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Antioxidative Defence Responses in *Brassica juncea* Plants Exposed to Cadmium Toxicity

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Abstract: ***In the present study, effect of cadmium (Cd) metal on antioxidant potential of Brassica juncea plants was observed. Seeds of Brassica juncea var. RLC-1 were given the treatment of three different concentrations (0.2, 0.4 and 0.6mM) of Cd. Activities of antioxidative enzymes (dehydroascorbate reductase, monodehydroascorbate reductase and glutathione peroxidase) and level of antioxidants (tocopherol and flavonoid) of 90-days old plants was analyzed. Results revealed that antioxidant potential of B. juncea plants were found to improve in terms of enhanced activities of enzymes and antioxidant content under Cd stress. Therefore, present piece of work suggested that treatment of Cd activated defence strategies in Brassica juncea plants to overcome the stress.***

Keywords: *Brassica juncea*, heavy metal stress, antioxidants, antioxidative enzymes

1. Introduction

In the agricultural soils, metals naturally found in low concentrations as infrequent element. Among metals, cadmium (Cd) is generally released into the environment from farming practices and industrial processes [1, 2]. Cd is easily uptaken by plant roots due to its highly toxic nature and transported through xylem into above aerial parts [3, 4]. It may cause serious threats to human health by entering into the food chain [5, 6]. Although it is a nonredox metal, but also considered as a strong phytotoxic metal leads to growth inhibition and plant death [1]. Sensitivity of plants varies towards Cd doses. As most of the plants are sensitive to low concentrations of Cd which further cause alteration in the chloroplast ultrastructure, antioxidant enzymes, disturbs the calvin cycle, photosynthesis and uptake and distribution of macro and micronutrients [7, 8]. Through the overproduction of reactive oxygen species (ROS), Cd produces oxidative stress in plants, further leads to oxidative burst. To protect themselves from ROS, plants possess a wide range of defence strategies which includes efficient antioxidative enzymes and low molecular weight antioxidants [9].

Brassica juncea is an oilseed crop, mainly acts as a food crop and also used for its medicinal purposes. The components of this crop include antioxidants like carotenes, flavonoids, lutein, indoles, and zea-xanthin[10], which help in the motivation of cellular defence system and biological system against oxidative damage. Therefore, present investigation was designed to study the effect of Cd stress on antioxidative defence system of *Brassica juncea* plants.

2. Materials and Methods

To study the effects of Cd metal on *Brassica juncea* plants, a field experiment was conducted in Botanical Garden of Guru Nanak Dev University, Amritsar. 20 X 20 feet area was taken for the experimentation and soil: manure in a ratio of 3:1 was added into it. The certified and disease free seeds of *Brassica juncea* L. var. RLC-1 were purchased from Punjab Agricultural University, Ludhiana, Punjab and surface sterilized with 0.01% mercuric chloride solution, followed by the repeated washing of sterile double distilled water (DDW). Different treatments of Cd metal were given (0, 0.2, 0.4 and 0.6 mM Cd). Plants were then harvested after 90-days of germination to study following parameters:

2.1 Antioxidants

2.1.1 Total flavonoid Content

Total flavonoid content was estimated by method given by Kim et al. [11].

2.1.2 Tocopherol content

Vitamin E was estimated by the method given by Martinek [12]. 0.5 ml of plant extract was mixed with 0.5 ml of absolute ethanol and 0.5 ml double distilled water. 0.5 ml of xylene was added to it and mixture was centrifuged at 3,000 rpm for 10 minutes. 0.5 ml xylene (top) was mixed with 0.5 ml of TPTZ reagent and added to the mixture and absorbance was taken at 600nm. 1 mg $100ml^{-1}$ concentration was used as a standard for tocopherol content determination.

2.2 Estimation of activities of antioxidative enzymes

2.2.1 Dehydroascorbate Reductase (DHAR) Activity

DHAR activity was measured by the method of Dalton et al. [13]. The reaction mixture consists of 1.5 ml phosphate buffer, 300 µL glutathione reduced, 300 µL dehydroascorbate and 400 µL enzyme extract. Increase in absorbance was recorded at 265 nm and enzyme activity was determined by extinction coefficient 14 mM^{-1} cm^{-1}.

2.2.2 Monodehydroascorbate reductase (MDHAR) activity

MDHAR activity was estimated by Hossain et al. [14] method. Reaction mixture was followed by 1.8 ml of phosphate buffer, 300 µL EDTA, 200 µL NADH, 250 µL ascorbate oxidase and 300 µL enzymes extract. Enzyme activity was determined by extinction coefficient 6.22 mM^{-1} cm^{-1}. Decrease in absorbance was measured at 340 nm.

2.2.3 Glutathione Peroxidase (GPOX) activity

GPOX activity was analyzed according to the method of Flohe and Gunzlar [15]. GPOX stimulates the production of GSSG from GSH and H2O2. GR cause reduction of GSSH and NADPH oxidation is measured at 340 nm. In 1 ml of reaction mixture 500 µl PPB, 100 µl EDTA, 100 µl NADPH and 100 µl H_2O_2 was added in a test tube. Then 50 µl of enzyme extract was added in it. Decrease in absorbance due to oxidation of NADPH was measured after 1 minute.

Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA) for scrutinizing the effect of Cd metal on various experiments. The *Fisher LSD post hoc test* ($p \leq 0.05$) was applied for the comparisons against control values using Assistat version 7.7 beta.

3. Results and Discussion

Flavonoid content decreased with Cd metal treatment from 0.2mM to 0.4mM Cd (3.88 to 2.87mg g^{-1} FW) (Fig 1). In 0.6mM Cd stressed plants, flavonoid content was further increased (2.97mg g^{-1} FW). With increasing Cd toxicity, tocopherol content enhanced in dose dependent manner. Maximum value of tocopherol was seen in 0.6mM Cd stressed plants (9.39mg g^{-1} FW) (Fig 2). In 0.2mM and 0.4mM Cd stress vit-E content got enhanced (8.18 and 8.81mg g^{-1} FW respectively), which was lower than 0.6mM Cd treatment. The non-enzymatic radical-scavengers have been considered as the major antioxidants for the removal of H_2O_2 in plant cells and consequently reducing the accumulation of the free radicals[16]. The results are supported by the findings of Yusuf et al. [17], where tocopherol was noticed to enhance due to Cd and NaCl toxicity in *Brassica juncea* plants.

A continuous increase in the activity of dehydroascorbate reductase (DHAR) was recorded with the increasing doses of Cd as compared to control plants (Fig 3). Maximum DHAR activity was observed in 0.6mM Cd treated plants (15.67UA mg^{-1} protein). Activity of monodehydroascorbate reductase (MDHAR) was altered slightly differently by Cd toxicity (Fig 4). Control plants showed minimum enzyme activity as compared to metal treated plants (11.51UA mg^{-1} protein). Activity of MDHAR first enhanced from 11.51 to 13.34UA mg^{-1} protein in 0.2mM Cd treated plants. Maximum enzyme activity was noticed in 0.6mM Cd treatment, where it got enhanced from from 11.51 to 14.19UA mg^{-1} protein. Activity of glutathione peroxidase (GPOX) was increased due to Cd toxicity as compared to control (Fig 5). Enzyme activity was found to enhance with the metal treatment from 5.72 to 8.63UA mg^{-1} protein in 90 days old plants exposed to 0.4mM Cd stress. MDHAR helps in regeneration of ascorbic acid and DHAR reproduces it by utilizing glutathione reduced (GSH) to form glutathione oxidised (GSSG) at the cost of nicotinamide adenine dinucleotide phosphate (NAD(P)H) and scavenge the reactive oxygen species. Similarly a rise in the activities of antioxidant enzymes was reported in *Nasturtium officinale* subjected to Ni stress [18].

4. Conclusions

This study confirms that under Cd toxicity activate the antioxidant potential of *Brassica juncea* plants in terms of activities of antioxidative enzymes and level of antioxidants, which protect the plants from heavy metal stress by scavenging free radicals.

5. Future Scope of Study

The present study will contribute in understanding the physiological mechanisms of plants and give rise to development of stress-tolerant varieties in response to various environmental stresses.

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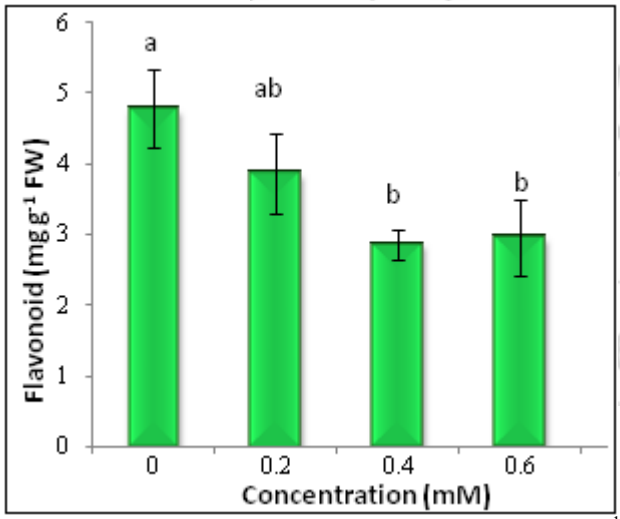

Figure 1: Effect of Cd metal on Flavonoid content (mg g^{-1} FW) in 90 days plants of *Brassica juncea*.

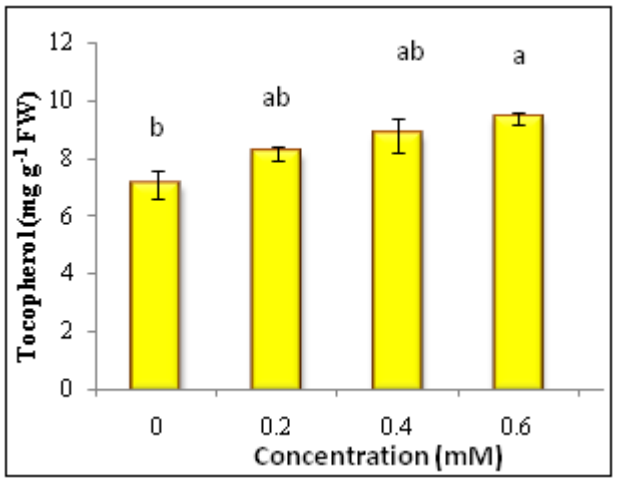

Figure 2: Effect of Cd metal on Tocopherol content (mg g^{-1} FW) in 90-days old Plants of *Brassica juncea*.

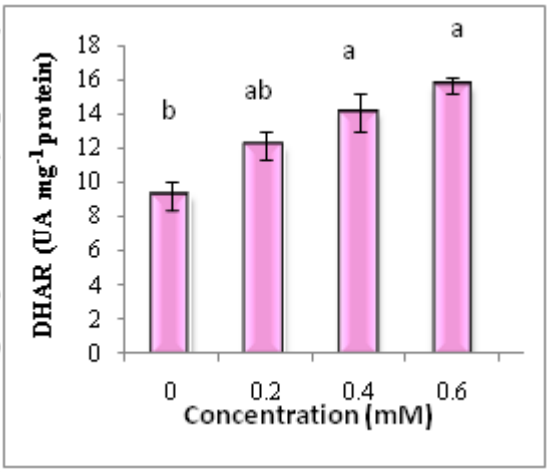

Figure 3: Effect of Cd metal on DHAR activity (UA mg^{-1} protein) in 90-days old Plants of *Brassica juncea*.

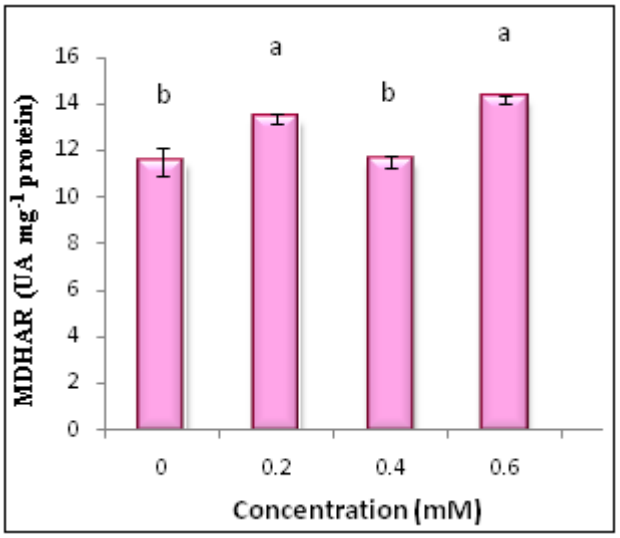

Figure 4: Effect of Cd metal on MDHAR activity (UA mg^{-1} protein) in 90-days old Plants of *Brassica juncea*.

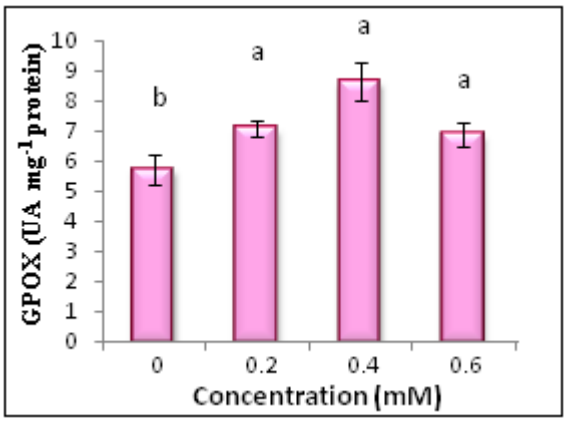

Figure 5: Effect of Cd metal on GPOX activity (UA mg^{-1} protein) in 90-days old Plants of *Brassica juncea*.

Author Profile

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