \( (r^2 = 0.959) \) and GR \( (r^2 = 0.954) \) in leaf for Pb; proline \( (r^2 = 0.940) \), APX \( (r^2 = 0.875) \) and GST \( (r^2 = 0.971) \) in root and GR \( (r^2 = 0.775) \) in leaf for Cr; and APX \( (r^2 = 0.864) \) in root and SOD \( (r^2 = 0.835) \) in leaf for Al applications.

All the doses of Cr and Pb caused a considerable increase in leaf GR activity compared to control with the highest level being at 16 ppm for Cr and 8 ppm for Pb. In Al applications a continuous increase was seen between the doses starting from 4 ppm. The similar trend with considerable increase at all doses was also the case for Al in root GR activity (Figures 3 and 4). The highest leaf GR activity was observed in 8 ppm for Pb (1.573), 16 ppm for Cr (0.503), and 64 ppm for Al (0.738). The response of the plant to Pb doses was higher compared with that of Al and Cr. The highest activity for Cr was at 32 ppm at root and 16 ppm for leaf. In Pb treatments, root GR activity increased at all doses reaching the maximum rate at 64 ppm (0.584), however, it was the case for leaf at 8 ppm (1.573). It indicates the translocation of Pb to leaves other than accumulating in root tissues. Positive correlations among proline, CAT, GST, and GR in root tissues, as well as proline, CAT, SOD, and GR in leaf tissues suggest that they have a primary role as a whole in detoxification of Pb. At the same time, GR and SOD in root, and proline, CAT and GR in leaf tissues seem to serve to eliminate Al and Cr toxicity.

Leaf GST activity for all doses of Al, Cr, and Pb indicated a significant increase compared to control. The maximum level reached at 4 ppm for Al (2.718) and Pb (0.857), and 8 ppm for Cr (2.184) applications. At subsequent doses of all three elements a gradual decrease was observed. Root GST response of the plant was higher for all elements compared with that of leaf. The maximum root GST activities for Al (4.099) and Cr (4.269), and Pb (0.748) treatments were reached at 16 and 64 ppm, respectively (Figures 3 and 4). GST activity was positively correlated with MDA \( (r^2 = 0.916) \), Proline \( (r^2 = 0.839) \), APX \( (r^2 = 0.963) \) in root for Al; CAT \( (r^2 = 0.971) \), MDA \( (r^2 = 0.762) \), Proline \( (r^2 = 0.924) \), and APX \( (r^2 = 0.847) \) in root, and SOD \( (r^2 = 0.961) \) and APX \( (r^2 = 0.965) \) in leaf for Cr; and CAT \( (r^2 = 0.966) \), Proline \( (r^2 = 0.916) \), SOD \( (r^2 = 0.755) \), GR \( (r^2 = 0.917) \) in root and APX \( (r^2 = 0.854) \) in leaf for Pb applications.

**Conclusions**

In conclusion, considering all data in the present study in coordination with the studies in literature it was clear that plant responses to heavy metal stress are multigenic and mostly involve cell detoxification through the antioxidative scavenging mechanisms. A common observation is that GST indicated a positive correlation with proline, CAT, MDA, and APX activity for Al and Cr treatments in root tissues. Furthermore, the only positive correlation at Al and Cr applications in leaf was observed between proline and MDA. It was seen that Al, Cr, and Pb caused prominent oxidative stress in *Sorghum bicolor* and production of ROS interfere with chlorophyll production. Other than Al and Pb, Cr levels induced considerable increase in SOD, APX, and GST activity especially in leaf and significantly demonstrated its effect in chlorophyll biosynthesis. The antioxidative enzyme activities in leaf was higher compared to that in root indicating the higher level of lipid peroxidation in leaf, and explain higher tolerance to heavy metal stress. Despite these, induction of antioxidant enzymes may not be enough to protect sorghum cells from heavy metal toxicity; however, it has great importance to find out the additive role of phenolic compounds. For all these reasons, further studies are required to verify the role of phenolics in antioxidative response mechanism in sorghum plant.

Higher antioxidant enzyme activity in leaf compared to root reveals that heavy metals are probably translocated to shoot parts of the plant suggesting that sorghum plant can efficiently be used in phytoremediation of heavy metal contaminated areas. Thus, it should be focused on field studies to determine the phytoremediation capacity of sorghum species.

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