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Effect of 24-Epibrassinolide and 28-Homobrassinolide on Some Biochemical Parameters in *Raphanus sativus* L. Plants under Chromium Stress

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ABSTRACT

The effects of 24-epibrassinolide (24-EBL) and 28-homobrassinolide (28-HBL) on protein content and the activities of Polyphenol oxidase (PPO; EC 1.10.3.1) and glutathione peroxidase (EC 1.11.1.9) were studied in the 75-days old *Raphanus sativus* L. cv 'Pusa Chetaki' plants grown under chromium (Cr) metal stress. Surface sterilized seeds of *R. sativus* were pre-treated with different concentrations (0 M, 10^{-11} M, 10^{-9} M and 10^{-7} M) of 24-EBL and 28-HBL for 8 h and grown in the field. The soil was treated with different concentrations (0, 0.5, 1.0 mM) of Cr metal. Seventy-five days old plants were harvested for further analysis of biochemical parameters. Cr metal treatment enhanced the protein content and PPO activity in both roots and leaves of radish plants. However, the activity of Glutathione peroxidase (GPOX) declined. The 24-EBL and 28-HBL treatments further increased the protein content and activities of antioxidant enzymes under Cr-stressed plants.

Keywords: brassinosteroids, antioxidants, heavy metal stress, radish, glutathione peroxidase, reactive oxygen species

Abbreviations: BR, brassinosteroid; **24-EBL**, 24-epibrassinolide; **GPOX**, Glutathione peroxidase; **28-HBL**, 28-homobrassinolide; **PPO**, Polyphenol oxidase; **ROS**, reactive oxygen species

INTRODUCTION

Plants produce numerous steroids and sterols (Geuns 1978; Jones and Roddick 1988; Janeczko and Skoczowski 2005). Brassinosteroids (BRs) are polyhydroxy steroids with significant growth-promoting activity (Bhardwaj *et al.* 2008; Sharma *et al.* 2010). In plants, BRs promote cell elongation, division, differentiation, disease resistance, stress tolerance and senescence throughout the plant life cycle (Clouse 2002; Bajguz and Hayat 2008). BRs also provide resistance to plants under biotic and abiotic stresses (Khripach *et al.* 2000) like high and low temperature (Dhaubhadel *et al.* 1999), drought (Li and Van Staden 1998), salt (Sasse *et al.* 1995), infection; pesticides (Sasse 1999) and heavy metals (Sharma *et al.* 2010) stress. Heavy metal toxicity is one of the major abiotic stresses leading to hazardous health effects in animals and plants (Maksymiec 2007). At higher concentrations, these metals are toxic and severely interfere with physiology and biochemical functions of plants (Parmar and Chanda 2005; Salvatore *et al.* 2008; Triantaphylidès and Havaux 2009). These have been demonstrated to induce oxidative stress through formation of reactive oxygen species (ROS).

Chromium (Cr) phytotoxicity results in the inhibition of seed germination, degraded pigment status, nutrient balance and induces oxidative stress in plants (Panda and Choudhury 2005). Also, Cr metal toxicity triggers the formation of ROS and catalyses the Haber-Weiss reaction (Shanker *et al.* 2005; Halliwell and Guteridge 2006). Over-production of ROS is highly toxic and can oxidize biological macromolecules such as nucleic acids, proteins and lipids, thereby disturbing the membrane permeability (Stohs and Bagchi 1995; Schutzendubel and Polle 2002) and causes oxidative stress. Antioxidant enzymes play an important role in protective mechanisms against ROS and like many other biochemical systems, their effectiveness varies with the type of plant and metal involved (Ozdemir *et al.* 2004; Almeida *et al.* 2005; Hayat *et al.* 2007). The influence of BRs on the response of antioxidant enzymes of plants under stress conditions have been studied recently (Hayat *et al.* 2007; Bhardwaj *et al.* 2008; Sharma *et al*. 2010).

Raphanus sativus is a widely used plant with culinary and medicinal importance, and has a protective role against environmental mutagens and their eventual use as therapeutics (Ghayura and Gilani 2007; Alquasoumi *et al*. 2008). *Raphanus* plants facing stressful conditions viz. heavy metal stress show reduced growth and development (Sharma *et al*. 2010). Recently, considerable efforts have been given to find out the possible roles of two most biologically active BRs *viz*. 24-epibrassinolide (24-EBL) and 28-homobrassinolide (28-HBL) in stress protection mechanisms (Khripach *et al.* 2000; Krishna 2003; Hayat *et al.* 2007; Bhardwaj *et al.* 2008). Keeping this in view, the importance of *R. sativus* in diverse ways and the role of BRs in stress amelioration, the present investigation was designed to study the effects of 28-HBL and 24-EBL on protein content and specific activity of Polyphenol oxidase (PPO) and Glutathione peroxidase (GPOX) enzyme in roots and leaves of 75-days-old *R. sativus* plants.

MATERIALS AND METHODS

Field experiment

To study the modulative effects of 28-HBL and 24-EBL on plant responses to oxidative burst produced due to heavy metal toxicity, a seasonal field experiment was carried out from December, 2008 to February, 2009 in the Experimental Fields of the Botanical Garden, Guru Nanak Dev University, Amritsar, India. Certified seeds of *R. sativus* cv 'Pusa Chetaki' were procured from the Department of Plant Breeding, Punjab Agricultural University, Ludhiana. The seeds were surface sterilized with 0.4% sodium hypochlorite

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Table 1 Effect of 28-HBL and 24-EBL on protein content (mg/g f.w.) in leaves and roots of *Raphanus sativus* L. plants under chromium metal stress (mean ± S.E.)

Treatments		Protein content in leaves			Protein content in roots		
		Control (DW)	**0.5 mM Cr**	**1.0 mM Cr**	**Control (DW)**	**0.5 mM Cr**	**1.0 mM Cr**
Control (DW)		8.106 ± 0.951	8.958 ± 0.58	17.106 ± 2.157	5.646 ± 0.512	4.866 ± 0.347	6.636 ± 0.631
Effect of 28-HBL	10^{-11} M 28-HBL	10.488 ± 1.787	4.827 ± 1.168	18.438 ± 1.01	4.518 ± 0.312	6.38 ± 0.502	8.727 ± 0.175
	10^{-9} M 28-HBL	12.99 ± 1.305	7.506 ± 0.835	10.956 ± 0.598	6.216 ± 0.56	5.106 ± 0.516	6.177 ± 0.435
	10^{-7} M 28-HBL	26.68 ± 2.148	12.387 ± 1.888	15.357 ± 1.447	7.086 ± 0.266	4.967 ± 0.657	6.858 ± 0.115
	F value (HSD)	34.32* (7.130)	6.8652* (5.750)	5.8458* (6.894)	2.006 (2.918)	1.020 (1.996)	8.4553* (1.842)
Effect of 24-EBL	10^{-11}M 24-EBL	11.775 ± 1.21	10.308 ± 1.237	14.514 ± 1.037	5.436 ± 0.026	5.106 ± 0.251	4.107 ± 0.435
	10^{-9} M 24-EBL	3.708 ± 0.0529	6.012 ± 1.213	15.084 ± 0.301	5.268 ± 0.822	5.016 ± 0.705	4.05 ± 0.646
	10^{-7} M 24-EBL	4.488 ± 0.489	6.231 ± 0.7179	16.98 ± 1.452	6.288 ± 0.7	5.817 ± 0.129	4.467 ± 0.249
	F value (HSD)	2.465 (7.028)	71.912* (1.393)	39.46* (3.704)	2.006 (2.918)	1.020 (1.996)	8.455* (1.842)

* Indicates statistically significant values at $P \leq 0.05$, DW indicates distilled water.

for 15 min, followed by repeated rinses in sterile distilled water. The seeds were soaked in the different concentrations of 28-HBL and 24-EBL (Sigma-Aldrich) (0, 10^{-11}, 10^{-9}, 10^{-7} M) for 8 h. The 28-HBL and 24-EBL pre-treated seeds were then sown in the experimental field. The field's soil was treated with different concentrations (0, 0.5 and 1.0 mM) of Cr. The Cr (Cr III) was given in the form of K_2CrO_4 (Hi-media, Mum- bai, India). Though Cr VI is more phytotoxic than Cr III but Cr III is reported to have more catalytic activity in Fenton reaction (Panda and Choudhury 2005). A factorial experiment was designed containing three replicates of each treatment of both BRs and Cr metal alone and in combinations; each replicate, with 5 plants each, was carried out in the experimental field. Biochemical analyses were performed both on the roots and leaves of 75-days-old *R. sativus* plants.

Biochemical analyses

1. Preparation of extracts

The roots and third leaves from the apex of radish plants were removed for further biochemical analyses. 5 g of each organ was homogenized in 50 mM phosphate buffer [pH 7.0, 1 mM EDTA, 1 mM PMSF, 0.5% (v/v) Triton X-100 and 2% (w/v) polyvinylpyrrolidone (PVP-30)] in a pre-chilled mortar and pestle. The homogenate was centrifuged at 15,000 × *g* for 20 min at 4°C. The supernatant was further used for assessing the protein content and activities of PPO and GPOX.

2. Protein content

Total soluble protein content was estimated by the Lowry *et al.* (1951) method using bovine serum albumin as a standard. A graph of absorbance vs concentration for standard solutions of protein was plotted and the amount of protein in the sample was calculated from the graph. The amount of protein was expressed as mg/g fresh tissue.

3. PPO assay

PPO (EC 1.10.3.1) activity was assayed as described by Kumar and Khan (1982) with modifications. The assay mixture for PPO contained 1 ml of 0.1 M phosphate buffer (pH 7.0), 0.5 ml of 0.1 M catechol and 0.25 ml of enzyme extract. This was incubated for 2 min at 25°C after which the reaction was stopped by adding 0.5 ml of 2.5 N H_2SO_4. The absorbance of the purpugallin formed was read at 495 nm. In the blank, 2.5 N H_2SO_4 was added at zero time to the assay mixture. PPO activity was expressed in Unit mg^{-1} protein (U = change in 0.1 absorbance min^{-1} M^{-1} protein).

4. GPOX assay

GPOX (EC 1.11.1.9) activity was measured according to the method given by Flohe and Gunzlar (1984) with modifications. This method is based on the principle that GPOX catalyzes the formation of GSSG from GSH and hydrogen peroxide (H_2O_2). GSSH is commonly reduced by excess GR and simultaneously, the oxidation of NADPH was measured at 340 nm. 1 ml of reaction mixture was placed in a cuvette containing 500 µl NADPH, 100 µl H_2O_2 and 50 µl enzyme extract from the root/leaf sample. After 1 min, the decrease in absorbance due to NADPH oxidation was monitored at 340 nm. The activity was expressed in U mg^{-1} protein. One U is defined as the change in absorbance by 0.1 min^{-1} mg^{-1} protein.

Statistical analysis

Statistical analysis was performed by calculating the mean value, standard error and using one-way analysis of variance (ANOVA), followed by calculations of F and HSD values by two-way ANOVA. The values are presented as mean ± S.E. Significant differences between the control and treatments was evaluated at $P \leq 0.05$ (Bailey 1995).

RESULTS

Cr metal stress significantly affected the protein content and activities of GPOX and PPO in both roots and leaves of radish plants. Seed-presoaking treatment with 28-HBL and 24-EBL significantly enhanced the protein content and activities of antioxidant enzymes under Cr-stressed plants (**Tables 1-3**). Overall, in both roots and leaves, 28-HBL alone was a more effective treatment than 24-EBL alone in comparison to untreated plants.

Effect on protein content

Protein content increased significantly with an increase in Cr metal concentration in both roots and leaves of *R. sativus* plants (**Table 1**). 24-EBL and 28-HBL significantly enhanced the levels of protein under Cr stress. 28-HBL was a more effective treatment than 24-EBL compared to the control.

1. Protein content in leaves

Maximum increase in protein content was observed at 1 mM Cr stress in leaves (17.106 mg/g fresh weight (FW)). However, seed-presoaking with 28-HBL at 10^{-7} M (15.357 mg/g FW) and 10^{-11} M (18.438 mg/g FW) at 1 mM stress further enhanced the total soluble proteins significantly. In contrast, 10^{-7} M of 24-EBL alleviated the protein content and it was found to be maximum (16.98 mg/g FW) at 1.0 mM Cr. To conclude, 10^{-7} M of HBL and 10^{-11} of 24-EBL were the most effective concentrations to enhance protein content alone and in combination with Cr stress in leaves of radish plants. Overall, 28-HBL alone increased the protein content approximately 3.5 times more than the control whereas 24-EBL treatment showed a 1.4-fold increase. Hence, 28-HBL was more effective than 24-EBL when compared to the control.

2. Protein content in roots

A similar trend was followed in roots: maximum increase in protein levels was observed at 1 mM Cr (6.636 mg/g FW). Protein content decreased significantly to a minimum

Table 2 Effect of 28-HBL and 24-EBL on specific activity of Polyphenol oxidase (PPO) (unit activity/mg protein) enzyme in leaves and roots of *Raphanus sativus* L. plants under chromium metal stress (mean ± S.E.).

Treatments		Activity of PPO in leaves			Activity of PPO in roots		
		Control (DW)	**0.5 mM Cr**	**1.0 mM Cr**	**Control (DW)**	**0.5 mM Cr**	**1.0 mM Cr**
Control (DW)		0.048 ± 0.001	0.042 ± 0.0075	0.0214 ± 0.004	0.049 ± 0.002	0.075 ± 0.006	0.062 ± 0.002
Effect of	10^{-11} M 28-HBL	0.045 ± 0.007	0.027 ± 0.001	0.025 ± 0.0012	0.058 ± 0.001	0.092 ± 0.001	0.097 ± 0.002
28-HBL	10^{-9} M 28-HBL	0.053 ± 0.005	0.063 ± 0.0053	0.041 ± 0.0041	0.087 ± 0.002	0.038 ± 0.001	0.102 ± 0.001
	10^{-7} M 28-HBL	0.022 ± 0.006	0.039 ± 0.001	0.033 ± 0.002	0.103 ± 0.001	0.115 ± 0.002	0.075 ± 0.001
	F value (HSD)	51.248* (0.009)	4510.9* 0.012)	54.831* (0.006)	3713.8* (0.036)	690.02*(0.014)	324.68*(0.016)
Effect of	10^{-11}M 24-EBL	0.02 ± 0.0012	0.027 ± 0.002	0.016 ± 0.002	0.047 ± 0.002	0.099 ± 0.008	0.144 ± 0.002
24-EBL	10^{-9} M 24-EBL	0.139 ± 0.00045	0.083 ± 0.002	0.0423 ± 0.002	0.098 ± 0.001	0.079 ± 0.002	0.104 ± 0.002
	10^{-7} M 24-EBL	0.093 ± 0.002	0.06 ± 0.002	0.027 ± 0.003	0.11 ± 0.003	0.063 ± 0.007	0.208 ± 0.006
	F value (HSD)	517.8* (0.011)	6198.9* 0.011)	4138.3* (0.03)	24.18* (0.036)	0.874 (0.494)	323.29*(0.016)

* Indicates statistically significant values at $P \leq 0.05$, DW indicates distilled water.

Table 3 Effect of 28-HBL and 24-EBL on specific activity of glutathione peroxidase (GPOX) (unit activity/mg protein) (GPOX) enzyme in leaves and roots of *Raphanus sativus* L. plants under chromium metal stress (mean ± S.E.).

Treatments		Activity of PPO in leaves			Activity of PPO in roots		
		Control (DW)	**0.5 mM Cr**	**1.0 mM Cr**	**Control (DW)**	**0.5 mM Cr**	**1.0 mM Cr**
Control (DW)		0.0019 ± 0.00005	0.0008 ± 0.00002	0.00093 ± 0.00002	0.0077 ± 0.0004	0.0024 ± 0.0001	0.007 ± 0.0004
Effect of 28-	10^{-11} M 28-HBL	0.00092 ± 0.00004	0.0009 ± 0.000032	0.0009 ± 0.00004	0.0066 ± 0.0003	0.0020 ± 0.00012	0.015 ± 0.0015
HBL	10^{-9} M 28-HBL	0.0008 ± 0.00004	0.0004 ± 0.00001	0.0001 ± 0.00006	0.0123 ± 0.0005	0.0061 ± 0.0002	0.0055 ± 0.0002
	10^{-7} M 28-HBL	0.0005 ± 0.00002	0.001 ± 0.00003	0.0001 ± 0.00005	0.0006 ± 0.0002	0.0034 ± 0.0004	0.0027 ± 0.0001
	F value (HSD)	0.847 (0.004)	1.193 (0.002)	0.742 (0.039)	2.006 (2.918)	1.020 (2.918)	8.455* (1.842)
Effect of	10^{-11} M 24-EBL	0.0007 ± 0.00008	0.0014 ± 0.00045	0.0004 ± 0.00004	0.0027 ± 0.00002	0.0087 ± 0.0006	0.0187 ± 0.0011
24-EBL	10^{-9} M 24-EBL	0.00057 ± 0.00004	0.00196 ± 0.0001	0.0021 ± 0.00032	0.0053 ± 0.00001	0.0072 ± 0.00005	0.0008 ± 0.0002
	10^{-7} M 24-EBL	0.00098 ± 0.00003	0.0012 ± 0.00043	0.0035 ± 0.00005	0.0016 ± 0.00015	0.0039 ± 0.0006	0.0014 ± 0.00021
	F value (HSD)	0.201 (0.002)	0.615 (0.003)	11.204* (0.006)	0.420 (3.343)	1.021 (1.996)	5.636* (2.470)

* Indicates statistically significant values at $P \leq 0.05$, DW indicates distilled water.

(4.866 mg/g FW) by 0.5 mM Cr treatment and increased by 1 mM Cr treatment showing an overall enhancement in content. 28-HBL treatment at 10^{-7} M significantly enhanced the protein content to a maximum in the control (7.086 mg/g FW) while 10^{-11} M 28-HBL was effective in ameliorating the stress caused by Cr (**Table 1**). The protein content was increased 1.25- and 1.2-fold by 28-HBL or 24-HBL, respectively as compared to the control. Overall, 24-EBL at 10^{-7} M improved protein levels in roots of *R. sativus* plants most under Cr metal stress (**Table 1**).

Effect on PPO activity

Specific activity of PPO decreased as Cr stress in leaves increased whereas in roots an increasing trend was observed under Cr stress in *R. sativus* plants (**Table 2**). Furthermore, seed-presoaking treatments of both BRs effectively modulated the activity of PPO in radish plants under Cr stress. In leaves, 24-EBL was more effective than 28-HBL compared to the control whereas in roots, the opposite trend was observed.

1. Activity of PPO in leaves

The activity of PPO was observed to decrease to lowest decline at 1 mM Cr (0.0214 unit activity/mg protein). Further treatment of 28-HBL at 10^{-9} M concentration, significantly increased the specific activity of PPO to the maximum (0.063 unit activity/mg protein) at 0.5 mM Cr. Similarly, 10^{-9} M of 24-EBL significantly enhanced the specific activity of PPO to the maximum (0.083 unit activity/mg protein) at 0.5 mM Cr metal stress. 10^{-11}M 28-HBL Overall, 10^{-9} M treatment of both 28-HBL and 24-EBL was observed to have maximum significant enhancing effect in specific activity of PPO in radish leaves. Treatment of 24-EBL alone resulted in 3.9-fold (approximately) increase in PPO activity whereas 28-HBL showed a 1.1-fold increase compared to the control.

2. Activity of PPO in roots

At 0.5 mM Cr concentration maximum increase (0.075 unit activity/mg protein) in the specific activity of PPO was observed. A similar increasing trend was followed in roots when 24-EBL and 28-HBL treatments were given to radish plants under Cr stress. It was observed that 10^{-7} M 28-HBL and 10^{-9} 24-EBL were most effective concentrations in alleviating the PPO activity in *R. sativus* roots. When effect of both BRs was compared, 28-HBL showed 3-fold increase whereas 24-HBL resulted in a 2.2-fold increase as compared to the control.

Effect on GPOX activity

With increase in Cr stress, the specific activity of GPOX was observed to decrease significantly in both the roots and leaves in radish plants. However, both 24-EBL and 28-HBL significantly enhanced the activity of GPOX under Cr stress in both roots and leaves of radish plants (**Table 3**). When compared together both BRs (24-EBL and 28-HBL alone) were not found effective in altering activity of GPOX as compared to the control.

1. Activity of GPOX in leaves

Maximum decline in specific activity of GPOX was observed at 0.5 mM Cr (0.0008 unit activity/mg protein) in leaves. It was observed that 10^{-7} M of 28-HBL and 10^{-7} M of 24-EBL significantly increased the specific activity of GPOX effectively in leaves. When both the treatments of BRs were compared, there was no significant change (in GPOX activity) caused by both 24-EBL and 28-HBL alone as compared to the control.

2. Activity of GPOX in roots

Similarly, lowest activity of GPOX was noticed at 0.5 mM Cr (0.0024 unit activity/mg protein) in roots. The treatment of 10^{-9} M of both 28-HBL and 24-EBL were noticed to be most effective in control whereas 10^{-11} M of both 28-HBL and 24-EBL showed maximum enhancing effect in 0.5 mM Cr and 1 mM Cr. There was no significant increase/decrease in activity of GPOX was observed when treatments of 24-EBL and 28-HBL alone were evaluated as compared to the control.

DISCUSSION

The present investigation revealed that seed-presoaking treatments of both 28-HBL and 24-EBL significantly alleviated the effects of Cr stress in *R. sativus* plants by enhancing the levels of soluble proteins and the activities of PPO and GPOX enzymes. Cr toxicity is well-documents by Shanker *et al.* (2005) in terms of reduced growth and development of Cr-stresses plants. Cr-induced ROS causes plant membrane damage, ultra-structural modifications in organelles, impaired metabolic activities (enzyme activities) and growth retardations (Panda 2007). Similarly, in present study, overall decrease in activities of PPO and GPOX were noticed in *R. sativus* plants under Cr stress as compared to control (**Tables 2, 3**). To overcome ROS-induced damaging effects, the plant defence system is well-equipped with both enzymatic and non enzymatic mechanisms to scavenge free radicals (Foyer and Noctor 2003). Antioxidant enzymes like PPO and GPOX play defensive role in protecting plant against oxidative stress. They catalyze oxidation of hydroxyphenols to their quinine derivatives which then simultaneously polymerize. GPOX in plants belongs to the phospholipids hydroperoxide glutathione peroxidase family. The function of glutathione peroxidases, such as fatty acid hydroperoxides is reduction of alkyl hydroperoxides, such as fatty acid hydroperoxides (Foyer *et al.* 1997).

Cr-phytotoxicity resulted in overall increase in protein content, which was further increased under application of 28-HBL in both roots and leaves of *R. sativus* L. (**Table 1**). These findings are in coherence with the previous report of Hayat and Ahmad (2003) in *Triticum aestivum* where 28-HBL increased the protein content. Similarly, 28-HBL was reported to enhance the protein content in *Oryza sativa* (Anuradha and Rao 2007; Maheshwari and Dubey 2008) and *Vigna radiata* L. (Jaleel *et al.* 2007) under heavy metals stress. In *Chlorella vulgaris*, BRs increased the contents of DNA, RNA and protein (Bajguz 2000). It suggests the possible role of BRs-mediated regulation of certain genes at transcriptional and translational levels.

Present findings revealed that both 28-HBL and 24-EBL increased the activity of PPO in radish plants. These observations are also consistent with earlier findings of Jaleel *et al.* (2007) in *Arachis hypogea*, Azooz *et al.* (2009) *Vigna radiata* and Hornero-Mendez *et al.* (2002) in *Gordaland manzanilla.* In the metabolism of polyphenols, the enzyme PPO is involved (Khripach *et al.* 2000), therefore any alteration in activity of PPO may be considered as one of the important factor correlated with increased plant protection under stress. In assessment of GPOX, overall decrease in specific activity was observed in study material (**Table 3**). However, seed-presoaking treatments with both BRs resulted in enhanced activity of GPOX enzyme under Cr stress. These results are supported by Dixit *et al.* (2001) in *Pisum sativum* L., where GPOX activity decreased in roots and remained unmodified in leaves. Altered activities of these enzymes further suggest that 28-HBL and 24-EBL treated plants were less affected by Cr metal than the control plants (**Tables 2, 3**). Also, present study reveals that 28-HBL alone is more effective treatment than 24-EBL alone in comparison to untreated plants. The difference in the activities of these two important BRs may be attributed to structural differences from each other (Khripach *et al*. 2000). 28-HBL differs from 24-EBL by the substitution at C-24 and its configuration at C-24.

An enhancement in the protein content and in the activity of antioxidant enzyme PPO under metal stress, suggests that higher antioxidant enzyme activity have a role in imparting tolerance against chromium metal stress. Decrease in specific activity of GPOX may be attributed to its ability to scavange hydroperoxides. It also functions to eliminate lipid hydroperoxides from cellular membranes, where oxidative stress leads to their lipid peroxidation (Vorobets 2006). BRs also regulate the cell wall elongation by enhancing the activities of cell wall loosening enzymes via activation of H^+-ATPase (Khripach *et al.* 2000; Haubrick and Assmann, 2006). Hence, BRs-regulated stress protection is a consequence of multifarious biochemical response regulated by both enzymatic and non-enzymatic antioxidant defence system in plants. Thus, the present findings suggest the possible ameliorative role of BRs (28-HBL and 24-EBL) in modulating the activities of key antioxidant enzymes under Chromium stress.

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