Scientific Journal for Damietta Faculty of Science 5 (1) 2015, 117-123 ISSN Print 2314-8594
ISSN Online 2314-8616

Enhanced Catechol Oxidase and Peroxidase Activities as Possible Markers for Measuring Chromium Tolerance in Sorghum (Sorghum bicolor L.) Genotypes

Hajo Elzein Elhassan [1*], Abdel Wahab Hassan Abdalla[2], El Busra El Shiekh. El Nur[3]

[1] Environment, Natural Resources and Desertification Research Institute, National Centre for Research P. O. Box 6096 Khartoum Sudan
[2] Department of Agronomy, Faculty of Agriculture, University of Khartoum, Khartoum, Sudan
[3] Department of Botany, Faculty of Science, University of Khartoum, Khartoum, Sudan

Received: 24 May 2015 / Accepted: 20 September 2015

[*] Corresponding author: elhassanhajo@yahoo.com

Abstract

The aim of the present study was to estimate the effect of chromium on catechol oxidase and peroxidase activities in ten sorghum genotypes grown in soil amended by different concentrations of chromium. 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., Seeds were sown in polyethylene bags filled with 2 kg soil (clay and sand, 2:1). Two weeks after sowing, the seedlings were irrigated with eight levels of chromium (Cr-VI) concentrations (0, 2.5, 5, 10, 20, 30, 40, and 50 mg/l.). Samples for assaying enzyme (catechol oxidase and peroxidase) activities were taken three times (on the 8, 12, and 15 day) following chromium application. The results revealed that the level of enzyme activity of most genotypes was increased significantly with increasing chromium concentration. The magnitude of increase in enzymatic activity was also dependent on the genotype and duration of time after chromium treatment. These findings suggest that, the activities of both enzymes might be used as indicator for selecting chromium tolerant sorghum genotypes.

Keywords: chromium, catechol oxidase, peroxidase, sorghum, genotypes.

Introduction

Chromium is important for metallurgical industry. Chromium salts are used in many industrial processes and product such as, steel production, electroplating, leather tanning, metal finishing, inhibition of metal corrosion, textile paints and pigment manufacture, catalysts applications, drilling muds, fungicides and nuclear weapons production. Therefore, chromium salts are frequently present in effluents of those industries and in municipal sewage (Wong *et al.*, 2001; Zayed and Terry 2003; Nath *et al.*, 2005; Babel and Opiso, 2007; Venkateswaran *et al.*, 2007)

Chromium (Cr) occurs in nature in bound forms that constitute 0.1- 0.3 mg kg^{-1} of the Earth's crust and has several oxidation states from Cr(-II) to Cr(+VI) (Zayed and Terry, 2003). It is unique among the heavy metals because of its existence in two environmentally important oxidation states: trivalent (Cr III) and hexavalent (Cr VI) (Srivastava *et al.*, 1999). Chromium is recognized as an essential element for humans and animals (Mertz, 1967), but not for plants (Huffman and Allaway, 1973; Liu *et al*., 1992), although some investigations report that it is beneficial to plant growth (Zheng *et al.*, 1987).

Symptoms of Cr phytotoxicity include inhibition of seed germination or of early seedling development, reduction of root growth, leaf chlorosis and depressed biomass (Sharma *et al*., 1995). Chromium significantly affects the metabolism of plants such as barley (*Hordeumvulgare*) (Ali *et al*., 2004), citrullus (Dube *et al*., 2003), cauliflower (Chatterjee and Chatterjee, 2000), vegetable crops (Zayed *et al*., 1998), wheat (*Triticum aestivum*cv. HD2204) (Sharma *et al*., 1995) and maize (*Zea mays*) (Sharma and Pant, 1994). Chromium toxicity in plants also leads to leaves chlorosis, tissue necrosis, decreases enzyme activity, causes membrane damage, diminished photosynthesis and changing of chloroplast (Jain *et al.,* 2000; Parmar *et al.*, 2002; Du *etal.,* 2003; Dube *et al.*, 2003; Zayed and Terry 2003; Scoccianti *et al.*, 2006).Therefore the aim of the present study was to investigate the effects of different concentrations of chromium on catechol oxidase and proxidase activity in ten sorghum genotypes seedling.

Materials and Methods

Seeds of 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. Seeds were germinated, on the third week of July, in polyethylene bags; each filled with 2 kg soil consisted of a mixture of clay and sand (2: 1). Eight chromium concentrations (0, 2.5, 5, 10, 20, 30, 40, and 50 mg l^{-1}.) were applied, using potassium dichromate as a source of chromium. Six seeds per pot were sown on the third week of July 2011 and routinely irrigated with tap water. Growth conditions two weeks after sowing, the plants were irrigated with the differences concentrations of chromium. The data were taken on $8^{th,}$ 12^{th}, and 15^{th} day after chromium application to measure the activities of catechol oxidase and peroxidase enzymes. Enzymes were extracted from plant leaves (second fully expanded leaf from the top) at harvest

Enzymes extraction:

Three g of fresh leaves of plants from each genotype were homogenized with 100 mM potassium phosphate buffer (pH 7.5) and 1 mM EDTA. The homogenate tissue was filtered through four layers of cheesecloth and then centrifuged at 13,000 rpm at 4°C for 10 minutes, using a Sigma Laboratory Refrigerated Centrifuge. The supernatant was used for the enzyme assays. Protein content was determined using bovine serum albumin as standard Bradford (1976).

Catechol oxidase (1.10.3.1):

Catechol oxidase activity was determined using substrate concentration as 3 mM catechol in 50 mM potassium phosphate buffer (pH 6.5) at 25° C by monitoring the increase of absorbance at 420 nm. Enzyme specific activity is expressed as U per mg protein (Tremoliere and Bieth1984)

Peroxidase (1.11.1.7):

Peroxidase activity was measured [19]. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, 40 mM H_2O_2, and 0.5 mM pyrogallol and 0.5 ml enzymes with final volume 3ml. Absorbance was read at 430 nm. Enzyme activity was expressed as µmol H_2O_2 destroyed min^{-1} mg^{-1} protein (Mandelman *et al*. 1998).

Statistical analysis:

Data were analyzed as complete randomized design with two factors. Analysis of variance (ANOVA) was performed. Means were separated using Duncan Multiple Range Test (Gomez and Gomez1984).

Results

The effect of chromium on catechol oxidase activity of ten sorghum genotypes on the $8^{th,}$ 12^{th} and $15^{th}$of treatment is shown in Tables 1, 2 and 3, respectively. In relation to corresponding controls,

catechol oxidase activity significantly increased with increasing chromium concentration. The (50 mgl^{-1}) of chromium elevated the enzyme activity by a range of 355 in L14 to1115% in L4 on the 8th of treatment; however, there was a decrease in L25, L32 and L34. Similarly, catechol oxidase activity was increased by 50 ppm of chromium on the 12th day by a range of 163 in L12 to337% in L32; however, a decrease was detected in L7. Also, 50 ppm of chromium increased catechol oxidase activity on the 15th day b\y a range of 149 % in L25 to2338% in L34

Table 4 represents the effect of chromium on peroxidase activity of ten sorghum genotypes on the 8thof treatment. As compared to control, peroxidase activity significantly increased with increasing chromium concentration throughout the entire period in spite of the decreases detected of the enzyme activity in L25, L32 and L34 genotypes. Similar increases were also detected on the 12th and 15thof treatment (Tables 5 and 6, respectively). The highest concentration (50 ppm) of chromium induced an increase of about 211% in Wad Ahmed to1200% in L14 on the 8th of treatment with a decrease in L25, L32 and L34. Peroxidase activity was increased by 50 ppm of chromium on the 12th day by a range of 103 in L7 to246% in L16. Also, 50 ppm of chromium increased peroxidase activity on the 15th day by a range of 217% in L25 to1800% in Tabat.

Table 1 Effect of chromium on catechol oxidase activity (u/mg protein) of the ten sorghum genotypes, after 8 days of chromium application (at eight different concentrations) to two-week-old seedlings.

Chromium concentrations (ppm)	Sorghum genotypes										Chromium means
	Tabat	Wad aAhmed	L4	L7	L12	L14	L16	L25	L32	L34	
0	0.65^{i}	1.83^{cdefgi}	0.64^{i}	0.68^{hi}	0.99^{ghi}	0.61^{i}	0.50^{i}	$3.13^{bcdefghi}$	$3.11^{bcdefghi}$	$1.92^{cdefghi}$	1.41^{c}
2.5	1.50^{defghi}	1.88^{cdefgi}	0.79^{hi}	1.01^{ghi}	0.68^{hi}	0.81^{hi}	0.71^{hi}	1.46^{defghi}	3.79^{bcdefg}	$2.23^{cdefghi}$	1.49^{c}
5	1.52^{defghi}	$2.24^{cdefghi}$	1.18^{efghi}	0.89^{hi}	1.05^{fghi}	1.56^{defghi}	0.65^{i}	1.40^{defghi}	$2.56^{bcdefghi}$	$1.70^{cdefghi}$	1.47^{c}
10	$2.19^{cdefghi}$	$2.81^{bcdefghi}$	$1.85^{cdefghi}$	1.44^{defgh}	$1.78^{cdefghi}$	1.23^{efghi}	1.31^{efghi}	1.68^{defghi}	1.17^{efghi}	1.10^{fghi}	1.66^{c}
20	$2.10^{cdefghi}$	$2.55^{bcdefghi}$	$2.32^{cdefghi}$	$2.41^{bcdeefghi}$	$2.84^{bcdefghi}$	1.56^{defghi}	0.92^{ghi}	1.23^{efghi}	0.76^{hi}	0.90^{ghi}	1.76^{bc}
30	$2.15^{cdefghi}$	$3.71^{bcdefgh}$	$3.03^{bcdefghi}$	$2.36^{cdefghi}$	4.27^{abcd}	$1.91^{cdefghi}$	1.65^{defghi}	1.10^{fghi}	0.73^{hi}	0.92^{ghi}	2.18^{bc}
40	4.00^{bcde}	4.04^{bcde}	$3.63^{bcdefgh}$	3.91^{bcdef}	4.35^{abcd}	$1.81^{cdefghi}$	$2.08^{cdefghi}$	0.78^{hi}	0.66^{hi}	0.67^{hi}	2.59^{ab}
50	4.64^{abc}	4.28^{abcd}	7.14^{a}	3.76^{bcdefg}	5.25^{ab}	$2.17^{cdefghi}$	$2.35^{cdefghi}$	0.75^{hi}	0.92^{ghi}	0.61^{i}	3.19^{a}
Genotypes means	2.34^{abc}	2.92^{a}	2.57^{ab}	2.06^{abcd}	2.65^{ab}	1.46^{cd}	1.27^{d}	1.44^{cd}	1.71^{bcd}	1.26^{d}	

Potassium dichromate was used as a source of chromium. The soil used was clay: sand (2:1). Statistical analysis was done using Duncan multiple range test (DMRT). Values with the same superscript letters are not significantly different at $p < 0.05$

Table 2 Effect of chromium on catechol oxidase activity (u/mg protein) of the ten sorghum genotypes at the 12th day after chromium application (at eight different concentrations) to two-week-old seedlings

Chromium concentrations (ppm)	Sorghum genotypes										Chromium means
	Tabat	Wad ahmed	L4	L7	L12	L14	L16	L25	L32	L34	
0	0.76^{a}	1.39^{a}	0.86^{a}	3.66^{a}	1.24^{a}	0.80^{a}	0.94^{a}	1.23^{a}	1.20^{a}	1.43^{a}	1.35^{d}
2.5	0.80^{a}	1.48^{a}	0.82^{a}	3.60^{a}	1.55^{a}	0.73^{a}	0.91^{a}	1.55^{a}	1.16^{a}	2.33^{a}	1.49^{cd}
5	0.94^{a}	1.49^{a}	1.54^{a}	3.72^{a}	1.61^{a}	0.89^{a}	0.95^{a}	1.43^{a}	1.21^{a}	2.74^{a}	1.65^{bcd}
10	0.94^{a}	1.59^{a}	1.58^{a}	3.03^{a}	1.59^{a}	0.95^{a}	1.14^{a}	1.57^{a}	1.61^{a}	2.51^{a}	1.65^{bcd}
20	0.97^{a}	1.77^{a}	1.90^{a}	2.40^{a}	1.71^{a}	1.17^{a}	1.10^{a}	1.58^{a}	1.56^{a}	2.68^{a}	1.68^{bcd}
30	1.33^{a}	1.95^{a}	2.00^{a}	2.33^{a}	1.80^{a}	1.47^{a}	1.21^{a}	1.68^{a}	2.13^{a}	2.72^{a}	1.86^{bc}
40	1.20^{a}	2.21^{a}	2.21^{a}	2.02^{a}	1.85^{a}	1.87^{a}	1.89^{a}	2.45^{a}	2.36^{a}	2.74^{a}	2.08^{b}
50	1.49^{a}	2.25^{a}	2.37^{a}	2.17^{a}	1.83^{a}	2.14^{a}	2.60^{a}	3.62^{a}	4.04^{a}	4.26^{a}	2.68^{a}
Genotypes means	1.06^{e}	1.77^{bcd}	1.66^{bcd}	2.87^{a}	1.65^{bcd}	1.25^{de}	1.34^{cd}	1.89^{bc}	1.91^{b}	2.68^{a}	

Potassium dichromate was used as a source of chromium. The soil used was clay: sand (2:1). Statistical analysis was done using Duncan multiple range test (DMRT). Values with the same superscript letters are not significantly different at $p < 0.05$

Table 3 Effect of chromium on catechol oxidase activity (u/mg protein) of the ten sorghum genotypes at the 15th day after chromium application at eight different concentrations) to two-week-old seedlings

Chromium concentrations (ppm)	Sorghum genotypes										Chromium means
	Tabat	Wad ahmed	L4	L7	L12	L14	L16	L25	L32	L34	
0	0.11^{a}	0.45^{a}	0.40^{a}	0.28^{a}	0.56^{a}	0.42^{a}	0.44^{a}	1.56^{a}	0.99^{a}	0.13^{a}	0.53^{e}
2.5	0.08^{a}	0.61^{a}	0.54^{a}	0.64^{a}	0.67^{a}	0.50^{a}	0.45^{a}	1.57^{a}	0.83^{a}	1.14^{a}	0.70^{e}
5	0.22^{a}	0.87^{a}	0.63^{a}	1.30^{a}	0.67^{a}	0.61^{a}	0.43^{a}	1.50^{a}	1.57^{a}	1.38^{a}	0.92^{e}
10	0.20^{a}	1.60^{a}	0.73^{a}	1.38^{a}	0.73^{a}	1.30^{a}	0.48^{a}	2.00^{a}	1.71^{a}	1.47^{a}	1.16^{cd}
20	0.08^{a}	2.60^{a}	1.10^{a}	1.44^{a}	0.77^{a}	1.26^{a}	0.69^{a}	2.09^{a}	2.04^{a}	1.96^{a}	1.40^{bc}
30	0.44^{a}	2.59^{a}	1.39^{a}	1.58^{a}	1.05^{a}	1.36^{a}	1.04^{a}	2.20^{a}	2.15^{a}	2.56^{a}	1.64^{b}
40	0.44^{a}	2.77^{a}	1.78^{a}	1.38^{a}	1.02^{a}	1.49^{a}	1.50^{a}	2.24^{a}	2.53^{a}	2.52^{a}	1.77^{ab}
50	0.44^{a}	3.27^{a}	2.04^{a}	1.87^{a}	1.68^{a}	2.37^{a}	1.85^{a}	2.33^{a}	2.91^{a}	3.04^{a}	2.18^{a}
Genotypes means	0.25^{c}	1.84^{a}	1.08^{b}	1.24^{b}	0.89^{b}	1.16^{b}	0.86^{b}	1.94^{a}	1.84^{a}	1.78^{a}	

Potassium dichromate was used as a source of chromium. The soil used was clay: sand (2:1). Statistical analysis was done using Duncan multiple range test (DMRT). Values with the same superscript letters are not significantly different at $p < 0.05$

Table 4 Effect of chromium on perooxidase activity (u/mg protein) of the ten sorghum genotypes at the 8th day after chromium application at eight different concentrations) to two-week-old seedlings

Chromium concentrations (ppm)	Sorghum genotypes										Chromium means
	Tabat	Wad ahmed	L4	L7	L12	L14	L16	L25	L32	L34	
٠	0.22^{ijk}	$0.83^{defghijk}$	0.21^{ijk}	0.16^{jk}	0.41^{ghijk}	0.03^{k}	0.13^{jk}	$0.81^{defghijk}$	$0.73^{defghijk}$	$0.88^{defghijk}$	0.44^{c}
2.5	0.48^{fghijk}	$0.97^{defghijk}$	0.23^{ijk}	0.39^{ghijk}	0.31^{hijk}	0.07^{k}	0.11^{jk}	$0.64^{efghijk}$	$0.76^{defghijk}$	$0.57^{efghijk}$	0.45^{c}
5	$0.60^{efghijk}$	$0.91^{defghijk}$	0.20^{ijk}	0.27^{hijk}	0.32^{hijk}	0.22^{ijk}	0.18^{ijk}	0.41^{ghijk}	$0.67^{efghijk}$	$0.56^{efghijk}$	0.43^{c}
10	$0.96^{defghijk}$	1.36^{bcdefg}	0.39^{ghijk}	0.40^{ghijk}	$0.62^{efghijk}$	0.22^{ijk}	0.29^{hijk}	0.34^{ghijk}	0.35^{ghijk}	0.26^{hijk}	0.52^{bc}
20	$0.91^{defghijk}$	$1.30^{bcdefgh}$	$0.70^{efghijk}$	$0.52^{efghijk}$	1.53^{abcde}	0.23^{ijk}	0.22^{ijk}	0.35^{ghijk}	0.26^{hijk}	0.27^{hijk}	0.63^{bc}
30	$1.04^{defghijk}$	1.46^{abcdef}	$0.75^{defghijk}$	$0.65^{efghijk}$	$1.13^{cdefghij}$	0.28^{hijk}	0.27^{hijk}	0.17^{ijk}	0.28^{hijk}	0.28^{hijk}	0.63^{bc}
40	$1.21^{bcdefghi}$	1.59^{abcde}	$0.73^{defghijk}$	$0.87^{defghijk}$	2.20^{ab}	0.29^{hijk}	0.47^{fghijk}	0.20^{ijk}	0.18^{ijk}	0.19^{ijk}	0.79^{ab}
50	2.10^{abc}	1.75^{abcd}	$0.91^{defghijk}$	$1.26^{bcdefgh}$	2.40^{a}	0.36^{ghijk}	$0.58^{efghijk}$	0.15^{jk}	0.23^{ijk}	0.15^{jk}	0.99^{a}
Genotypes mean	0.94^{a}	1.27^{a}	0.52^{bc}	0.57^{b}	1.11^{a}	0.21^{c}	0.28^{bc}	0.38^{bc}	0.43^{bc}	0.40^{bc}	

Potassium dichromate was used as a source of chromium. The soil used was clay: sand (2:1). Statistical analysis was done using Duncan multiple range test (DMRT). Values with the same superscript letters are not significantly different at $p < 0.05$

Table 5 Effect of chromium on perooxidase activity (u/mg protein) of the ten sorghum genotypes at the 12th day after chromium application at eight different concentrations) to two-week-old seedlings

Chromium concentrations (ppm)	Sorghum genotypes										Chromium means
	Tabat	Wad ahmed	L4	L7	L12	L14	L16	L25	L32	L34	
0	0.11^{a}	0.09^{a}	0.09^{a}	0.34^{a}	0.11^{a}	0.09^{a}	0.13^{a}	0.12^{a}	0.21^{a}	0.14^{a}	0.14
2.5	0.12^{a}	0.11^{a}	0.09^{a}	0.20^{a}	0.13^{a}	0.07^{a}	0.17^{a}	0.15^{a}	0.14^{a}	0.20^{a}	0.14
5	0.13^{a}	0.16^{a}	0.18^{a}	0.21^{a}	0.12^{a}	0.11^{a}	0.14^{a}	0.14^{a}	0.15^{a}	0.26^{a}	0.16
10	0.13^{a}	0.14^{a}	0.17^{a}	0.23^{a}	0.13^{a}	0.18^{a}	0.14^{a}	0.14^{a}	0.16^{a}	0.25^{a}	0.17
20	0.13^{a}	0.13^{a}	0.20^{a}	0.27^{a}	0.14^{a}	0.14^{a}	0.15^{a}	0.15^{a}	0.18^{a}	0.23^{a}	0.17
30	0.17^{a}	0.13^{a}	0.23^{a}	0.30^{a}	0.15^{a}	0.18^{a}	0.17^{a}	0.16^{a}	0.31^{a}	0.25^{a}	0.20
40	0.21^{a}	0.15^{a}	0.20^{a}	0.29^{a}	0.22^{a}	0.29^{a}	0.18^{a}	0.18^{a}	0.29^{a}	0.28^{a}	0.23
50	0.21^{a}	0.14^{a}	0.23^{a}	0.35^{a}	0.20^{a}	0.20a	0.32a	0.19^{a}	0.36^{a}	0.32^{a}	0.25
Genotypes means	0.15^{c}	0.13^{c}	0.18^{bc}	0.27^{a}	0.15^{c}	0.16^{c}	0.17^{bc}	0.15	0.23^{ab}	0.24^{a}	

Potassium dichromate was used as a source of chromium. The soil used was clay: sand (2:1). Statistical analysis was done using Duncan multiple range test (DMRT). Values with the same superscript letters are not significantly different at $p < 0.05$

Table 6 Effect of chromium on perooxidase activity (u/mg protein) of the ten sorghum genotypes at the 15th day after chromium application at eight different concentrations) to two-week-old seedlings

Chromium concentrations (ppm)	Sorghum genotypes										Chromium means
	Tabat	Wad ahmed	L4	L7	L12	L14	L16	L25	L32	L34	
0	0.01^{a}	0.08^{a}	0.05^{a}	0.12^{a}	0.08^{a}	0.08^{a}	0.06^{a}	0.15^{a}	0.12^{a}	0.03^{a}	0.08^{e}
2.5	0.01^{a}	0.11^{a}	0.06^{a}	0.15^{a}	0.09^{a}	0.10^{a}	0.07^{a}	0.21^{a}	0.13^{a}	0.13^{a}	0.11^{de}
5	0.02^{a}	0.14^{a}	0.10^{a}	0.17^{a}	0.10^{a}	0.09^{a}	0.17^{a}	0.20^{a}	0.18^{a}	0.13^{a}	0.13^{de}
10	0.03^{a}	0.17^{a}	0.12^{a}	0.19^{a}	0.12^{a}	0.15^{a}	0.35^{a}	0.24^{a}	0.28^{a}	0.14^{a}	0.18^{cd}
20	0.03^{a}	0.27^{a}	0.15^{a}	0.19^{a}	0.13^{a}	0.18^{a}	0.35^{a}	0.25^{a}	0.28^{a}	0.15^{a}	0.20^{bc}
30	0.04^{a}	0.29^{a}	0.18^{a}	0.19^{a}	0.33^{a}	0.20^{a}	0.37^{a}	0.25^{a}	0.27^{a}	0.28^{a}	0.24^{bc}
40	0.08^{a}	0.31^{a}	0.23^{a}	0.19^{a}	0.34^{a}	0.25^{a}	0.38^{a}	0.31^{a}	0.28^{a}	0.32^{a}	0.27^{b}
50	0.18^{a}	0.41^{a}	0.23^{a}	0.26^{a}	0.53^{a}	0.24^{a}	0.42^{a}	0.48^{a}	0.42^{a}	0.37^{a}	0.35^{a}
Genotypes means	0.05^{c}	0.22^{ab}	0.14^{b}	0.18^{ab}	0.21^{ab}	0.16^{b}	0.27^{a}	0.26^{a}	0.25^{a}	0.19^{ab}	

Potassium dichromate was used as a source of chromium. The soil used was clay: sand (2:1). Statistical analysis was done using Duncan multiple range test (DMRT). Values with the same superscript letters are not significantly different at $p < 0.05$

Discussion

The eight chromium concentrations used in this study generally induced significant differences in catechol oxidase activities on the 8, 12 and 15 days of treatments, and those of peroxidase after 8th and 15th days. Generally, the levels of both enzymes were progressively increased with the increase in chromium concentrations. The H_2O_2 levels were increased in both roots and leaves of sorghum treated with either 50 µM hexavalent chromium or 100 µM trivalent chromium Chatterjee and Chatterjee, (2000) who found that H_2O_2 levels was increased in both roots and leaves of sorghum treated with either 50 µM hexavalent chromium or 100µM trivalent chromium. The activity of antioxidant enzymes was increased even at low heavy metal concentrations Gwozdz *et al*. (1997) found that at lower heavy metal concentrations, activity of antioxidant enzymes was increased. The present results suggest that, the levels of activities of catechol oxidase and peroxidase enzymes increased following treatment with different concentrations of hexavalent chromium. This elevation after the 8th, 12th and 15th day from exposure might be a mechanism of the plant for the enzymes to engage in antioxidant defense. The plant cells have evolved antioxidant defense mechanisms to combat the danger posed by the presence of reactive oxygen species. These include enzymatic mechanisms involving antioxidant enzymes (Meloni *et al*., 2003). Modification of the plant antioxidant defense system has been reported to enhance tolerance to oxidative stress (Rai *et al*., 2004; Mishra *et al*., 2006a, b; Meng *et al*., 2007; Dazy *et al*., 2008). Alteration of antioxidant enzymes may be due to the synthesis of new iso enzyme for enhancement of the activity of pre existing enzymes for the metabolism of ROS (Kang *et al*., 1999). Significant differences were detected among the ten sorghum genotypes in both enzymes, of growth period after chromium treatment application with exception. Generally, with exception of the decreased levels of catechol and peroxidase in L25, L32 and L34 on the 8th day of treatment, the levels of both enzymes in the other genotypes increased with the increase in the chromium concentrations. Similar results were reported by Samantary (2002) who found that, the enzyme activity varied among the chromium tolerant and chromium sensitive ones in mung bean plant. Furthermore Divya (1999) reported a decrease in the activity of ascorbate peroxidase with increase in cadmium concentration in radical and plumule of pea seedling and decrease was prominent in susceptible than the tolerant variety. The decline in activity of this oxidative and antioxidant enzyme has been ascribed to inhibition of enzyme biosynthesis and the denaturation of enzyme proteins (Mohapatra, 1995).

Conclusion

The present results indicate a fluctuation in the activity of both catechol oxidase and peroxidase. Such fluctuation seemed to depend on genotypes and/or the elapsed time following treatment with Cr. Therefore, the differential changes in these antioxidant enzymes might point at a differential degree of stress induced in sorghum genotypes by Cr, So, the changes in activity of catechol oxidase and peroxidase might be in relation with plant tolerance to Cr. Hence, the variation in the

enzymatic activity might be used as markers for oxidative stress and tolerance of sorghum to Cr phytotoxicity, the point that needs further investigation.

References

Ali NA, Ater M, SunaharaGl, Robidoux PY (2004) Phytotoxicity and bioacuumulation of copper and chromiumusing barley (HordeumVulgare L.) in spiked artificial and natural forestsoils. Ecotoxicology and environmental safety. 57: 363-374

Babel S, Opiso EM (2007) Removal of Cr from synthetic wastewater by sorption into volcanic ash soil.Int. J. Environ. Sci. Tech., 4 (1), 99-107

Bradford MM (1976) A rapid and sensitive method for quantitation of microgram quantities of protein utilization the principle of protein- dye binding.Analytical Biochemistry.2:248 – 254.

Chatterjee J,Chatterjee C (2000) Phytotoxicity of cobalt, chromium and copper in cauliflower. Environmental Pollution 109: 69–74.

Dazy M, Beraud E, Cotelle S, Meux E, Masfaraud JF,Ferard JF (2008)Antioxidant enzyme activities as affected by trivalent and hexavalent chromium species in FontinalisantipyreticaHedw.Chemosphere, 73: 281-290

Divya D (1999) Screening of pea cultivars for cadmium toxicity and mechanism of cadmium-calcium interaction. Ph.D. Thesis, India: C.C.S. Harayana Agricultural University, Hisar.

Du Y, He JH, Chen JJ, Wei XG, Yang XQ, WangSY, He WB (2003) Effects of heavy metals of Pb,Cd and Cr on the growth of vegetables and their uptake.Acta HorticulturaeSinica, 30 (1), 51-55

Dube B, Tewari K, Chatterjee J, Chatterjee C (2003) Excess chromium alters uptake and translocation of certain nutrients in citrullus. Chemosphere 53: 1147–1153.

Gomez, K.A. and Gomez, A. A. (1984) Statistical Procedures for Agricultural Research.John-Willey and Sons, New York.

Gwozdz EA, Przymusinski R, Rucinska R, Deckert J (1997) Plant cell responses to heavy metals: molecular and physiological aspects. Acta Physiol Plant.19:459 – 465.

Huffman EWD, Allaway WH (1973) Growth of plants in solution culture containing low levels of chromium. Plant Physiol., 52: 72-75.

Jain R, Srivastava S, Madan VK, Jain R (2000) Influence of chromium on growth and cell division of sugarcane. Indian J. Plant Physi., 5 (3), 228-231

Kang HG, Jun SH, Kim J, Kawaide H, Kamiya Y, An G (1999) Cloning and molecular analyses of a gibberellin 20-oxidase gene expressed specifically in developing seeds of watermelon. Plant Physiol.121: 373–382.

Liu DH, Jiang WS, Li MX (1992) Effects of trivalent and hexavalent chromium on root growth and cell division of Allium cepa. Hereditas, 117: 23-29.

MandelmanD, Jamal J, Poulos TL (1998) Identification of two electron transfer sites in ascorbate peroxidase using chemical modification, enzyme kinetics and crystallography.Biochemistry, 73: 17610 – 17617.

Meloni DA, Oliva MA, Martinez CA, Cambrai AJ (2003) Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. Environmental and Experimental Botany. 49: 69–76.

Meng QM, Zouj Z, Jinng WS, Liu DH (2007) Effect of Cu2+concentration on growth, antioxidant enzyme activity and malondialdehyde content in garlic (Allium sativum L.). Acta BiologicaCracoviensia Series Botanica .49: 95–101.

Mertz, W. 1967.Biological role of chromium.Fed. Proc., 26: 186-193

Mishra S, Srivastava S, Tripathi RD, Govindarajan R, Kuriakose SV, Prasad MNV (2006a) Phytochelatin synthesis and response of antioxidants during cadmium stress inBacopamonnieriL. Plant Physiol. Biochem. 44, 25–37.

Mishra S, Srivastava S, Tripathi RD, Kumar R, Seth CS, Gupta DK (2006b) Lead detoxification by coontail (Ceratophyllumdemersum L.) involves induction of phytochelatins and antioxidant system in response to its accumulation. Chemosphere, 65, 1027–1039.

Mohapatra S (1995) Chromium toxicity and water stress interaction in green gram Vigna radiate L. during seed germination and seedling growth. M.Phil. Thesis, India: Utkal University. Bhubaneshwar.

Nath K, Saini S, Sharma YK (2005) Chromium in tannery industry effluent and its effect on plant metabolism and growth. J. Environ. Biol., 26 (2), 197- 204

Parmar NG, Vithalani SD, Chanda SV (2002) Alteration in growth and peroxidase activity by heavy metals in Phaseolusseedlings.Acta Physiolog.Plant. 24 (1), 89-95

Rai V, Vajpayee P, Mehratra S (2004) Effects of Chromium accumulation on photo-synthetic pigments, oxidative stress defense system, nitrate reduction, proline level and eugenol content of Ocimumtenuiflorum L., Plant Sci. 167: 1159–1169.

Samantary S (2002) Biochemical responses of Cr-tolerant and Cr-sensitive mung bean cultivars grown on varying levels of chromium. Chemosphere. 47: 1065–1072

Scoccianti V, Crinelli R, TirilliniB,Mancinelli V, Speranza A (2006) Uptake and toxicity of Cr (III) in celery seedlings. Chemosphere. 64 (10), 1695-1703

Sharma DC, Pant RC (1994) Chromium uptake its effectson certain plant nutrients in Maize (Zea mays L. cv. Ganga 5). Journal of Environmental Science andHealth, Part A 29: 941–948

Sharma DC, Chatterjee C, Sharma CP (1995) Chromium accumulation by barley seedlings (Hordeumvulgare L.). J. Exp. Bot. 25: 241-251

Srivastava S,Prakash S, Srivastava MM (1999) Chromium mobilization and plant availability – the impact of organic complexing agents. Plant Soil, 212: 203-208.

Tremoliere M, Bieth JB (1984) Determination of catechol oxidase activity using spectroscopic methods. Phyochemistry. 23: 501 – 505.

Venkateswaran P, Vellaichamy S, Palanivelu, K (2007) Speciation of heavy metals in electroplating industry sludge and wastewater residue using inductively coupled plasma. Int. J. Environ. Sci. Tech., 4 (4), 497-504

Wong JWC, Lai KM, Su DS, Fang M (2001) Availability of heavy metals for Brassica chinensisgrown in an acidic loamy soil amended with a domestic and an industrial sewage sludge. Water Air Soil Poll. 128 (3-4), 339-353

Zayed A, Lytle CM,Qian JH, Terry N (1998) Chromium accumulation, translocation and chemical speciation in vegetable crops, planta. 206: 293-299.

Zayed AM, Terry N (2003) Chromium in the environment: factors affecting biological remediation. Plant Soil, 249: 139-156.

Zhen Z Q, FengWH, Bian SP, Zheng JM, Zhang LZ, Xing SL (1987) Study on fate of pollutant chromium in the Agro-Ecosystem. J. Environ. Sci., 8: 14-19.

الملخص العربي

عنوان البحث: الاستدلال بنشاط الكاتيكول أكسديز والبيرأكسديز كمؤشر لمقاومة عنصر الكروم في بعض الأنماط الوراثية لنبات للذرة الرفيعة (Sorghum bicolor)

هجو الزين الحسن[١]، عبدالوهاب حسن عبدالله[٢]، والبشرى الشيخ النور[٣]

[١] معهد أبحاث البئية والموارد الطبيعية والتصحر، المركز القومي للبحوث، الخرطوم، السودان
[٢] قسم المحاصيل الحقلية، كلية الزراعة، جامعة الخرطوم، الخرطوم، السودان
[٣] قسم النبات، كلية العلوم، جامعة الخرطوم، الخرطوم، السودان

هدفت الدراسة الحالية الى تقييم أثر عنصر الكروم على نشاط انزيمي الكاتيكول أكسديز والبير أكسديز لعشرة طرز وراثية من نبات الذرة الرفيعة. (طابت وود أحمد و L4و L7و L12و L14و L16و L25 و L32 و L34) والتى تم جلبها من قسم المحاصيل الحقلية، كلية الزراعة، جامعة الخرطوم. وقد تمت زراعة البذور في اكياس بولثيرين يحتوى كل منها على ٢ كجم من تربة خليط من الطين والرمل (١:٢). وبعد إسبوعين من الزراعة تم ري بادرات النباتات بثمانية تركيزات مختلفة من عنصر الكروم هي(٠ و ٢,٥ و ٥ و ١٠ و ٢٠ و ٣٠ و ٤٠ و ٥٠ ملجم / لتر). و قد أخذت عينات النباتات عند اليوم الثامن والثاني عشر والخامس عشر بعد إضافة الكروم لدراسة تأثيره على نشاط انزيمي كاتيكول أكسديز وبيرواكسديز. وأوضحت النتائج ان النشاط الإنزيمي يزداد بزيادة تركيز الكروم وأن معدل الزيادة يعتمد أساسا على الطراز الوراثي والفترة الزمنية التى تعقب المعاملة. وخلصت هذه الدراسة الى انه يمكن ان الاستدلال بتأثر نشاط الإنزيمين قيد الدراسة بالكروم كمؤشر لانتخاب أصناف مقاومة لعنصر الكروم.