Research Article

Alteration in the Level of Osmoprotectants and Antioxidant analysis by Confocal Microscopy in *Brassica juncea* Subjected to Metal Stress

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ABSTRACT

Heavy metals are major environmental contaminants in today's world. Oxidative stress is produced due to the accumulation of these heavy metals beyond critical levels. This stress is usually subjugated by antioxidant defence system and compatible solutes. Thus the present investigation has been mainly focused to find out the effects of cadmium metal on the level of osmoprotectants and antioxidant in the seedlings of *Brassica juncea*. Seedlings of *Brassica* were exposed for 7 days to different concentrations (0, 0.2, 0.4, and 0.6mM) of cadmium to analyze the level of total osmolytes, sugars and glutathione. Results of the present study revealed the increased osmolytes content and sugar uptake with increasing metal doses. Qualitative analysis by HPLC showed the activation of various sugars under different concentrations of metal. Confocal microscopic study of roots revealed that glutathione level enhanced with increasing metal doses. In conclusion, the antioxidants and osmoprotectants play defensive role in plants that protects them by scavenging free radicals, generated during stress conditions.

Keywords: *Brassica juncea*, heavy metal, osmoprotectant, antioxidant.

INTRODUCTION

In today's world, agricultural soils are contaminated by heavy metal toxicity like Zn, Ni, Cd, Cu, As Co, Cr and Pb. Regular use of phosphatic fertilizers, sewage sludge application, dust from smelters, wrong watering practices in agricultural lands and industrial waste lead to the soil contamination[1]. Generation of reactive oxygen species (ROS) is the primary response of plants when exposed to the critical levels of heavy metals. Heavy metal toxicity triggers the overproduction of ROS and generation of oxidative stress in plants[2]. Disturbances in the metabolism of essential elements, antioxidant system and disruption in the electron transport chain are the indirect consequences of metal toxicity. Lipid peroxidation or increase in malondialdehyde level is one of the most deleterious effects produced by heavy metals exposure in plants that can directly cause biomembrane deterioration[3].

Plants have an extensive range of defence strategies including efficient antioxidant system and compatible solutes to protect themselves from ROS[4]. Some previous and recent studies noticed an increase in antioxidant defence machinery in plants to protect them from Cd stress[5].

Glutathione (GSH) molecules act as substrate for the synthesis of phytochelatins that play key role in the detoxification of metal by chelation.

Natural antioxidants donate a hydrogen atom or an electron to the free radical and help in quenching of oxygen-derived free radicals and also neutralize free radicals through different models system[6].

Thus the aim of the present work is to observe the responses of plant regarding their total osmolytes content, sugar (quantitatively and qualitatively) and glutathione level through anatomical studies in 7 days old seedlings of *Brassica juncea* treated with Cd metal.

MATERIALS AND METHODS

The study material for the experimentation included plants of *Brassica juncea* L. var. RLC-1. The certified and disease free seeds were procured from Punjab Agricultural University, Ludhiana, Punjab. Uniform sized seeds of *Brassica* were surface sterilized with 0.01% mercuric chloride ($HgCl_2$) for 1 minute followed by three-four rinses in sterile distilled water. Surface sterilized seeds were germinated in *Whatman No.1* filter paper lined glass petriplates (10 cm diameter, 30 seeds per Petriplate) containing the solution of Cd (0, 0.2, 0.4 and 0.6 mM).

Control seedlings were supplemented with distilled water only. Each treatment was replicated 3 times. The experiment was performed under controlled conditions (25°C ± 0.5°C, 16 h photoperiod, 175 µmol m^{-2} s^{-1} light intensity).

Total Osmolytes Content

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

Quantitative Estimation of Sugars

Total sugars were quantitatively detected by the method given by Scott and Melvin[7].

Qualitative Estimation of Sugars

High performance liquid chromatography (HPLC) was used to estimate the sugars in 7-days old seedlings. Seedlings were dried in hot air oven at 80°C and crushed to make fine powder. 2 g of powered sample was mixed with 5 ml of DDW and incubated for 12 h at room temperature.

Then samples were filtered with 0.22 micron pore sized filter paper. 20 µl supernatant was used for the analysis of sugars by HPLC.

Glutathione Estimation by Confocal Microscope

Localization of glutathione was observed in the roots of 7-days old seedlings of *B. juncea*. 1 cm section of roots were cut and placed in the 100µM solution of monochlorobimane (MCB) dye.

After 20 minutes of staining, slides of stained roots were prepared and observed under confocal microscope in 20X magnification.

Multiline argon gas laser is used for MCB to excite the electrons at the wavelength 405 nm.

RESULTS AND DISCUSSION

Total Osmolytes Content

Lowest content of total osmolytes was found in control seedlings (166.97m mol/Kg). Osmolytes were noticed to enhance with metal treatment (Table 1).

Maximum osmolytes were observed in 0.6mM Cd treatment (189.5m mol/Kg) in comparison to 0.2mM Cd (170.6 m mol/Kg) and 0.4mm Cd (183.37 m mol/Kg).

Total Sugars

Treatment of Cd enhanced the accumulation of total sugars in 7 days old seedlings of *B. juncea* (Table 1). Elevation in sugar content was noticed from 5.01 to 8.71µ mol g^{-1} FW.

Least sugar content was noticed in control seedlings (5.01µ mol g^{-1} FW), which further increased with 0.2mM Cd (7.39µ mol g^{-1} FW) and 0.4mM Cd (6.26µ mol g^{-1} FW) treatment.

Maximum value of sugar content was recorded in the seedlings exposed to 0.6mM Cd stress (8.71µ mol g^{-1} FW).

Table 1: Effect of Cd metal on Total Osmolytes and Total Sugar Content in 7 days old Seedlings of *B. juncea*

Treatments	Total Osmolytes (m mol/Kg)	Total Sugar Content (µ mol g^{-1} FW)
0.0 mM	166.97 ± 3.17^{c}	5.01 ± 0.14^{c}
0.2mM	170.6 ± 2.56^{bc}	7.39 ± 0.47^{ab}
0.4mM	183.37 ± 3.9^{ab}	6.26 ± 0.27^{bc}
0.6mM	189.5 ± 1.04^{a}	8.71 ± 0.31^{a}

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 (The *Tukey post hoc test, p≤0.05*) and signify the effect of Cd metal on total osmolytes and total sugar content.

Qualitative Analysis of Sugars

Treatment of Cd enhanced the accumulation of total sugars in 7 days old seedlings of *B. juncea* (Figs. 1-4). Elevation in sugar content was noticed from 5.01 to 8.71µ mol g^{-1} FW.

Least sugar content was noticed in control seedlings (5.01µ mol g^{-1} FW), which further increased with 0.2mM Cd (7.39µ mol g^{-1} FW) and 0.4mM Cd (6.26µ mol g^{-1} FW) treatment.

Maximum value of sugar content was recorded in the seedlings exposed to 0.6mM Cd stress (8.71µ mol g^{-1} FW).

During Cd toxicity compatible solutes such as total osmolytes and sugars were observed to get accumulated in *Brassica juncea* seedlings. Under stress conditions, Δ1-pyrroline-5-carboxylate synthase enzyme get stimulated, which further cause increase in content of compatible solutes. Osmolytes like glycine betaine is also accumulated more during the stress conditions as it is formed by choline and GB substrates.

Two-step oxidation of choline is stimulated via the toxic intermediate betaine aldehyde and these reactions are catalysed by choline monooxygenase (CMO) and NAD+-dependent betaine aldehyde dehydrogenase (BADH), which is activated under stress conditions[8]. These osmolytes provide protection against stress, they act as antioxidant by scavenging ROS and stabilizing the membranes[9]. ROS such as HO• reacts with sugars and oxidizes them to release formic acid, that is the main breakdown product[10]. Mannitol was found to accumulate in the chloroplasts; it showed an increased tolerance to oxidative stress in transgenic tobacco. As mannitol causes the removal of HO• and therefore increased mannitol is the indicator of enhanced stress protection.

These results are in coherence with the observations of Choudhary[11], where Cr toxicity caused rise in the level of GB in *Raphanus sativus*. Similarly, in *Cajanus cajan*, proline content was recorded to enhance due to Al stress[12]. The present work was also supported by the findings of Gengmao[13], where NaCl stress increased the accumulation of total sugars in *Salvia miltiorrhiza*.

Estimation of Glutathione by Confocal Microscope

Anatomical studies of roots of 7-days old seedlings showed the blue colour stains, which indicated the localization of glutathione (Figs. 5-8). It was noticed that intense staining of MCB dye was observed with increasing concentrations of Cd, which revealed the enhanced glutathione in metal stressed seedlings in comparison to control. During Cd stress, glutathione level was found to enhance in *B. juncea*.

Report suggested that level of GSH increased under Cu and Cd stress in *Cleome gynandra*[14]. Similar trends in the level of ascorbic acid and GSH were observed in *Raphanus sativus* seedlings exposed to Cr stress[11].

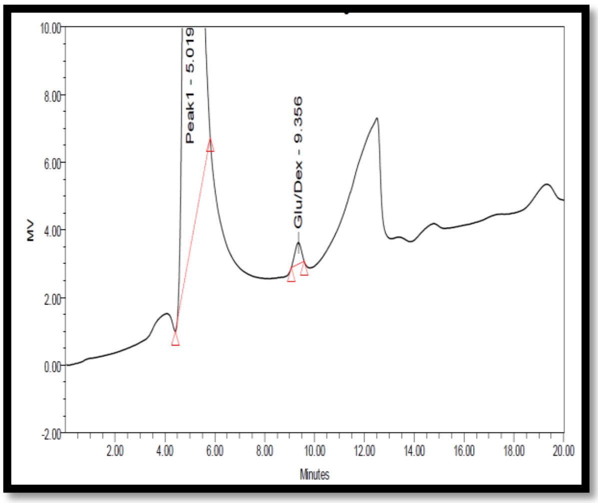

Figure 1: HPLC analysis of Sugars in 7-days control seedlings of *Brassica juncea*

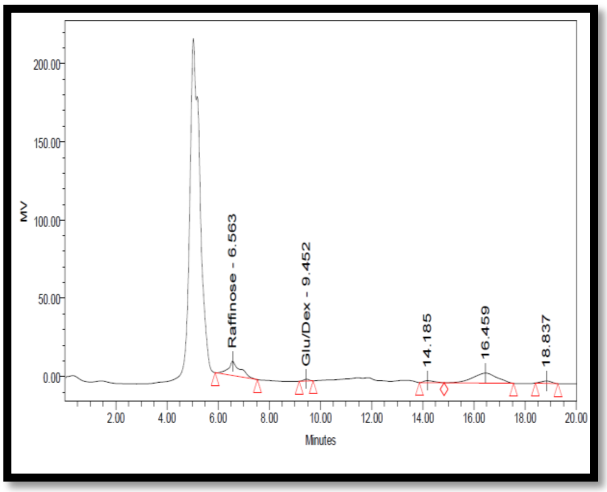

Figure 2: HPLC analysis of Sugars in 7-days old seedlings of *Brassica juncea* treated with 0.2mM Cd

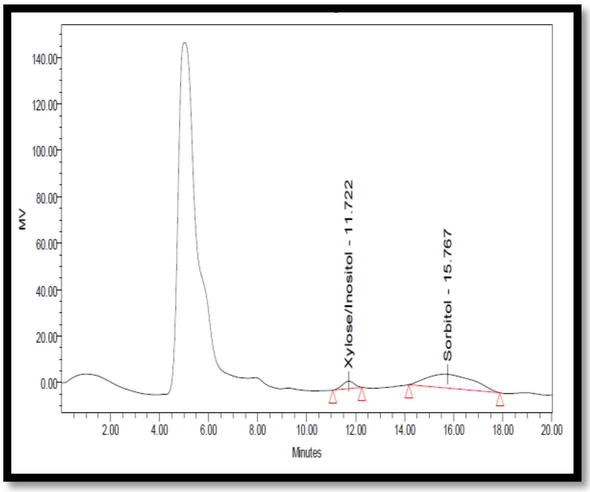

Figure 3: HPLC analysis of Sugars in 7-days old seedlings of *Brassica juncea* treated with 0.4mM Cd

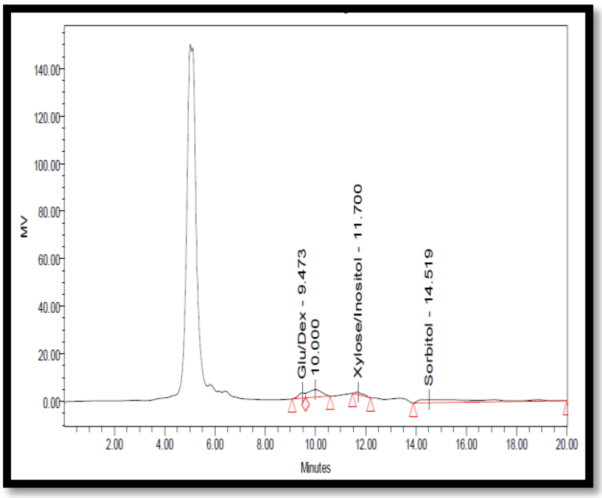

Figure 4: HPLC analysis of Sugars in 7-days old seedlings of *Brassica juncea* treated with 0.6mM Cd

Results of present work were also supported by the findings of Mohamed and Akladious[15], where antioxidants like GSH were recorded to enhance under drought conditions in *Glycine max*. In the alleviation of oxidative stress posed by ROS, improved antioxidant system acts as a key player[16].

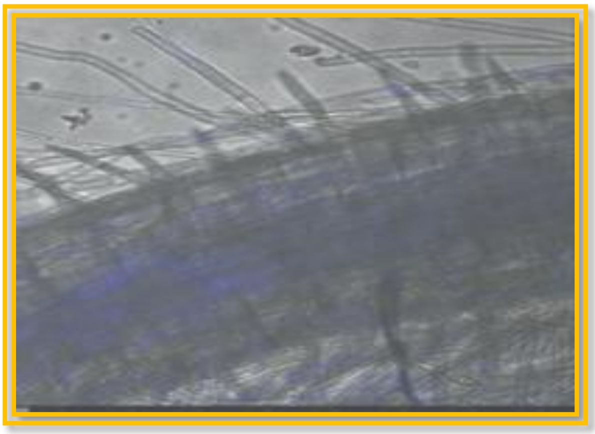

Figure 5: Localization of Glutathione in 7-days old Seedlings of *Brassica juncea*

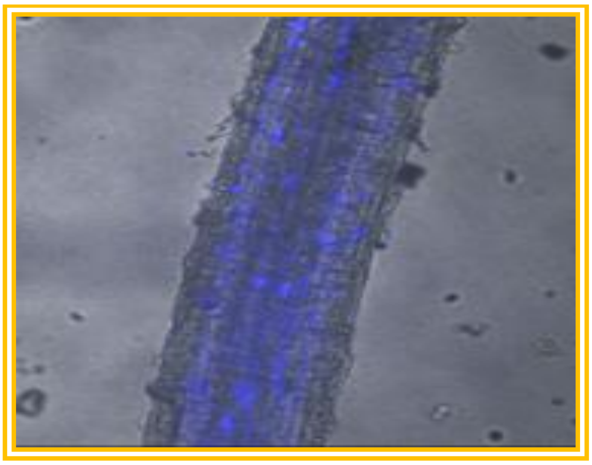

Figure 6: Localization of Glutathione in 7-days old Seedlings of *Brassica juncea* Subjected to 0.2mM Cd Stress

CONCLUSION

It is concluded from present study that with increasing concentrations of Cd, various defence strategies of *B. juncea* became activated to combat the stress like osmolytes, sugars and antioxidants. Plants subjected to Cd metal stress got potential to scavenge the free radicals.

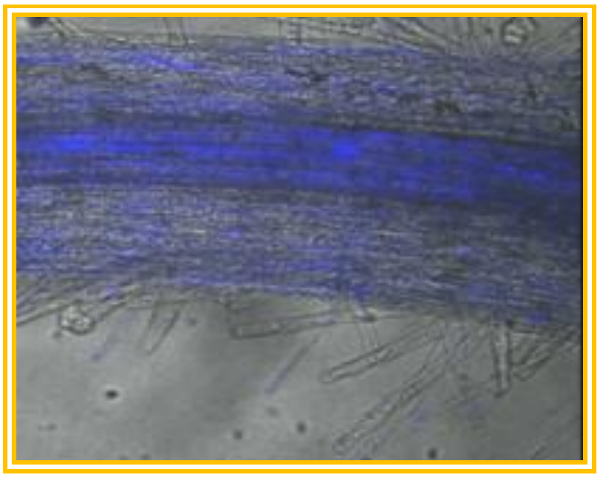

Figure 7: Localization of Glutathione in 7-days old Seedlings of *Brassica juncea* Subjected to 0.4mM Cd Stress

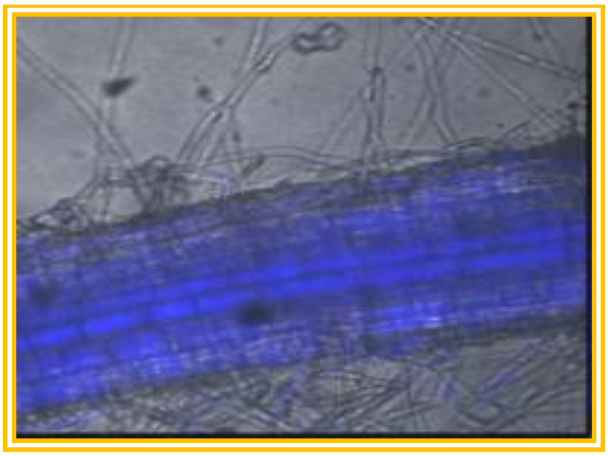

Figure 8: Localization of Glutathione in 7-days old Seedlings of *Brassica juncea* Subjected to 0.6mM Cd Stress

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