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Physiological Mechanisms of *Brassica Juncea* L. Plants Exposed to Cadmium Metal Stress

KEYWORDS: Cadmium toxicity, Brassica juncea, Osmolytes, Antioxidative defence system

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ABSTRACT *Plants of Brassica juncea L. var. RLC-1 were exposed for 90 days to different concentrations (0, 0.2, 0.4 and 0.6mM) of cadmium (Cd) to analyze the growth (root length, shoot length and number of leaves), level of osmoprotectants (total osmolytes, proline and glycine-betaine content) and activities of antioxidative enzymes (ascorbate peroxidase, glutathione peroxidase and polyphenol oxidase. Results of the present study revealed the decreased growth of Brassica with increasing metal doses. Level of osmolytes was positively influenced by Cd stress and activities of antioxidative enzymes were altered significantly in response to metal treatment. In conclusion, level of osmolytes and antioxidative defence system of plants got activated due to heavy metal stress, which protects the plants by scavenging free radicals.*

Introduction
Heavy metal pollution is emerging environmental threat to the world. Heavy metal concentration is rising in the environment due to various natural sources such as forest fires, wind-blown dust, decaying vegetation and sea spray and certain anthropogenic activities like use of phosphate fertilizer, metal production, mining and wood production, etc. 1 Cadmium (Cd) is considered as one of the most toxic metals due to its high water solubility and phytotoxicity. 2 Main symptoms of Cd toxicity are leaf chlorosis, stunted growth and unspecific necrosis which cause death of plants. 3 Cd leads to alteration in metabolic processes of plants such as photosynthesis, nutritional status and respiration. 4 It triggers the generation of reactive oxygen species (ROS) like hydrogen peroxide, superoxide radicals and hydroxyl ions and consequently leads to oxidative burst. To ameliorate heavy metal stress, plants have evolved certain defence strategies like antioxidative defence system and accumulation of compatible solutes. 5 Brassica juncea is an edible crop and also hyperaccumulator of Cd metal. Therefore, present investigation was undertaken to study the growth, antioxidative defence system and level of osmoprotectants in 90-days old plants of B. juncea.

Material and Methods
Field experiment was conducted in Botanical Garden of Guru Nanak Dev University, Amritsar. The surface sterilized seeds were sown in different blocks of field, in which different treatments of Cd metal was given (0, 0.2, 0.4 and 0.6 mM Cd). Plants were then harvested after 90-days of germination.

Growth Parameters
Root length, Shoot length and number of leaves of 90-days old plants were observed.

Estimation of Osmolytes:
Total osmolyte content:
Total osmolyte content was analyzed by using vapor pressure osmometer (VPO) (Vapro 5600).

Proline Content:
Proline was estimated by the method proposed by Bates et al. 6

Glycine betaine (GB) content:
GB content was estimated by following the method of Grieve and Grattan. 7

Estimation of activities of Antioxidative Enzymes:
Activities of antioxidative enzymes were determined by the standard methods of Nakano and Asada (1981) for ascorbate peroxidase (APOX), Carlberg and Mannervik (1975) for glutathione reductase (GR) and Kumar and Khan (1982) for polyphenol oxidase (PPO). 8-10

Statistical analysis
Each experiment was conducted in three replicates. Data was expressed in Mean±SE. To check the statistical significant difference between the treatments, one way-ANOVA was carried out by using Assistat version 7.7 beta.

Results
Reduced growth was noticed in terms of root length, shoot length and mean number of leaves Brassica juncea. Root length (6.92Cm), shoot length (44.17 Cm) and number of leaves (11) were highest in untreated control, whereas decline in growth parameters (3.6 Cm, 30.66 Cm, 7.67 respectively) were observed with increasing the concentration of Cd metal (Table 1).

Significant rise in total osmolyte content was found with the treatment of Cd metal. Least value of osmolytes was observed in control plants. Total osmolytes were noticed 1.94 folds higher in 0.4mM Cd (352.02m mol/Kg) treated plants with respect to its control (181.26m mol/Kg). 0.4mM Cd treated plants showed the maximum increase (20.61µ mol g^{-1} FW) in proline content as compared to control plants (12.21µ mol g^{-1} FW). Results revealed that GB content was lowest in control plants (6.35µ mol g^{-1} FW), whereas highest GB was found in 0.2mM Cd treatment (12.27µ mol g^{-1} FW) (Table 2).

Control plants showed minimum APOX activity as compared to metal treated plants (8.43UA mg^{-1} protein). Maximum enzyme activity was noticed in 0.6mM Cd treatment (14.21UA mg^{-1} protein). GR activity was reduced from 10.97 to 10.74UA mg^{-1} protein in 0.2mM Cd treatment. Enzyme activity then enhanced from 10.97 to 15.3UA mg^{-1} protein

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in 0.4mM Cd and from 10.97 to 14.47UA mg^{-1} protein in 0.6mM Cd stress. Similarly, activity of PPO also decreased in 0.2mM Cd (6.88UA mg^{-1} protein), which got increased in 0.4mM (8.49UA mg^{-1} protein) and 0.6mM Cd (7.12UA mg^{-1} protein) treatment (Table 3).

Table 1. Effect of Cd on Root Length, Shoot Length and number of leaves of 90 days old B. juncea Plants

Treatments	Root Length (Cm)	Shoot Length (Cm)	Number of Leaves
0.0 mM	6.92±0.19 [a]	44.17±2.03 [a]	11.0±0.58 [a]
0.2mM	4.79±0.49 [b]	38.71±1.12 [ab]	8.67±0.33 [b]
0.4mM	3.63±0.28 [b]	35.28±1.44 [bc]	7.67±0.33 [b]
0.6mM	3.6±0.1 [b]	30.66±1.19 [c]	7.67±0.33 [b]

Table 2. Effect of Cd on Proline, Glycine-betaine and Total Osmolyte Content in 90 days old B. juncea Plants

Treatments	Proline (μ mol g^{-1} FW)	Glycine-betaine (μ mol g^{-1} FW)	Total Osmolytes (m mol/Kg)
0.0 mM	12.21±0.70 [c]	6.35±0.87 [b]	181.26±6.63 [b]
0.2mM	16.51±0.89 [b]	12.27±0.76 [a]	269.45±18.67 [ab]
0.4mM	20.61±0.73 [a]	10.11±0.85 [a]	352.02±26.28 [a]
0.6mM	16.78±1.06 [ab]	9.03±0.39 [ab]	257.33±22.28 [b]

Table 3. Effect of Cd metal on Protein Content, Specific Activities of APOX, GR and PPO in 90 days old B. juncea Plants

Treatments	APOX (UA mg^{-1} protein)	GR (UA mg^{-1} protein)	PPO (UA mg^{-1} protein)
0.0 mM	8.43± 0.71 [b]	10.97± 0.82 [b]	7.48± 0.49 [ab]
0.2mM	10.44± 0.68 [b]	10.74± 0.53 [b]	6.88± 0.47 [c]
0.4mM	8.63± 0.44 [b]	15.3± 0.53 [a]	8.49± 0.62 [a]
0.6mM	14.21± 0.94 [a]	14.47± 0.44 [a]	7.12± 0.07 [ab]

Data presented in mean ± SE. Different letters (a, b, c & d) within various concentrations of Cd (0, 0.2, 0.4 and 0.6mM) are significantly different (Fisher LSD post hoc test, $p \leq 0.05$) and signify the effect of Cd metal on Enzyme activities.

Discussion

Growth of Brassica juncea plants was observed to inhibit due to Cd toxicity. Metal mostly accumulates in the roots of plants and its translocation is regulated by root cell wall. 11 Due to its harmful effects on the roots, nutrition and water supply is blocked, which negatively affects the growth and physiology of aerial parts of plants. 12 There is increased accumulation of osmolytes under stress in present study. Osmolytes like proline and glycine-betaine play key role in cellular compatibility and osmotic adjustment to maintain normal metabolism of plants during stress. 13 They also protect the plants by scavenging ROS produced during stress and also provide protection to cellular machinery. 14 Activities of antioxidative enzymes namely APOX, GR and POD were increased in the plants treated with Cd in Brassica juncea plants. ROS-scavenging antioxidant enzymes such as APOX, GR etc. maintain the balance between generation and detoxification of ROS. 15 APOX helps in removal of H_2O_2. 16 GR is a member of flavoenzyme family that induces the NADPH dependent reduction of glutathione disulphide (GSSG) to glutathione (GSH), which further reduces dehydroascorbate to ascorbate. 17 Therefore, it is concluded from the investigation that Cd toxicity caused retardation in the growth of 90 days old Brassica juncea. However, defence strategies of plants such as antioxidative defence system and osmolytes got activated to combat the metal stress.

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