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Impact of Chromium on the Oxidative Defense System of *Brassica juncea* L. cv. 'Pusa Jai Kissan' under Hydroponic Culture

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ABSTRACT

Brassica juncea is a medicinally important plant and is commonly used as a diuretic, stimulant and to treat arthritis. Seed are used for the treatment of tumors and stomach disorders. *B. juncea* can hyperaccumulate cadmium and many other soil trace elements like selenium, chromium, iron and zinc food supplements. Chromium (Cr)-induced oxidative damage and changes in the contents of proline and glutathione in leaves of *B. juncea.* L. cv. 'Pusa Jai Kissan' were investigated after 3 and 5 days of treatment under hydroponic culture. Cr was supplied as $K_2Cr_2O_7$. The main response was an increase in superoxide dismutase activity and proline content which subsequently reduced the activity of catalase and glutathione content in plants.

Keywords: antioxidants, catalase, proline, superoxide dismutase
Abbreviations: CAT, catalase; **Cr**, chromium; **GTH**, glutathione content; **ROS**, reactive oxygen species. **SOD** superoxide dismutase

INTRODUCTION

Brassica juncea belongs to the Cruciferae family and is known as Indian mustard (English name). This plant is reported to be an anodyne, aperitif, diuretic, emetic, rubefacient, stimulant and is a folk remedy for arthritis, foot-ache, lumbago, and rheumatism (Duke and Wain 1981). Seeds are used to treat tumors in China while its roots are used as a galactagogue in Africa. Ingestion may impart a body odour which repels mosquitoes (Burkill 1966). Believed to be aperient and tonic, its volatile oil is used as a counter-irritant and stimulant. Leaves of this plant are said to relieve headache (Burkill 1966). The seeds are used for abscesses, colds, lumbago, rheumatism, and stomach disorders in Korea. Chinese eat the leaves in soups to treat bladder inflammation or hemorrhaging. Mustard oil is used for skin eruptions and ulcers (Perry 1980). In Africa, the leaves are cooked as a vegetable (Grubben and Denton 2004).

The rapid development and evolution of metal-base industries have lead to contamination of environment with heavy metals especially chromium (Cr). Heavy metals cannot be destroyed but can only be transformed from one oxidation state or organic complex to another (Marques *et al*. 2009). It is easily absorbed by plants from the soil and atmosphere, accumulates in their organs and shows cytotoxic and phytotoxic effects (Parmar and Chanda 2005). Cr is toxic to plants and does not play any role in plant metabolism (Dixit *et al*. 2002). Evidence also indicates that chromosomal abnormalities (micronuclei) and genomic instability (microsatellite instability) are possible by induction of Cr(VI) (Wise *et al*. 2008). Metabolic alterations by exposure to Cr have also been described in plants either by a direct effect on enzymes or other metabolites or by its ability to generate reactive oxygen species (ROS) which may cause oxidative stress. The potential of plants with the capacity to accumulate or to stabilize Cr compounds for bioremediation of Cr contamination has gained interest in recent years (Shanker *et al*. 2005). Cr phytotoxicity can induce the production of ROS like superoxide radical (O_2^-), OH^-, alkoxy radical (RO^-), singlet oxygen ($^1O_2^-$) and toxic H_2O_2 (Brensgen *et al.* 2001) and these ROS produced are detoxified by both enzymatic catalase (CAT), peroxidase (POX), superoxidase (SOD) and gluthatione reductase (GR) and non-enzymatic ascorbate (ASC), glutathione, α-tocopherol (α-TOC) and carotenoids (CAR). The oxidative system, if not detoxified, causes serious damage to cholorophyll, protein, membrane lipids and nucleic acids (Alcher *et al*. 1997). Cr(VI) is a strong oxidant with a high redox potential in the range of 1.33-1.38 eV accounting for a rapid and high generation of ROS and its resultant toxicity (Shanker *et al.* 2004). Cr increases radical growth (Panda *et al*. 2002) and is reported to affect Hill's reaction affecting both dark and light reactions (Krupa and Baszynki 1995; Zeid *et al*. 2001). *B. juncea* has been widely used in phytoremediation because of its capacity to accumulate high levels of Cr and other metals such as lead (Zaier *et al.* 2010). The efficiency removal of copper from soil by *B. juncea* (L.) Czern and *Bidens alba* (L.) DC. var *radiata* was reported (Naiyanan and Winaipanich 2006).

MATERIALS AND METHODS

Mustard seeds were germinated in paper towels and germinated seedlings of similar size were placed in half-strength Hoagland's solution (Hoagland and Arnon 1950) containing (in mM): 2.4 Ca $(NO_3)_2$, 1.0 KH_2PO_4, 3.0 KNO_3, 1.0 $MgSO_4$ and 0.5 NaCl and (in μM) 23.1 H_3BO_3, 4.6 $MnCl_2$, 0.38 $ZnSO_4$, 0.16 $CuSO_4$, 0.052 H_2MoO_4 and 44.8 $FeSO_4$ (as ferric sodium-EDTA complex) on perforated polystyrene floats. The nutrient solution was bubbled with sterile air and changed on alternate days. The experiment was conducted in a completely randomized design with five replications. Growth chamber conditions were: photosynthetic photon flux density of 430 μM m^{-2} s^{-2}, 14-h photoperiod and 60% relative humidity. After day 7, plants were subjected to three Cr treatments.

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Research Note

Table 1 Effect of various concentrations of chromium on SOD activity (EU mg protein h^{-1}), catalase activity (EU mg protein min^{-1}), total glutathione content (μmol g^{-1} fw) (*GSH*) and proline (μg g^{-1} fw).

Parameter	TREATMENT (μM)			
	T0	**T1**	**T2**	**T3**
	Mean ± SD (PV)	**Mean ± SD (PV)**	**Mean ± SD (PV)**	**Mean ± SD (PV)**
SOD activity after 3 days	1.036 ± 0.115 (0.0)	1.699 ± 0.045 (64.4)	1.238 ± 0. 242 (45.2)	1.36 ± 0.058 (51.4)
SOD activity after 5 days	0.773 ± 0.02 (0.0)	0.951 ± 0.005 (23.1)	0.852 ± 0.011 (10.2)	1.220 ± 0.03 (50.3)
Catalase activity after 3 days	0.687 ± 0.067 (0.0)	0.577 ± 0.0092 (8.4)	0.600 ± 0.48 (1.74)	0.47 ± 0.030 (21.2)
Catalase activity after 5 days	0.433 ± 0.038 (0.0)	0.391 ± 0.136 (10.3)	0.310 ± 0.069 (17.5)	0.298 ± 0.043 (22.2)
Glutathione content after 3 days	1061.6 ± 5.02 (0.0)	1046 ± 4.88 (12.08)	988.7 ± 4.70 (16. 3)	880.10 ± 5.99 (15.09)
Glutathione content after 5 days	1008.4 ± 4.08 (0.00)	828.8 ± .5.01 (17.06)	760 ± 2.25 (18.23)	701 ± .4.06 (20.0)
Proline concentration after 3 days	0.320 ± 0.120 (0.0)	0.778 ± 0.04 (60.5)	1.32 ± 0.112 (72.1)	1.29 ± 0.080 (68.9)
Proline concentration after 5 days	0.976 ± 0.289 (0.0)	1.011 ± 0.096 (51.0)	1.532 ± 0.120 (63.0)	2.01 ± 0.0126 (74.5)

Data represents average of three samples analysed ± S.D, Values in brackets represent % variation, compared to control. Plants were subjected to three concentrations of Cr i.e., T1 = 25 μM, T2 = 50 μM and T3 = 100 μM; T0 = control.

Cr was supplied as $K_2Cr_2O_7$. Plants (15/treatment) were subjected to three concentrations of Cr (T_1 = 25 μM, T_2 = 50 μM, T_3 = 100 μM). One set of seedlings was kept without Cr i.e. T_0 and served as control. SOD (EC 1.15.1.1) activity was estimated by the method of Dhindsa *et al*. (1981), CAT oxididoredutase (EC 1.11.1.6) activity in leaves following the method of Aebi (1984), Glut (EC 1.8.1.7) content as per Anderson (1985) and proline content by the method of Bates *et al*. (1973). All results represent the mean ± SE of three replicates per treatment.

RESULTS AND DISCUSSION

Proline concentration and SOD activity increased as Cr concentration increased 3-5 days after treatment (**Table 1**). In contrast, Glut content and CAT activity decreased as Cr concentration increased 3-5 days after treatment (**Table 1**).

A common feature of different stress factors is their potential to increase the production of ROS in plant tissues. To prevent damage, plants possess an antioxidative system composed of low molecular weight antioxidants like Glut and Pro and protective antioxidant enzymes like SOD and CAT (Asada and Takahashi 1987; Aguirre and Borneo 2010). Heavy metals generate toxic ROS such as H_2O_2, O_2^-, OH^- and O_2^- which degrade important cellular components by inducing oxidative stress (Dietz *et al.* 1999). CAT is an important heme-containg enzyme that catalyses the dismutation of H_2O_2 to H_2O and O_2 and is localized in peroxisomes. It is an important enzyme required for ROS detoxification of CAT in response to Cr and has been studied in many crops like rice, wheat, green gram and even in lower mosses (Choudhury and Panda 2004; Panda and Patra 2004). Sen *et al*. (1994) observed a decrease in CAT activity and increase in peroxidase activity at concentrations above 10 μg L^{-1} Cr(VI). In most cases a decline in CAT activity was registered (Panda and Patra 1998, 2000, 2002; Panda 2003). In the present study, total CAT content in plants in response to Cr decreased since Cr is a heme-containing enzyme that affects iron uptake in dicots (Guerinot and Yi 1994). Glut and Glut-reductase are important components of the ascorbate-Glut cycle, which plays an important role in detoxification of ROS. In the present study there was a marked decrease in total Glut content. De Vos *et al.* (1997) also reported a decrease in Glut content in *Silene cucubalis* after exposure to a heavy metal (copper). Several authors have observed oxidation of different cellular thiols such as GSH, glutathione and cysteine by Cr(VI) in *in vitro* studies. Dichromate reacts with GSH at the sulphydryl group forming an unstable gluthione-CrO_3^- complex (Brauer and Wetterhahn 1991). Thiolate complexes of Cr(VI) with γ–glutamylcysteine, *N*-accetylcysteine and cysteine have also been described (Brauer *et al*. 1996). The inter-conversion of reduced and oxidized forms of Glut to maintain the redox status of the cells so as to scavenge free radicals could have caused a decrease in GSH (Shanker *et al*. 2004b).

SOD converts superoxide and hydroxyl radicals into H_2O_2 which is degraded into water and molecular oxygen by CAT or peroxidase. SOD plays a central role in defense against oxidative defense (Beyer 1994). In this study up regulation of SOD occurred in response to Cr; high SOD activity might be in direct response to the generation of super oxide radicals by Cr-induced blockage of the electron transport chain in the mitochondria. The decrease in the activity of SOD as the concentration of external Cr increased might be because of the inhibitory effect of Cr ions on the enzyme itself (Schiavon *et al*. 2008). One of the strategies that plants have evolved to counteract toxic effects of heavy metal stress is through accumulation of an organic solute like Pro which is an imminoacid that accumulates under stress conditions. Accumulation of Pro in plants under stress results due to its active synthesis from glutamate. Pro accumulation helps to conserve nitrogenous compounds and protect plant under stress. An observed increase of Pro in plants treated with Cr is not unexpected, since metabolism reaction of these compounds used may represent a stress situation comparable to various environmental stresses (Aspinall and Paleg 1981; Sumira *et al*. 2010). Under Zn stress a marked decrease in Proline content was found by Prasad and Saradhi (1995) in *Brassica* and *Cajanus.*

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