World Journal of Agricultural Sciences 6 (4): 425-433, 2010
ISSN 1817-3047

The Antioxidative Response System in *Brassica juncea* L. cv. pusa Jai Kisan. Exposed to Hexavalent Chromium

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Abstract: Being non-degradable, chromium persists in environment and once mixed in soil; it undergoes transformation into various mobile forms before ending into environmental sink. In this context, a study was carried out to investigate the effects of chromium on the oxidative defence system in *Brassica juncea* L. Pusa jai kisan grown under hydroponic conditions. Different concentrations of P otassium dichromate (5, 10, 15, 20, 25 μM), was applied to 30 days old *B. juncea* plants and harvested after 3rd and 5th day of treatment for estimation of anti-oxidant enzyme activities such as Ascorbate peroxidase, (APX,1.11.1.11), superoxide dismutase (SOD,1.15.1.1), glutathione reductase (GR, 1.6.4.2) and catalase (CAT, 1.11.1.6)] and also the content of non- ezymatic components (Ascorbate and glutathione) . A significant increase in ascorbate peroxidase and superoxide dismutase activity has been observed corresponding to increasing Cr concentrations. Catalase activity was decreased with increasing chromium concentrations. While, in case of non-enzymatic anti-oxidants, total glutathione and total ascorbate content was decreased with increasing chromium concentrations. The biochemical aspects of *B. juncea* like photosynthetic pigments was decreased with increasing chromium concentrations. However, the osmolyte like Proline accumulation was increased with increasing chromium applied.

Abbreviation: Σ Extinction Coefficient; μg Microgram; Nmolnanomole; EU Enzyme unit

Key words: Chromium toxicity · *Brassica juncea* · Protein · SOD · APX · Oxidative Stress

INTRODUCTION

India is the one of the largest producers of leather and nearly 80% of tanneries engaged in chrome tanning process. Leather tanning, electroplating and stainless-steel industries contribute to most of chromium contamination [1]. Chromium is broad line heavy metal which phytotoxic above certain threshold levels [2]. Cumulative chromium production has been estimated to be 105.4 million tonnes globally in 2000 and has been significantly increased since 1950 [3]. Currently, the regulatory limit of chromium in environment in U.S. is 50μg/ Kg. However, actual Cr concentration in soil can reach levels between 5 to 4g/ Kg [4]. Chromium exists in variable oxidation states, trivalent Cr^{3+} and Hexavalent chromium Cr^{6+}. Hexavalent chromium is found to more hazardous to animals and plants due its high mobility, toxicity as well as carcinogenic and mutagenic properties [5].

The bio toxicity of Cr^{6+} is largely a function of its ability to cross biological membranes, its powerful oxidising capabilities and its interference with electron transport in respiration and photosynthesis [6]. Chromium is strong oxidant with a high redox potential in the range of 1.33-1.38 eV accounting for a rapid and high generation of reactive oxygen species (ROS) and its resultant effects on photosynthesis in terms of carbon dioxide fixation, photophosphorylation and enzyme activities [7, 8]. Chromium phytoxicity can result in inhibition of seed germination, degrade proteins and pigment status, nutrient balance, antioxidative stress in plants [9-14]. Active production of free radicals has been reported in many plants exposed to chromium stress, leading to DNA damage, protein and initiated lipid per oxidation [15]. Adequate defence against oxygen toxicity requires efficient scavenging ofboth O^{2-} and H2O2 [16], superoxide radicals (O^{2-} and H_2O_2 are by-products of oxidation metabolism [17]. Their toxicity is attributed to

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interaction with hydrogen peroxide to form highly reactive hydroxyl radicals (OH^-) which are thought to be largely responsible for mediating oxygen toxicity in vivo [18]. The enzymatic antioxidants components e.g. SOD, CAT, POD and glutathione can neutralize free radicals and may reduce or even help some of potential damage they cause [19-20].

MATERIALS AND METHODS

The seeds of Indian Mustard *Brassica juncea* L.cv. Pusa Jai kisan , *Compositae,* were obtained from Indian Agricultural Research Institute (IARI, New Dehli). Experiments were carried out in Hydroponic chamber of Department of Botany, Jamia Hamdard . The seeds were first sterilized with $HgCl_2$, thereafter transferred to petriplates for germination. The germinated seedlings were transferred to Hoagland solution. Potassium dichromate as source of Hexavalent chromium was provided to all the seedling in following doses Control, 5μM, 10μM, 15μM, 20μM and 25μM. Each experiment was repeated two times with 5 replicates following three and five days after treatment. Seedlings were collected three and five days after treatment to analyze the effects of Cr^{6+} on the biochemical, enzymatic and non-enymatic parameters of *Brassica juncea*.

The total ascorbate (Asc-DHAs) and ascorbate (total ascorbate-DHAs) were assayed separately and absorbance was recorded at 525 nm [21]. A standard curve in the range of 0-100 nmoles of Asc was used for calibration. Values in both cases were corrected for absorbance by eliminating the supernatant in the blank prepared separately for ascorbate and total ascorbate. The difference of total ascorbate and the ascorbate gave the amount of the pools were expressed in $nmoles g^{-1}{}_{fw}{}^{-1}$.

Proline content in leaf samples was estimated [22] by using Proline (BSA, Sigma, USA). The amount was expressed in $\mu g^{-1} g$ fw. using acid ninhydrin, glacial acetic acid and toluene as chemical agents to be added to the supernatant of the fresh leaf homogenate . The toluene layer was separated from mixture and absorbance recorded at 520 nm, on Beckmans spectrophotometer, using toluene as blank.

CAT activity in leaves was measured by the method [23], monitoring the disappearance of H_2O_2 at 240 nm on UV -Vis Spectrophotometer (Model DU 640, Beckman, USA), calculated by using the extinction coefficient (Σ) 0.036 $mM^{-1}{}_{cm}{}^{-1}$ and expressed in enzyme unit (mg/protein). One unit enzyme is the amount necessary to decompose 1μmol of H_2O_2 per min at 25°C.

The activity of SOD was measured by the method [24]. It was assayed by its ability to inhibit the photochemical reduction of Nitroblue tetrazolium (NBT), the reaction buffer , methionine , enzyme extract , $NaCO_3$, NBT, EDTA, riboflavin and distilled water . Fifty percent reduction of NBT was considered as one unit of enzyme activity which was expressed as Enzyme Unit (EU)mg/protein/h.

APX activity was estimated following the method [25]. And it was determined from the decrease in the absorbance at 290 nm due to its enzymatic breakdown calculated by using extinction coefficient (Σ) 2.8 m/M/cm and expressed in Enzyme unit (mg/protein). One unit of enzyme is the amount necessary to decompose 1μmol of substrate per minute at 25°C.

GR activity was determined [26] and modified by [27]. The method involved monitoring of the glutathione dependant oxidation of NADPH at 340 nm on a DU 640, UV-Vis Spectrophotometre. GR activity was calculated by using extinction coefficient (Σ) 6.2m/M/cm and expressed in enzyme unit (mg/protein). One unit of enzyme is the amount necessary to decompose 1μmol NADPH per min at 25°C.

Glutathione Content: Reduced (GSH), oxidised (GSSG) and total glutathione (GSH + GSSG) were determined by the glutathione recycling method [28]. Fresh leaf (0.05 g) was homogenized in 2 ml of 5 % sulphosalicylic acid at 4°C. The homogenate was centrifuged at 10,000 rpm for 10 min. To a 0.5 ml of supernatant, 0.6 ml of reaction buffer (0.1 M Na-phosphate, pH 7, 1 mM EDTA) and 40 μl of 0.15 %n 5,5-dithiobis-2-nitrobenzoic acid (DTNB) were added and read at 412 nm after 2 min. To the same, 40 μl of 0.4 % NADPH and 2 μl of glutathione reductase (GR; 0.5 enzyme unit) were added and reaction was run for 30 min at 25°C. The samples were again read at 412 nm to determine the total glutathione.

Soluble protein content was determined following the method of Bradford [29].

Hiscox and Isrealtams [30] method was used to estimate the pigment concentration in the samples while as various concentrations in mg/g fw were calculated by using the following formulae given by MacLachlan and Zalik [31] for chlorophyll a, Duxbury and Yentsch [32], for chlorophyll b, Arnon [33] for total chlorophyll.

Statistical Analysis: Values in the text and tables indicate mean values±S.E of two independent experiments. The significance of differences between control and each treatment was analyzed using Statistical Program Minitab.

RESULTS

Chlorophyll Content: Compared to control, chlorophyll *a, b* and total chlorophyll were decreased with increasing chromium concentration as well as with duration of time.

The total chlorophyll content was significantly affected . It has been noticed that 75 % of chlorophyll *a* decreased at 25 µM concentration of C r applied. Total chlorophyll degradation was increased from 50.67 to 64.6% in Cr treated plants as observed in Table 1. The maximum reduction in chlorophyll took place with T5 (25 µM) compared to respective control . The decrease in Chlorophyll was more significant among 5 DAT plants starting from 15 µM concentrations.

Protein and Proline Contents: With increasing concentrations of chromium in growing media , the protein content of leaves decreased. However, protein content showed remarked decrease after 10 µM. A slight reduction of 4.34% soluble protein was observed for *Brassica* at 5 µM. There was continuous degradation of protein after 10µM with maximum decrease observed at 25µM in both 3 and 5 DAT. A protein decrease of 12.76 and 49% was found in 3 and 5 DAT plants respectively as shown in Table 2.

Compared to control, Proline content showed significant increase with increasing concentration of chromium and duration of treatment. The maximum enhancement taking place at T5 (25µM) after 5 days treatment. The proline increased from 42.0 to 97 % in Cr treated plants. There was strong proportionality between levels of proline and chromium applied.

Anti-oxidant Enzymes: The changes in antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), Ascorbate peroxidise (APX) activity in 5 and 3DAT as showed in Tables 3, 4 and 5.

The CAT acitivity decreased upon chromium treatment . CAT activity showed significant change in relation to control . At 25µM Cr maximum decrease was noticed, while as slight decrease was observed at 10 µM in 3 DAT. The CAT activity decreased at maximum of 33.29 and 46.5% in 3 and 5 DAT as observed in Table 3. CAT is an important enzyme involved in detoxification in plants. The reduction in CAT activity was consistent both in 3 and 5 days after Cr treated plants. Exposure of chromium to developing *Brassica* seedlings decreased after 3 and 5 day of Cr treatment at 5, 10, 15 and 25µM.

SOD activity was increased with increasing concentration of chromium concentration. At Cr concentration of 20µM and 25µM, higher SOD activity as observed in Table 4. The gain was maximum with T5 (25µM) during 3 days Cr^{6+} treatment followed by same trend among 5DAT plants.

Table 1: Effect of Cr (VI) on the photosynthetic pigments mgg-1fw in *Brassica juncea* seedlings determined after 3 and 5 days of treatment with different Cr(VI) concentration.

Treatment duration	Parameters (mg/g fr.wt)	T0 0	T1 5µM	T2 10µM	T3 15µM	T4 20µM	T5 25µM
3DAT	Chlorophyll a	0.47±0.04	0.45±0.03	0.36±0.04	0.28±0.03	0.25±0.03	0.22±0.05
		0	-4.25	(23.40)*	(40.4)**	(46.8)**	(53.19)**
	Chlorophyll b	0.28±0.01	0.25±0.01	0.22±0.01	0.19±0.02	0.17±0.02	0.12±0.04
		0	(10.72)*	(21.42)*	(32.14)**	(39.28)**	(57.14)**
	Total Chlorophyll	0.75±0.01	0.72±0.01	0.61±0.01	0.55±0.01	0.51±0.01	0.37±0.01
		0	(4)*	(18.6)*	(26.6)**	(32.0)**	(50.67)**
5DAT	Chlorophyll a	1.27±0.05	1.17±0.03	0.9±0.03	0.58±0.03	0.52±0.03	0.31±0.04
		0	(7.87)*	(29.13)*	(54.34)**	(59.02)**	(75.5)**
	Chlorophyll b	0.43±0.02	0.40±0.01	0.38±0.04	0.34±0.01	0.32±0.01	0.23±0.01
		0	(6.97)*	(11.62)*	(20.93)**	(25.58)**	(46.51)**
	Total Chlorophyll	1.67±0.02	1.62±0.01	1.35±0.02	0.96±0.02	0.89±0.01	0.59±0.01
		0	(2.99)*	(19.16)**	(42.51)**	(46.70)**	(64.6)**

P≤0.05

The values represent mean ±S.E (n=5)

CD at 5%

Treatments: 2.516*

Table 2: Effect of Cr (VI) on the protein and proline content mgg-1fw in *Brassica juncea* determined 3 and 5 days after treatment.

Treatment duration	Parameters (mg/g fr.wt)	T0 0	T1 5µM	T2 10µM	T3 15µM	T4 20µM	T5 25µM
3DAT	Proline (mg/g fr.wt.)	2.38±0.03	3.38±0.07	3.7±0.04	3.78±0.02	3.93±0.03	4.18±0.02
		(0.00	(-42.0)*	(-55.4)*	(-58.82)**	(-65.10)**	(-75.6)**
	Protein(mg/fr.wt.)	21.15±0.07	20.23±0.05	20.04±0.08	19.35±0.16	19.14±0.291	8.45±0.25
		0	(4.34)*	(5.25)**	(8.51)**	(9.50)**	(12.76)**
5DAT	Protein (mg/ gfr.wt.)	22.0±1.57	20.0±1.57	17±2.3	16.28±0.05	14.2±0.06	11.22±0.02
		0	(9.09)*	(22.7)**	(26)**	(35.4)**	(49)**
	Proline(mg/g fr.wt.)	3.31±0.07	4.09±1.2	5.11±1.9	5.41±0.03	5.58±0.03	6.59±0.02
		0	(-23.45)	(-54.3)*	(-63.4)**	(-68.5)**	(-99.0)**

The values represent mean ±S.E (n=5)

CD at 5%

Treatments: 1.119

Table 3: Effect of Cr (VI) on acivity of catalase (mmol mg^{-1} protein min^{-1}) in *Brassica juncea* seedlings determined after 3 and 5 days of treatment with different Cr(VI) concentrations.

Parameter	Treatments					
Catalase (mmol mg-1protein min^{-1})	T0	T1	T2	T3	T4	T5
	0	5µM	10µM	15µM	20µM	25µM
3DAT	9.04±0.04	8.85±0.07	6.98±0.03	6.72±0.04	6.58±0.05	6.03±0.06
	0	(2.10)*	(25.6)**	(27.2)**	(33.29)**	(22.7)**
5DAT	8.9±0.05	7.86±0.05	6.3±0.02	5.45±0.02	4.84±0.04	4.76±0.04
	0	(0.11)*	(29.2)**	(38.7)**	(45.6)**	(46.5)**

P≤0.05

The values represent mean ±S.E (n=5)

CD at 5%

Treatments: 0.456*

Table 4: Effect of Cr (VI) on acivity of SOD (EU mg^{-1} Protein h^{-1}) in *Brassica juncea* seedlings determined after 3 and 5 days of treatment with different Cr(VI) concentrations.

Parameter	Treatments					
SOD	T0	T1	T2	T3	T4	T5
(EU mg^{-1} Protein h^{-1})	0	5µM	10µM	15µM	20µM	25µM
3DAT	0.39±0.03	0.55±0.05	0.57±0.08	0.59±0.03	0.65±0.03	0.69±0.03
	0	(-41.0)	(-46.0)**	(-51.2)**	(-66.67)**	(-76.9)**
5DAT	0.45±0.03	0.50±0.04	0.53±0.02	0.54±0.02	0.59±0.03	0.63±0.02
	0	(-5)*	(-17.78)**	(-20)**	(-31.12)**	(-40)**

P≤0.05

The values represent mean ±S.E (n=5

CD at 5%

Treatments: 0.614*.

Table 5: Effect of Cr (VI) on acivity of APX acivity (µ mol mg^{-1} Protein min^{-1}) in *Brassica juncea* seedlings determined after 3 and 5 days of treatment with different Cr(VI) concentrations.

Parameter	Treatments					
APX (µ mol mg^{-1} Protein min^{-1})	T0	T1	T2	T3	T4	T5
	0	5µM	10 µM	15 µM	20 µM	25 µM
3DAT	0.23±0.01	0.27±0.02	0.32±0.01	0.37±0.02	0.38±0.01	0.43±0.01
	0	(-17.39)*	(-39.13)**	(-60.8)**	(-65.28)**	(-86.9)**
5DAT	0.28±0.05	0.51±0.02	0.53±0.02	0.54±0.02	0.64±0.01	0.67±0.01
	0	(-82.1)*	(-89.2)**	(-92.8)**	(-128.57)**	(-139.2)**

P≤0.05

The values represent mean ±S.E (n=5)

CD at 5%

Treatments : 0.007*

Table 6: Effect of Cr (VI) on total ascorbate content (n mol g^{-1} fr.wt.) in *Brassica juncea* seedlings determined after 3 and 5 days of treatment with different Cr(VI) concentrations.

		T0	T1	T2	T3	T4	T5
	PARAMETERS	0	5μM	10μM	15μM	20μM	25μM
3DAT	Ascorbate	34.7±1.72	32.2±20.4	29.9±1.3	27.8±0.95	24.8±0.85	21.4±2.1
	(n mol g^{-1} fr.wt.)	0	-7.2	(13.8)**	(19.8)**	(28.5)**	(38.32)**
		15.9±1.8	15.6±2.1	12.5±0.9	12.0±0.2	11.9±0.3	11.6±2.1
	Dehydroascorbate	0	(1.88)*	(21.3)**	(24.5)**	(25.12)**	(27.04)**
		42.7±1.I6	42.0±1.6	41.7±1.19	40.8±1.6	34.4±1.6	30.3±1.07
	Total ascorbate	0	(1.63)*	(2.34)*	(4.44)**	(21.94)**	(29.03)**
5DAT	Ascorbate	71.3±3.26	64.7±1.23	52.6±2.07	47.3±1.23	39.2±0.01	32.6±1.00
		0	(9.25)*	(26.23)**	(33.67)**	(45.02)**	(54.27)**
	Dehydroascorbate	25.8±0.82	23.3±0.7	19.6±0.36	15.8±0.72	12.3±1.52	9.3±0.48
		0	(9.68)*	(24.03)*	(38.75)**	(52.32)**	(63.9)**
		76.4±0.87	72.9±0.69	69.4±0.62	64.4±0.84	62.2±0.9	56.8±1.6
	Total Ascorbate	0	(4.58)*	(9.42)*	(15.7)**	(18.5)**	(25.65)**

*P≤0.05

The values represent mean ±S.E (n=5)

CD at 5%Treatments 0.0108

Table 7: Effect of Cr (VI) on total glutathione content (n mol g^{-1} fr.wt.) in *Brassica juncea* seedlings determined after 3 and 5 days of treatment with different Cr(VI) concentrations.

	Parameters	T0	T1	T2	T3	T4	T5
Treatment duration	(n mol g^{-1} fr.wt.)	0	5μM	10μM	15μM	20μM	25μM
3DAT	GSH	33.7±1.72	30.2±20.4	25.9±1.3	23.8±0.95	22.8±0.85	21.4±2.1
		0	-7.2	(13.8)**	(19.8)**	(28.5)**	(38.32)**
	GSSG	40.7±1.I6	41.0±1.6	39.7±1.19	38.8±1.6	34.4±1.6	30.3±1.07
		0	(1.63)ns	(2.34*	(4.44)**	(21.94)**	(29.03)**
	Total Glutathione	71.3±3.26	64.7±1.23	52.6±2.07	47.3±1.23	39.2±0.01	32.6±1.00
		0	(9.25)*	(26.23)*	(33.67)**	(45.02)**	(54.27)**
5DAT	GSH	25.8±0.82	23.3±0.7	19.6±0.36	15.8±0.72	12.3±1.52	9.3±0.48
		0	(9.68)*	(24.03)**	(38.75)**	(52.32)**	(63.9)**
	GSSG	76.4±0.87	72.9±0.69	69.4±0.62	64.4±0.84	62.2±0.9	56.8±1.6
		0	(4.58)*	(9.42)**	(15.7)**	(18.5)**	(25.65)**
	Total Glutathione	76.4±0.87	72.9±0.69	69.4±0.62	64.4±0.84	62.2±0.9	56.8±1.6
		0	(4.58)*	(9.42)**	(15.7)**	(18.5)**	(25.65)**

Significant at 5%

** Significant at 1%

Non-significant.

CD at 5%

Treatments: 0.376.

In *Brassica* leaves APX activity was increased with increasing chromium concentration in medium as represented by data in Table 5. A rapid up gradation of APX started at 15 μM and 20μM of Cr applied. APX activity at 20μM increased significantly among 3 and 5 days after treatment.

Ascorbate content showed decreased with respect to Cr treatment applied. The maximum decline was observed with T5 (25μM) treatment. The decrease was more significant in 5 DAT. There was significant decrease of 54.27 and 25.65% among 3 and 5 days after treatment at highest concentration of 25μM as shown in table 6.

Total glutathione or GSH pool under chromium stress decreased in time and dosage dependant manner. Generally GSSG activity in leaves was increased from 7.2 to 29.03% among 3 days after treatments while as among 5 days after treatment plants showed more significant decrease with maximum degradation at 25 μM (Table 7). The ratio of oxidised a scorbate to reduced ascorbate as well as ratio of oxidised glutathione to reduced glutathione was observed consistent of chromium and duration of exposure to chromium applied.

DISCUSSION

Elevated concentration of chromium can induce oxidative stress in *Brassica juncea* as shown in Table 3,4,5 and 6. Total Chlorophyll content declined under metal treatment. A decline in chlorophyll *a, b* and total chlorophyll has been reported in many plants under heavy metal stress [12,14,34].

The decline in chlorophyll pigment as result of Cr stressor indicates inhibitory esffect of Cr on pigment biosynthesis which might be metal specific. The decrease in total chlorophyll, chlorophyll *a* and chlorophyll *b* have been well documented under Cr stress in plants [12,13,35]. The decrease in photosynthetic activity may be due to exposure of plants to chromium induced decrease in absorption per reaction centre without any significant alteration in electron transport [36]. Growth parameters namely, shoot length and dry weight were sensitive to much lower dichromate concentrations and did react more quickly than biochemistry related parameters chlorophyll fluorescence and pigment concentration [37] Chromium possesses capacity to degrade Σ-aminolevulinic acid dehydratase, an important enzyme involved in chlorophyll biosynthesis thereby affects aminolevulinic acid (ALA) utilization [38]. Hexavalent Chromium replaces Mg^{2+} from active sites of many enzymes and deplete chlorophyll content [38].

Chromium decrease protein in dose and time dependent manner. Degradation of proteins in plants can result in inhibition of nitrate reductase activity [39, 40]. The correlation between nitrate and protein is well documented in plants [41]. The significant decrease in the sulphate uptake rates was observed in Cr treated plants in *Brassica juncea* which was accompanied by repression of root low affinity sulphate transporter. This is reason for decrease of protein activity and attained levels of reducing sugars in Cr treated *Brassica* plants [42].

An increase in proline content may be linked with stimulated protein oxidation or an impaired protein synthesis . Proline in plants acts as osmoregulator , a soluble nitrogen sink, a signal of senescence and an indicator of plant resistance to plant stress. Proline affects the solubility of various protein and this protects them against denaturation under stressful conditions [43].

To mitigate and repair the damage caused by ROS, plants have evolved complex antioxidant system (enzymatic and Non-enzymatic). In present study, Chromium treated *Brassica* seedlings showed a significant increase in SOD activity. The increased SOD activity in Cr treated plants accounted for its role in scavenging superoxide radicals [44]. The increase in SOD activity under Chromium stress indicates the constant detoxification of superoxide radicals that have been generated. Increase in SOD activity was first reported earlier in moss *Taxum naplanese* under Pb, Cr, Zn, Cu and As toxicity and also a lichen *Diploshistes musocoum.*

Decrease in catalase activity and increase of peroxidise activity at concentration at 25µM while as at lower concentration enzymatic are least affected. The increase in a antioxidant enzyme activity observed might have been. The increase in antioxidant enzyme activity observed might have been in direct response to generation of superoxide radical by Cr6+ induced blockage of Electron Transport Chain (ETC) in mitochondria . The decrease in activity of enzyme as concentration of external chromium increased might be because of inhibitory effect of Cr ions on enzyme system itself . Decrease in CAT activity may be due to flux of superoxide radicals that inhibit CAT biosynthesis [45]. Decline in catalase activity is regarded as general response to many stresses and is suppressed due to inhibition of enzyme synthesis or change in assembly of enzyme subunits [46,47]. Catalase is Heme-containing enzyme, Hence the decrease in catalase activity may also be due to active competition between Iron and chromium uptake or absorption by plant roots.

Non- enzymatic cellular oxidants, like Ascorbate and glutathione undergo alteration under oxidative stress [48-49]. Marked decrease in GSH pool under chromium stress may be observed because of oxidation of different cellular thiols such as GSH and Cysteine by Cr^{6+} in vitro studies [49]. Dichromate reacts GSH at the sulfahydryl groups to form an unstable glutathione-CrO_3 complex [49]. Thiolate complexes with Cr^{6+} with y-glutamyl cysteine, N- acetyl cysteine and cysteine have also been described [50]. The interconversions of reduced and oxidised form of glutathione to maintain redox status of cell as well as to scavenge free radicals could hace caused a decrease in GSH .A decrease in Ascorbate possibly due to the shortage of reductant to maintain the MDAR activities has been reported earlier [51]. Our results of decreased APX acitivity are supported by [52] who reported significant reduction in APX activity in *Ocimum tenuiflonimum.* Increase in APX activity is suggestive of its role in the detoxification of H2O2 [53] under abiotic stress.

CONCLUSION

The investigations highlighted involvement of Chromium in inducing oxidative stress in *Brassica juncea* reflected by physiological disturbances like decline in chlorophyll and in protein content indicated toxic effects of Chromium . Proper scavenging of free radicals by *Brassica juncea* is suggestive of its role as hyperaccumulator that provides the efficient Phytoremedation of Cr polluted soil. Phytoremedation of Chromium polluted environment by molecular and genetic techniques should be used to indentify gene that encode synthesis of Phytochelatins or Metallothiones in the detoxification of metals within plants. These technologies will prove useful in environmental cleanup procedures and subsequent restoration of soil fertility.

ACKNOWLEDGEMENTS

I will like to thank Dr. Mohmmed Iqbal, Head of department Botany for providing all facilities required for above accomplished work.

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