

# Effect Of Chromium on Antioxidant Enzyme activity in Some Sorghum (*Sorghum Bicolor* L.) Genotypes Plant

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**ABSTRACT:** The aim of this study was to investigate the effect of chromium on enzymatic activity of sorghum genotypes grown under polluted conditions. Ten sorghum genotypes, (Tabat, Wad Ahmed, L4, L7, L12, L14, L16, L25, L32 and L34) were obtained from the Department of Agronomy, Faculty of Agriculture University of Khartoum, grown in Polyethylene bags filled with 2 kg soil (clay and sand, 2:1). Two weeks after sowing, seedlings of grown plants were irrigated with eight levels of chromium (Cr VI) concentrations (0, 2.5, 5, 10, 20, 30, 40, and 50 mg/l.) for 15 days. Samples of plants were taken three times (at 8<sup>th</sup>, 12<sup>th</sup>, and 15<sup>th</sup> days) after chromium application to measure the enzymatic activity, namely, malate dehydrogenase, alcohol dehydrogenase and glutamate dehydrogenase. The results revealed that the level of enzyme activity increased significantly with increase in chromium concentration. Also the rate of increase in enzymatic activity, depend mainly on genotype and age of the plant. These findings suggest that, the activity of antioxidant enzymes of sorghum play a significant role in plant defense system.

**Key words:** chromium, malate dehydrogenase, alcohol dehydrogenase, glutamate dehydrogenase

## 1. INTRODUCTION

Chromium (Cr) is considered as one of most important heavy metal, concerning the risk of human health. Contamination of the environment by such metal has become of major concern, since it is used in many different industries, including metallurgy, electroplating, production of paints and pigments, tanning, wood preservation, chemical production, and pulp and paper production (Zayed and Terry, 2003). The problems associated with chromium contamination are of great significance, especially in agricultural production system (Gupta and Gupta, 1998). The presence of chromium in some agricultural soils could be attributed to the use of organic wastes fertilizer as well as application of wastewater for irrigation (Baxter *et al.* 1983). Chromium has many effects on certain enzyme such as catalase, peroxidase and cytochrome oxidase. An important response to chromium stress by aerobic cells is the generation of reactive oxygen species (ROS), such as superoxide radical (O<sub>2</sub><sup>-</sup>), hydroxyl radical (OH<sup>•</sup>), alkoxy radical (RO<sup>•</sup>), singlet oxygen (<sup>1</sup>O<sub>2</sub>), and toxic hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) molecules (Breusegemet *al.*, 2001). These ROS produced in the plant cells are scavenged by both enzymatic and nonenzymatic antioxidative systems. ROS, if not detoxified, make serious damage to chlorophyll, protein, membrane lipids and nucleic acids (Alscheret *al.*, 1997). The aim of this study was to investigate the effect of chromium on enzymatic activity of malate dehydrogenase, alcohol dehydrogenase and glutamate dehydrogenase enzymes in selected sorghum genotype plants.

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## 2. MATERIALS AND METHODS

### 2.1. Experimental methods:

A pot experiment was carried out to cultivate sorghum genotype plants under different concentration of chromium. The experiment was conducted in complete randomized design with three replicates for each time. Polyethylene bags were used in the experiment and they were filled with 2 kg soil which consisted of a mixture of clay and sand in 2:1 ratio. Ten sorghum genotypes, (Tabat, Wad Ahmed, L4, L7, L12, L14, L16, L25, L32 and L34) were obtained from the Department of Agronomy, Faculty of Agriculture University of Khartoum were used in the experiment. The treatment of chromium consisted of eight levels of chromium concentrations (0, 2.5, 5, 10, 20, 30, 40, and 50 mg/l.) Potassium dichromate was used as sources of chromium. 6 seeds per pots were sown on the third week of July 2011, and were irrigated with tap water for two weeks and then the seedlings were irrigated with different chromium concentration for growth period of 15 days. The data were taken from four plants of each pot after 8<sup>th</sup>, 12<sup>th</sup>, and 15<sup>th</sup> days after chromium application to measure enzymes activities.

### 2.2. Analytical methods

#### 2.2.1. Enzymes extraction:

Three g of frozen fresh leaves of plants from each treatment were washed in distilled water and homogenized with 100 mM potassium phosphate buffer (pH 7.5) and 1 mM EDTA. The homogenized plant material was filtered through four layers cheese cloth and centrifuged at 13,000 rpm at 4°C for 10 minutes using a sigma Laboratory Refrigerated Centrifuge. The taken supernatants were kept and used to form enzymatic assays.

#### 2.2.2. Measurement of Malate Dehydrogenase activity:

The measurement of Malate dehydrogenase activity was carried out according to method described by Asahi and Nishimura (1973), where an exact amount of 0.5 ml from each leaf extracts were mixed with 1.5 ml of 94 mM potassium phosphate buffer (pH 8.8), 0.5 ml of 3.4

mMnicotinamide adenine dinucleotide (NAD), and 0.5 ml of 33mM L-malate. the absorbance of each solution was read by Jenway visible spectrophotometer at 340 nm. One unit of enzyme activity for the oxidation of L-malate, is defined as amount that catalyses the conversion of 1 m mol of NAD<sup>+</sup> to NADH, per minute of reaction time at 25 °C. The level of enzyme activity was expressed as mmol NAD destroyed/min/mg protein.

### 2.2.3 Measurement of alcohol dehydrogenase activity:

Alcohol dehydrogenase was assayed in a reaction mixture contained 1 ml of 20 mM potassium phosphate buffer (pH 10.0), 0.5 ml of 1.0 mMnicotinamide adenine dinucleotide(NAD<sup>+</sup>), 0.5 ml of 200 mM (NH<sub>4</sub>)<sub>2</sub> SO<sub>4</sub>, 0.5 ml of 100 mM alcohol and 0.5 ml of leaf extract, in total volume 3 ml. the absorbance was read at 340 nm. The level of enzyme activity was expressed as mmol NAD destroyed/min/mg protein, according to Brady *et al.* (1990).

### 2.2.4. Measurement of glutamate dehydrogenase:

Glutamate dehydrogenase was measured according to method described by Sukalovic (1990) based on the change in absorbance at 340 nm. Assay mixture contained 1ml of 50 mM potassium phosphate buffer (pH 7.4), 0.5 ml of 0.6 mMnicotinamide adenine dinucleotide (NAD), 0.5 ml of 50 mM L-glutamate and 0.5 ml leaf extract in total volume 3 ml. One unit of enzyme activity is defined as the amount of enzyme catalyzing the conversion of 1 mmol NAD to NADH per minute. Enzyme specific activity is expressed as U per mg protein under standard assay condition.

### 2.3. Statistical analysis:

The obtained data was analyzed as complete randomized design with two factors. Analysis of variance (ANOVA) was performed according to procedure described by Gomez and Gomez (1984). Means were separated by using Duncan Multiple Range Test (DMRT)

## 3. RESULTS

The effect of chromium concentrations on malate dehydrogenase activity of ten sorghum genotypes, at 8<sup>th</sup>, 12<sup>th</sup> and 15<sup>th</sup> after chromium application, is shown in table 1, 2 and 3 respectively. Chromium concentration significantly increased malate dehydrogenase activity at 8<sup>th</sup>, 12<sup>th</sup> and 15<sup>th</sup> as compared with control. The 50 ppm indicating a 70, 67 and 350 % increase in malate activity, at 8<sup>th</sup>, 12<sup>th</sup> and 15<sup>th</sup> day, respectively as compared with the control. As for genotypes, the malate activity varied significantly among the ten sorghum genotypes, at different intervals. So far in all sorghum genotypes, the malate activity increased with increasing of the chromium concentrations; except the genotypes L16, L32 and L34 at 8<sup>th</sup> day, L16 and L25 at 12<sup>th</sup> day. The maximum increase was observed at 50 ppm treatment in Tabat genotype, which were (1300%) and (588%) at 8<sup>th</sup> and 12<sup>th</sup> day respectively, and (1675 %) at 15<sup>th</sup> day was scored by L34 genotype. On the other hand, the minimum increase at 50 ppm, of 5.5 % scored by L12, 67 % scored by L34 and 119 % scored by L14, at 8<sup>th</sup>, 12<sup>th</sup> and 15<sup>th</sup> day respectively, as compared with the control. The results obtained in table 4, 5 and 6, illustrated the effect of chromium on alcohol dehydrogenase of ten sorghum genotypes at 8<sup>th</sup>, 12<sup>th</sup> and 15<sup>th</sup> after chromium application

respectively. Hence, the alcohol activity increased significantly, when chromium concentration was increased. The alcohol activity at 50 concentration increased significantly by 52% at 8<sup>th</sup> day, 102.86 % at 12<sup>th</sup> day and 266.67 % at 15<sup>th</sup> day as compared to control. Regarding the sorghum genotypes, the alcohol activity varied significantly among the ten sorghum genotypes at 8<sup>th</sup>, 12<sup>th</sup> and 15<sup>th</sup> day. Moreover, the activity in all genotypes increased with the increased in chromium concentration, except of L12, L16, L32 and L34 at 8<sup>th</sup> day, which within the alcohol activity decreased when chromium concentration was increased. The maximum increase in alcohol activity at 50 ppm treatment, of 1292.31 scored by Tabat at 8<sup>th</sup> day, 222.22 and 528.57 % recorded by Wad Ahmed at 12<sup>th</sup> and 15<sup>th</sup> day respectively. However, the minimum increase at 50 ppm, of 9.68 %, 26.4 and 18.25 scored by L14, L7 and Tabat at 8<sup>th</sup>, 12<sup>th</sup> and 15<sup>th</sup> day respectively, as compared with the control. Table 7, 8 and 9 demonstrated the effect of chromium on glutamate activity of ten sorghum genotypes at 8<sup>th</sup>, 12<sup>th</sup> and 15<sup>th</sup> day respectively. The glutamate activity was significantly increased with increased in chromium concentration at 8<sup>th</sup>, 12<sup>th</sup> and 15<sup>th</sup> day. The 50 ppm indicating a 81.16, 96.97 and 400 % increase in glutamate activity, at 8<sup>th</sup>, 12<sup>th</sup> and 15<sup>th</sup> day respectively, as compared with the control. As for genotypes, the glutamate activity was significantly differing among the ten sorghum genotypes. The level of glutamate activity in all genotypes increased with increasing chromium concentration, with except of L16, (L32 and L34) at 8<sup>th</sup> day and (Tabat and L4) at 12<sup>th</sup> day, wherein the activity of glutamate was inhibited with increased in chromium concentration. The maximum increase in glutamate activity at 50 ppm, 1284.31%, 246.67% and 1883 % were scored by Tabat, L14 and L34 at 8<sup>th</sup> day, 12<sup>th</sup> and 15<sup>th</sup> day respectively. However, the minimum increase at 50 ppm, of 122.22 %, 59.52% and 172% scored by Wad Ahmed, L25 and L32 at 8<sup>th</sup>, 12<sup>th</sup> and 15<sup>th</sup> day respectively, as compared with the control.

## 4. DISCUSSION

The present study revealed that, the level of all enzymes was increase with increase in chromium concentrations. The decline in activity of antioxidant enzyme has been ascribed to inhibition of enzyme biosynthesis and the denaturation of enzyme proteins. This suggested that the activity of antioxidant enzyme recorded in sorghum indicated that the enzymes are play important role in antioxidant defense system to combat the danger posed by the presence of reactive oxygen species (ROS). Similar results were reported by (Samantaray, 1991) who found that Cr adversely affected several physiological activities, produced a severe stress reaction by increasing enzyme activities. Furthermore, Prasad, 1998; Shanker *et al.*, 2003a). Gwozdz *et al.*, (1997) found that at lower heavy metal concentrations, activity of antioxidant enzymes was increased. Also the result indicated that since the level of enzymes activity varied significantly among the ten sorghum genotypes, therefore the sorghum genotypes tolerance to chromium stress may varied. Similar results were reported by (Samantary, 2002) who found that, the enzyme activity varied among the Cr-tolerant and Cr-sensitive ones in mung bean plant. Also Divya (1999) reported a decrease in the activity of ascorbate peroxidase with increase in cadmium concentration in radicle and plumule of pea seedling and

decrease was prominent in susceptible than the tolerant variety.

**Table 1 Effect of chromium on malate dehydrogenase activity (u/mg protein) of the ten sorghum genotypes at the 8<sup>th</sup> day after chromium application**

Chromium concentrations (ppm)	Sorghum genotypes										Chromium means
	Tabat	Wad Ahmed	L4	L7	L12	L14	L16	L25	L32	L34	
0	0.13 <sup>a</sup>	0.89 <sup>a</sup>	0.34 <sup>a</sup>	0.77 <sup>a</sup>	2.36 <sup>a</sup>	0.28 <sup>a</sup>	0.71 <sup>a</sup>	0.26 <sup>a</sup>	1.02 <sup>a</sup>	1.04 <sup>a</sup>	0.78 <sup>a</sup>
2.5	0.59 <sup>a</sup>	0.94 <sup>a</sup>	0.49 <sup>a</sup>	0.54 <sup>a</sup>	1.16 <sup>a</sup>	0.30 <sup>a</sup>	0.83 <sup>a</sup>	0.90 <sup>a</sup>	0.89 <sup>a</sup>	1.00 <sup>a</sup>	0.76 <sup>a</sup>
5	0.82 <sup>a</sup>	0.86 <sup>a</sup>	0.72 <sup>a</sup>	0.96 <sup>a</sup>	1.70 <sup>a</sup>	0.34 <sup>a</sup>	0.76 <sup>a</sup>	1.01 <sup>a</sup>	0.99 <sup>a</sup>	0.62 <sup>a</sup>	0.88 <sup>a</sup>
10	1.26 <sup>a</sup>	1.16 <sup>a</sup>	0.97 <sup>a</sup>	1.24 <sup>a</sup>	1.71 <sup>a</sup>	0.42 <sup>a</sup>	0.57 <sup>a</sup>	1.14 <sup>a</sup>	0.63 <sup>a</sup>	0.30 <sup>a</sup>	0.94 <sup>a</sup>
20	1.27 <sup>a</sup>	1.42 <sup>a</sup>	1.27 <sup>a</sup>	1.81 <sup>a</sup>	1.94 <sup>a</sup>	0.49 <sup>a</sup>	0.47 <sup>a</sup>	1.12 <sup>a</sup>	0.45 <sup>a</sup>	0.33 <sup>a</sup>	1.06 <sup>a</sup>
30	1.50 <sup>a</sup>	1.74 <sup>a</sup>	1.64 <sup>a</sup>	1.90 <sup>a</sup>	2.02 <sup>a</sup>	0.51 <sup>a</sup>	0.33 <sup>a</sup>	1.38 <sup>a</sup>	0.34 <sup>a</sup>	0.27 <sup>a</sup>	1.16 <sup>a</sup>
40	1.34 <sup>a</sup>	1.35 <sup>a</sup>	1.89 <sup>a</sup>	2.02 <sup>a</sup>	2.24 <sup>a</sup>	0.40 <sup>a</sup>	0.22 <sup>a</sup>	1.35 <sup>a</sup>	0.18 <sup>a</sup>	0.22 <sup>a</sup>	1.12 <sup>a</sup>
50	1.82 <sup>a</sup>	1.51 <sup>a</sup>	2.57 <sup>a</sup>	2.30 <sup>a</sup>	2.49 <sup>a</sup>	0.56 <sup>a</sup>	0.16 <sup>a</sup>	1.40 <sup>a</sup>	0.23 <sup>a</sup>	0.26 <sup>a</sup>	1.33 <sup>a</sup>
Genotyps mean	1.09 <sup>bc</sup>	1.23 <sup>b</sup>	1.24 <sup>b</sup>	1.44 <sup>ab</sup>	1.95 <sup>a</sup>	0.41 <sup>d</sup>	0.51 <sup>d</sup>	1.07 <sup>bc</sup>	0.59 <sup>cd</sup>	0.51 <sup>d</sup>	

Means followed by the same letters are not significantly different at 0.05 level of probability according to DMRT.

**Table 2 Effect of chromium on malate dehydrogenase activity (u/mg protein) of the ten sorghum genotypes at the 12<sup>th</sup> day after chromium application**

Chromium concentrations (ppm)	Sorghum genotypes										Chromium means
	Tabat	Wad Ahmed	L4	L7	L12	L14	L16	L25	L32	L34	
0	0.09 <sup>a</sup>	0.36 <sup>a</sup>	0.25 <sup>a</sup>	1.41 <sup>a</sup>	0.27 <sup>a</sup>	0.20 <sup>a</sup>	0.25 <sup>a</sup>	0.57 <sup>a</sup>	0.34 <sup>a</sup>	0.53 <sup>a</sup>	0.43 <sup>a</sup>
2.5	0.23 <sup>a</sup>	0.41 <sup>a</sup>	1.71 <sup>a</sup>	0.92 <sup>a</sup>	0.43 <sup>a</sup>	0.18 <sup>a</sup>	0.25 <sup>a</sup>	0.56 <sup>a</sup>	0.50 <sup>a</sup>	0.77 <sup>a</sup>	0.60 <sup>a</sup>
5	0.24 <sup>a</sup>	0.45 <sup>a</sup>	0.46 <sup>a</sup>	0.97 <sup>a</sup>	0.35 <sup>a</sup>	0.21 <sup>a</sup>	0.23 <sup>a</sup>	0.46 <sup>a</sup>	0.42 <sup>a</sup>	0.79 <sup>a</sup>	0.46 <sup>a</sup>
10	0.25 <sup>a</sup>	0.52 <sup>a</sup>	0.52 <sup>a</sup>	1.13 <sup>a</sup>	0.35 <sup>a</sup>	0.32 <sup>a</sup>	0.25 <sup>a</sup>	0.42 <sup>a</sup>	0.44 <sup>a</sup>	0.85 <sup>a</sup>	0.50 <sup>a</sup>
20	0.29 <sup>a</sup>	0.46 <sup>a</sup>	0.51 <sup>a</sup>	1.23 <sup>a</sup>	0.45 <sup>a</sup>	0.51 <sup>a</sup>	0.27 <sup>a</sup>	0.34 <sup>a</sup>	0.76 <sup>a</sup>	0.86 <sup>a</sup>	0.57 <sup>a</sup>
30	0.33 <sup>a</sup>	0.48 <sup>a</sup>	0.60 <sup>a</sup>	1.30 <sup>a</sup>	0.45 <sup>a</sup>	0.61 <sup>a</sup>	0.30 <sup>a</sup>	0.28 <sup>a</sup>	0.78 <sup>a</sup>	0.88 <sup>a</sup>	0.60 <sup>a</sup>
40	0.36 <sup>a</sup>	0.50 <sup>a</sup>	0.57 <sup>a</sup>	1.38 <sup>a</sup>	0.54 <sup>a</sup>	0.66 <sup>a</sup>	0.59 <sup>a</sup>	0.26 <sup>a</sup>	0.73 <sup>a</sup>	0.90 <sup>a</sup>	0.65 <sup>a</sup>
50	0.62 <sup>a</sup>	0.90 <sup>a</sup>	0.57 <sup>a</sup>	1.46 <sup>a</sup>	0.46 <sup>a</sup>	0.57 <sup>a</sup>	0.66 <sup>a</sup>	0.28 <sup>a</sup>	0.81 <sup>a</sup>	0.89 <sup>a</sup>	0.72 <sup>a</sup>
Genotypes means	0.30 <sup>e</sup>	0.51 <sup>cde</sup>	0.65 <sup>bc</sup>	1.22 <sup>a</sup>	0.41 <sup>cde</sup>	0.41 <sup>cde</sup>	0.35 <sup>de</sup>	0.39 <sup>cde</sup>	0.60 <sup>bcd</sup>	0.81 <sup>b</sup>	

Means followed by the same letters are not significantly different at 0.05 level of probability according to DMRT.

**Table 3 Effect of chromium on malate dehydrogenase activity (u/mg protein) of the ten sorghum genotypes at the 15<sup>th</sup> day after chromium application**

Chromium concentrations (ppm)	Sorghum genotypes										Chromium means
	Tabat	Wad Ahmed	L4	L7	L12	L14	L16	L25	L32	L34	
0	0.06 <sup>a</sup>	0.11 <sup>a</sup>	0.13 <sup>a</sup>	0.09 <sup>a</sup>	0.34 <sup>a</sup>	0.21 <sup>a</sup>	0.13 <sup>a</sup>	0.29 <sup>a</sup>	0.22 <sup>a</sup>	0.07 <sup>a</sup>	0.16 <sup>f</sup>
2.5	0.03 <sup>a</sup>	0.18 <sup>a</sup>	0.19 <sup>a</sup>	0.18 <sup>a</sup>	0.32 <sup>a</sup>	0.21 <sup>a</sup>	0.16 <sup>a</sup>	0.32 <sup>a</sup>	0.26 <sup>a</sup>	0.20 <sup>a</sup>	0.20 <sup>e</sup>
5	0.04 <sup>a</sup>	0.16 <sup>a</sup>	0.24 <sup>a</sup>	0.29 <sup>a</sup>	0.24 <sup>a</sup>	0.29 <sup>a</sup>	0.15 <sup>a</sup>	0.32 <sup>a</sup>	0.33 <sup>a</sup>	0.22 <sup>a</sup>	0.23 <sup>d</sup>
10	0.05 <sup>a</sup>	0.18 <sup>a</sup>	0.28 <sup>a</sup>	0.29 <sup>a</sup>	0.22 <sup>a</sup>	0.27 <sup>a</sup>	0.37 <sup>a</sup>	0.33 <sup>a</sup>	0.39 <sup>a</sup>	0.39 <sup>a</sup>	0.28 <sup>d</sup>
20	0.05 <sup>a</sup>	0.23 <sup>a</sup>	0.29 <sup>a</sup>	0.34 <sup>a</sup>	0.24 <sup>a</sup>	0.30 <sup>a</sup>	0.79 <sup>a</sup>	0.45 <sup>a</sup>	0.41 <sup>a</sup>	0.37 <sup>a</sup>	0.35 <sup>c</sup>
30	0.09 <sup>a</sup>	0.35 <sup>a</sup>	0.35 <sup>a</sup>	0.36 <sup>a</sup>	0.60 <sup>a</sup>	0.29 <sup>a</sup>	0.83 <sup>a</sup>	0.40 <sup>a</sup>	0.40 <sup>a</sup>	0.82 <sup>a</sup>	0.45 <sup>b</sup>
40	0.14 <sup>a</sup>	0.42 <sup>a</sup>	0.42 <sup>a</sup>	0.33 <sup>a</sup>	0.70 <sup>a</sup>	0.34 <sup>a</sup>	0.42 <sup>a</sup>	0.78 <sup>a</sup>	0.42 <sup>a</sup>	0.96 <sup>a</sup>	0.49 <sup>b</sup>
50	0.30 <sup>a</sup>	0.57 <sup>a</sup>	0.62 <sup>a</sup>	0.41 <sup>a</sup>	1.50 <sup>a</sup>	0.46 <sup>a</sup>	0.61 <sup>a</sup>	0.90 <sup>a</sup>	0.60 <sup>a</sup>	1.23 <sup>a</sup>	0.72 <sup>a</sup>
Genotypes means	0.10 <sup>e</sup>	0.27 <sup>cd</sup>	0.32 <sup>abc</sup>	0.29 <sup>bcd</sup>	0.52 <sup>ab</sup>	0.29 <sup>bcd</sup>	0.43 <sup>abc</sup>	0.47 <sup>abc</sup>	0.38 <sup>abc</sup>	0.53 <sup>a</sup>	

Means followed by the same letters are not significantly different at 0.05 level of probability according to DMRT.

**Table 4 Effect of chromium on alcohol dehydrogenase activity (u/mg protein) of the ten sorghum genotypes at the 8<sup>th</sup> day after chromium application**

Chromium concentrations (ppm)	Sorghum genotypes										Chromium means
	Tabat	Wad Ahmed	L4	L7	L12	L14	L16	L25	L32	L34	
0	0.13 <sup>a</sup>	0.75 <sup>a</sup>	0.35 <sup>a</sup>	0.39 <sup>a</sup>	2.87 <sup>a</sup>	0.31 <sup>a</sup>	0.76 <sup>a</sup>	0.35 <sup>a</sup>	0.78 <sup>a</sup>	0.78 <sup>a</sup>	0.75 <sup>a</sup>
2.5	0.58 <sup>a</sup>	0.96 <sup>a</sup>	0.84 <sup>a</sup>	0.79 <sup>a</sup>	1.06 <sup>a</sup>	0.28 <sup>a</sup>	0.93 <sup>a</sup>	0.74 <sup>a</sup>	0.79 <sup>a</sup>	0.63 <sup>a</sup>	0.76 <sup>a</sup>
5	0.93 <sup>a</sup>	0.82 <sup>a</sup>	1.31 <sup>a</sup>	1.09 <sup>a</sup>	0.82 <sup>a</sup>	0.34 <sup>a</sup>	0.52 <sup>a</sup>	0.75 <sup>a</sup>	0.61 <sup>a</sup>	0.53 <sup>a</sup>	0.77 <sup>a</sup>
10	1.63 <sup>a</sup>	0.80 <sup>a</sup>	1.40 <sup>a</sup>	1.25 <sup>a</sup>	0.87 <sup>a</sup>	0.24 <sup>a</sup>	0.41 <sup>a</sup>	0.90 <sup>a</sup>	0.30 <sup>a</sup>	0.22 <sup>a</sup>	0.80 <sup>a</sup>
20	1.66 <sup>a</sup>	1.44 <sup>a</sup>	1.50 <sup>a</sup>	1.77 <sup>a</sup>	0.54 <sup>a</sup>	0.23 <sup>a</sup>	0.39 <sup>a</sup>	0.74 <sup>a</sup>	0.38 <sup>a</sup>	0.26 <sup>a</sup>	0.89 <sup>a</sup>
30	1.57 <sup>a</sup>	1.48 <sup>a</sup>	1.39 <sup>a</sup>	1.74 <sup>a</sup>	0.36 <sup>a</sup>	0.24 <sup>a</sup>	0.62 <sup>a</sup>	0.76 <sup>a</sup>	0.36 <sup>a</sup>	0.20 <sup>a</sup>	0.87 <sup>a</sup>
40	1.86 <sup>a</sup>	1.35 <sup>a</sup>	1.61 <sup>a</sup>	2.01 <sup>a</sup>	0.34 <sup>a</sup>	0.44 <sup>a</sup>	0.59 <sup>a</sup>	0.72 <sup>a</sup>	0.16 <sup>a</sup>	0.19 <sup>a</sup>	0.93 <sup>a</sup>
50	1.81 <sup>a</sup>	1.66 <sup>a</sup>	2.66 <sup>a</sup>	2.58 <sup>a</sup>	0.39 <sup>a</sup>	0.34 <sup>a</sup>	0.73 <sup>a</sup>	0.82 <sup>a</sup>	0.24 <sup>a</sup>	0.21 <sup>a</sup>	1.14 <sup>a</sup>
Genotypes means	1.27 <sup>ab</sup>	1.16 <sup>abc</sup>	1.38 <sup>a</sup>	1.45 <sup>a</sup>	0.91 <sup>abcd</sup>	0.30 <sup>e</sup>	0.62 <sup>cde</sup>	0.72 <sup>bcde</sup>	0.45 <sup>de</sup>	0.38 <sup>de</sup>	

Means followed by the same letters are not significantly different at 0.05 level of probability according to DMRT.

**Table 5 Effect of chromium on alcohol dehydrogenase activity (u/mg protein) of the ten sorghum genotypes at the 12<sup>th</sup> day after chromium application**

Chromium concentrations (ppm)	Sorghum genotypes										Chromium means
	Tabat	Wad Ahmed	L4	L7	L12	L14	L16	L25	L32	L34	
0	0.23 <sup>a</sup>	0.27 <sup>a</sup>	0.36 <sup>a</sup>	0.68 <sup>a</sup>	0.24 <sup>a</sup>	0.15 <sup>a</sup>	0.30 <sup>a</sup>	0.30 <sup>a</sup>	0.44 <sup>a</sup>	0.54 <sup>a</sup>	0.35 <sup>c</sup>
2.5	0.23 <sup>a</sup>	0.25 <sup>a</sup>	0.40 <sup>a</sup>	0.64 <sup>a</sup>	0.38 <sup>a</sup>	0.22 <sup>a</sup>	0.34 <sup>a</sup>	0.34 <sup>a</sup>	0.44 <sup>a</sup>	0.68 <sup>a</sup>	0.39 <sup>c</sup>
5	0.24 <sup>a</sup>	0.43 <sup>a</sup>	0.47 <sup>a</sup>	0.71 <sup>a</sup>	0.34 <sup>a</sup>	0.20 <sup>a</sup>	0.17 <sup>a</sup>	0.40 <sup>a</sup>	0.29 <sup>a</sup>	0.96 <sup>a</sup>	0.42 <sup>c</sup>
10	0.26 <sup>a</sup>	0.40 <sup>a</sup>	0.48 <sup>a</sup>	0.70 <sup>a</sup>	0.43 <sup>a</sup>	0.29 <sup>a</sup>	0.20 <sup>a</sup>	0.38 <sup>a</sup>	0.34 <sup>a</sup>	0.80 <sup>a</sup>	0.43 <sup>c</sup>
20	0.27 <sup>a</sup>	0.34 <sup>a</sup>	0.48 <sup>a</sup>	0.75 <sup>a</sup>	0.46 <sup>a</sup>	0.32 <sup>a</sup>	0.25 <sup>a</sup>	0.33 <sup>a</sup>	0.38 <sup>a</sup>	0.71 <sup>a</sup>	0.43 <sup>c</sup>
30	0.36 <sup>a</sup>	0.42 <sup>a</sup>	0.51 <sup>a</sup>	0.74 <sup>a</sup>	0.53 <sup>a</sup>	0.40 <sup>a</sup>	0.49 <sup>a</sup>	0.38 <sup>a</sup>	0.46 <sup>a</sup>	0.83 <sup>a</sup>	0.51 <sup>bc</sup>
40	0.40 <sup>a</sup>	0.62 <sup>a</sup>	0.53 <sup>a</sup>	0.71 <sup>a</sup>	0.50 <sup>a</sup>	0.43 <sup>a</sup>	0.82 <sup>a</sup>	0.49 <sup>a</sup>	0.63 <sup>a</sup>	0.91 <sup>a</sup>	0.61 <sup>ab</sup>
50	0.52 <sup>a</sup>	0.87 <sup>a</sup>	0.57 <sup>a</sup>	0.86 <sup>a</sup>	0.55 <sup>a</sup>	0.38 <sup>a</sup>	0.42 <sup>a</sup>	0.65 <sup>a</sup>	0.89 <sup>a</sup>	1.43 <sup>a</sup>	0.71 <sup>a</sup>
Genotypes means	0.31 <sup>b</sup>	0.45 <sup>b</sup>	0.48 <sup>b</sup>	0.72 <sup>a</sup>	0.43 <sup>b</sup>	0.30 <sup>b</sup>	0.37 <sup>b</sup>	0.41 <sup>b</sup>	0.48 <sup>b</sup>	0.86 <sup>a</sup>	

Means followed by the same letters are not significantly different at 0.05 level of probability according to DMRT.

**Table 6 Effect of chromium on alcohol dehydrogenase activity (u/mg protein) of the ten sorghum genotypes at the 15<sup>th</sup> day after chromium application**

Chromium concentrations (ppm)	Sorghum genotypes										Chromium means
	Tabat	Wad Ahmed	L4	L7	L12	L14	L16	L25	L32	L34	
0	0.16 <sup>a</sup>	0.14 <sup>a</sup>	0.14 <sup>a</sup>	0.24 <sup>a</sup>	0.31 <sup>a</sup>	0.18 <sup>a</sup>	0.16 <sup>a</sup>	0.32 <sup>a</sup>	0.24 <sup>a</sup>	0.17 <sup>a</sup>	0.21 <sup>d</sup>
2.5	0.15 <sup>a</sup>	0.15 <sup>a</sup>	0.16 <sup>a</sup>	0.30 <sup>a</sup>	0.33 <sup>a</sup>	0.17 <sup>a</sup>	0.14 <sup>a</sup>	0.39 <sup>a</sup>	0.34 <sup>a</sup>	0.24 <sup>a</sup>	0.24 <sup>d</sup>
5	0.31 <sup>a</sup>	0.31 <sup>a</sup>	0.22 <sup>a</sup>	0.38 <sup>a</sup>	0.39 <sup>a</sup>	0.27 <sup>a</sup>	0.16 <sup>a</sup>	0.40 <sup>a</sup>	0.41 <sup>a</sup>	0.25 <sup>a</sup>	0.31 <sup>cd</sup>
10	0.29 <sup>a</sup>	0.38 <sup>a</sup>	0.27 <sup>a</sup>	0.42 <sup>a</sup>	0.36 <sup>a</sup>	0.34 <sup>a</sup>	0.16 <sup>a</sup>	0.45 <sup>a</sup>	0.45 <sup>a</sup>	0.34 <sup>a</sup>	0.35 <sup>cd</sup>
20	0.32 <sup>a</sup>	0.40 <sup>a</sup>	0.23 <sup>a</sup>	0.44 <sup>a</sup>	0.41 <sup>a</sup>	0.41 <sup>a</sup>	0.65 <sup>a</sup>	0.49 <sup>a</sup>	0.49 <sup>a</sup>	0.35 <sup>a</sup>	0.42 <sup>bcd</sup>
30	0.43 <sup>a</sup>	0.44 <sup>a</sup>	0.29 <sup>a</sup>	0.43 <sup>a</sup>	0.28 <sup>a</sup>	0.40 <sup>a</sup>	0.79 <sup>a</sup>	0.42 <sup>a</sup>	0.49 <sup>a</sup>	0.81 <sup>a</sup>	0.48 <sup>abc</sup>
40	0.36 <sup>a</sup>	0.55 <sup>a</sup>	0.41 <sup>a</sup>	0.44 <sup>a</sup>	0.73 <sup>a</sup>	0.69 <sup>a</sup>	0.50 <sup>a</sup>	1.03 <sup>a</sup>	0.48 <sup>a</sup>	0.90 <sup>a</sup>	0.61 <sup>ab</sup>
50	0.29 <sup>a</sup>	0.88 <sup>a</sup>	0.49 <sup>a</sup>	0.50 <sup>a</sup>	1.53 <sup>a</sup>	0.74 <sup>a</sup>	0.74 <sup>a</sup>	0.91 <sup>a</sup>	0.67 <sup>a</sup>	0.94 <sup>a</sup>	0.77 <sup>a</sup>
Genotypes means	0.29 <sup>a</sup>	0.41 <sup>a</sup>	0.28 <sup>a</sup>	0.39 <sup>a</sup>	0.54 <sup>a</sup>	0.40 <sup>a</sup>	0.41 <sup>a</sup>	0.55 <sup>a</sup>	0.45 <sup>a</sup>	0.50 <sup>a</sup>	

Means followed by the same letters are not significantly different at 0.05 level of probability according to DMRT.

**Table 7 Effect of chromium on glutamate dehydrogenase activity (u/mg protein) of the ten sorghum genotypes at the 8<sup>th</sup> day after chromium application**

Chromium concentrations (ppm)	Sorghum genotypes										Chromium means
	Tabat	Wad Ahmed	L4	L7	L12	L14	L16	L25	L32	L34	
0	0.13 <sup>a</sup>	0.99 <sup>a</sup>	0.35 <sup>a</sup>	0.48 <sup>a</sup>	1.32 <sup>a</sup>	0.28 <sup>a</sup>	0.72 <sup>a</sup>	0.35 <sup>a</sup>	1.42 <sup>a</sup>	0.82 <sup>a</sup>	0.69 <sup>c</sup>
2.5	0.57 <sup>a</sup>	0.99 <sup>a</sup>	1.11 <sup>a</sup>	0.66 <sup>a</sup>	0.78 <sup>a</sup>	0.38 <sup>a</sup>	0.70 <sup>a</sup>	0.88 <sup>a</sup>	0.81 <sup>a</sup>	0.56 <sup>a</sup>	0.74 <sup>bc</sup>
5	0.78 <sup>a</sup>	1.05 <sup>a</sup>	1.72 <sup>a</sup>	0.75 <sup>a</sup>	0.73 <sup>a</sup>	0.33 <sup>a</sup>	0.69 <sup>a</sup>	0.97 <sup>a</sup>	0.79 <sup>a</sup>	0.51 <sup>a</sup>	0.83 <sup>bc</sup>
10	0.80 <sup>a</sup>	1.09 <sup>a</sup>	1.35 <sup>a</sup>	1.48 <sup>a</sup>	0.73 <sup>a</sup>	0.30 <sup>a</sup>	0.47 <sup>a</sup>	1.12 <sup>a</sup>	0.50 <sup>a</sup>	0.27 <sup>a</sup>	0.81 <sup>bc</sup>
20	0.86 <sup>a</sup>	1.50 <sup>a</sup>	1.38 <sup>a</sup>	2.09 <sup>a</sup>	0.55 <sup>a</sup>	0.41 <sup>a</sup>	0.48 <sup>a</sup>	1.05 <sup>a</sup>	0.45 <sup>a</sup>	0.31 <sup>a</sup>	0.91 <sup>abc</sup>
30	1.30 <sup>a</sup>	1.53 <sup>a</sup>	2.09 <sup>a</sup>	2.36 <sup>a</sup>	0.49 <sup>a</sup>	0.46 <sup>a</sup>	0.44 <sup>a</sup>	1.17 <sup>a</sup>	0.46 <sup>a</sup>	0.21 <sup>a</sup>	1.05 <sup>abc</sup>
40	1.80 <sup>a</sup>	1.48 <sup>a</sup>	2.34 <sup>a</sup>	2.38 <sup>a</sup>	0.50 <sup>a</sup>	0.64 <sup>a</sup>	0.35 <sup>a</sup>	1.21 <sup>a</sup>	0.20 <sup>a</sup>	0.22 <sup>a</sup>	1.11 <sup>ab</sup>
50	1.80 <sup>a</sup>	2.20 <sup>a</sup>	2.47 <sup>a</sup>	2.69 <sup>a</sup>	0.47 <sup>a</sup>	0.68 <sup>a</sup>	0.23 <sup>a</sup>	1.45 <sup>a</sup>	0.22 <sup>a</sup>	0.32 <sup>a</sup>	1.25 <sup>a</sup>
Genotypes means	1.01 <sup>bc</sup>	1.35 <sup>ab</sup>	1.60 <sup>a</sup>	1.61 <sup>a</sup>	0.70 <sup>cd</sup>	0.43 <sup>d</sup>	0.51 <sup>d</sup>	1.03 <sup>bc</sup>	0.61 <sup>cd</sup>	0.40 <sup>d</sup>	

Means followed by the same letters are not significantly different at 0.05 level of probability according to DMRT.

**Table 8 Effect of chromium on glutamate dehydrogenase activity (u/mg protein) of the ten sorghum genotypes at the 12<sup>th</sup> day after chromium application**

Chromium concentrations (ppm)	Sorghum genotypes										Chromium means
	Tabat	Wad ahmed	L4	L7	L12	L14	L16	L25	L32	L34	
0	0.34 <sup>a</sup>	0.19 <sup>a</sup>	0.47 <sup>a</sup>	0.47 <sup>a</sup>	0.28 <sup>a</sup>	0.15 <sup>a</sup>	0.31 <sup>a</sup>	0.42 <sup>a</sup>	0.29 <sup>a</sup>	0.42 <sup>a</sup>	0.33 <sup>c</sup>
2.5	0.30 <sup>a</sup>	0.23 <sup>a</sup>	0.43 <sup>a</sup>	0.66 <sup>a</sup>	0.44 <sup>a</sup>	0.19 <sup>a</sup>	0.42 <sup>a</sup>	0.47 <sup>a</sup>	0.39 <sup>a</sup>	0.79 <sup>a</sup>	0.43 <sup>bc</sup>
5	0.26 <sup>a</sup>	0.46 <sup>a</sup>	0.42 <sup>a</sup>	0.76 <sup>a</sup>	0.42 <sup>a</sup>	0.21 <sup>a</sup>	0.36 <sup>a</sup>	0.43 <sup>a</sup>	0.29 <sup>a</sup>	0.97 <sup>a</sup>	0.46 <sup>bc</sup>
10	0.24 <sup>a</sup>	0.44 <sup>a</sup>	0.37 <sup>a</sup>	0.67 <sup>a</sup>	0.53 <sup>a</sup>	0.33 <sup>a</sup>	0.37 <sup>a</sup>	0.50 <sup>a</sup>	0.38 <sup>a</sup>	0.89 <sup>a</sup>	0.47 <sup>bc</sup>
20	0.23 <sup>a</sup>	0.52 <sup>a</sup>	0.32 <sup>a</sup>	0.85 <sup>a</sup>	0.54 <sup>a</sup>	0.36 <sup>a</sup>	0.37 <sup>a</sup>	0.49 <sup>a</sup>	0.40 <sup>a</sup>	0.75 <sup>a</sup>	0.48 <sup>bc</sup>
30	0.21 <sup>a</sup>	0.54 <sup>a</sup>	0.32 <sup>a</sup>	0.83 <sup>a</sup>	0.61 <sup>a</sup>	0.45 <sup>a</sup>	0.38 <sup>a</sup>	0.49 <sup>a</sup>	0.66 <sup>a</sup>	1.04 <sup>a</sup>	0.55 <sup>ab</sup>
40	0.19 <sup>a</sup>	0.54 <sup>a</sup>	0.31 <sup>a</sup>	0.86 <sup>a</sup>	0.61 <sup>a</sup>	0.51 <sup>a</sup>	0.55 <sup>a</sup>	0.52 <sup>a</sup>	0.64 <sup>a</sup>	1.08 <sup>a</sup>	0.58 <sup>ab</sup>
50	0.17 <sup>a</sup>	0.63 <sup>a</sup>	0.31 <sup>a</sup>	0.90 <sup>a</sup>	0.59 <sup>a</sup>	0.52 <sup>a</sup>	0.65 <sup>a</sup>	0.67 <sup>a</sup>	0.74 <sup>a</sup>	1.33 <sup>a</sup>	0.65 <sup>a</sup>
Genotypes means	0.24 <sup>c</sup>	0.44 <sup>b</sup>	0.37 <sup>bc</sup>	0.75 <sup>a</sup>	0.50 <sup>b</sup>	0.34 <sup>bc</sup>	0.43 <sup>b</sup>	0.50 <sup>b</sup>	0.47 <sup>b</sup>	0.91 <sup>a</sup>	

Means followed by the same letters are not significantly different at 0.05 level of probability according to DMRT.

**Table 9 Effect of chromium on glutamate dehydrogenase activity (u/mg protein) of the ten sorghum genotypes at the 15<sup>th</sup> day after chromium application**

Chromium concentrations (ppm)	Sorghum genotypes										Chromium means
	Tabat	Wad Ahmed	L4	L7	L12	L14	L16	L25	L32	L34	
0	0.07 <sup>a</sup>	0.15 <sup>a</sup>	0.12 <sup>a</sup>	0.11 <sup>a</sup>	0.28 <sup>a</sup>	0.16 <sup>a</sup>	0.13 <sup>a</sup>	0.29 <sup>a</sup>	0.25 <sup>a</sup>	0.06 <sup>a</sup>	0.16 <sup>d</sup>
2.5	0.06 <sup>a</sup>	0.17 <sup>a</sup>	0.16 <sup>a</sup>	0.29 <sup>a</sup>	0.29 <sup>a</sup>	0.15 <sup>a</sup>	0.16 <sup>a</sup>	0.36 <sup>a</sup>	0.28 <sup>a</sup>	0.26 <sup>a</sup>	0.22 <sup>d</sup>
5	0.10 <sup>a</sup>	0.25 <sup>a</sup>	0.23 <sup>a</sup>	0.28 <sup>a</sup>	0.35 <sup>a</sup>	0.28 <sup>a</sup>	0.14 <sup>a</sup>	0.54 <sup>a</sup>	0.37 <sup>a</sup>	0.32 <sup>a</sup>	0.29 <sup>cd</sup>
10	0.10 <sup>a</sup>	0.45 <sup>a</sup>	0.26 <sup>a</sup>	0.34 <sup>a</sup>	0.33 <sup>a</sup>	0.29 <sup>a</sup>	0.41 <sup>a</sup>	0.42 <sup>a</sup>	0.34 <sup>a</sup>	0.37 <sup>a</sup>	0.33 <sup>cd</sup>
20	0.10 <sup>a</sup>	0.51 <sup>a</sup>	0.25 <sup>a</sup>	0.34 <sup>a</sup>	0.35 <sup>a</sup>	0.41 <sup>a</sup>	0.59 <sup>a</sup>	0.42 <sup>a</sup>	0.35 <sup>a</sup>	0.34 <sup>a</sup>	0.37 <sup>cd</sup>
30	0.14 <sup>a</sup>	0.65 <sup>a</sup>	0.31 <sup>a</sup>	0.34 <sup>a</sup>	0.59 <sup>a</sup>	0.45 <sup>a</sup>	0.65 <sup>a</sup>	0.44 <sup>a</sup>	0.40 <sup>a</sup>	0.78 <sup>aa</sup>	0.47 <sup>bc</sup>
40	0.16 <sup>a</sup>	0.82 <sup>a</sup>	0.51 <sup>a</sup>	0.33 <sup>a</sup>	0.70 <sup>a</sup>	0.87 <sup>a</sup>	0.56 <sup>a</sup>	0.96 <sup>a</sup>	0.44 <sup>a</sup>	0.96 <sup>a</sup>	0.63 <sup>ab</sup>
50	0.27 <sup>a</sup>	0.86 <sup>a</sup>	0.52 <sup>a</sup>	0.38 <sup>a</sup>	1.48 <sup>a</sup>	0.76 <sup>a</sup>	0.81 <sup>a</sup>	1.08 <sup>a</sup>	0.68 <sup>a</sup>	1.19 <sup>a</sup>	0.80 <sup>a</sup>
Genotypes means	0.13 <sup>d</sup>	0.48 <sup>avc</sup>	0.30 <sup>cd</sup>	0.30 <sup>cd</sup>	0.55 <sup>a</sup>	0.42 <sup>abv</sup>	0.43 <sup>abc</sup>	0.57 <sup>a</sup>	0.39 <sup>abc</sup>	0.53 <sup>ab</sup>	

Means followed by the same letters are not significantly different at 0.05 level of probability according to DMRT.

## ONCULSION

The present results suggest that, the activities of antioxidant enzymes we recorded in sorghum indicate that the enzymes are engaged in antioxidant defense as compared to the control. Modification of the plant antioxidant defense system has been reported to enhance tolerance to oxidative stress. Alteration of antioxidant enzymes may be due to the synthesis of new isoenzymes or enhancement of the activity of pre-existing enzymes for the metabolism of ROS. The varied among sorghum genotypes in enzymes activity indicated that the sorghum genotypes varied in tolerance to chromium stress, and this helps for selecting genotypes have a high efficiency to remediate chromium contaminated soil.

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