Journal of Advances in Developmental Research (IJAIDR)

E-ISSN: 0976-4844 ● Website: www.ijaidr.com ● Email: editor@ijaidr.com

Impact of Cadmium Stress on Germination, Seedling Growth, and Antioxidant Enzyme Activity in Indian Mustard

Atar Singh

NET - Chemistry
Village - Pidawali Post. Khohara Tehsil – Bayana, Bharatpur, Rajasthan.

Abstract:
Plants that accumulate the heavy metals are the best subjects to study the mechanisms of deleterious effects of toxic metals in the plant kingdom. Indian mustard (Brassica junicea) is found to accumulate moderate level of heavy metals like Cadmium, Lead, Nickel, Chromium etc. In this research article, 6 different concentration of cadmium (0µM, 200µM, 400µM, 600µM, 800µM, 1000µM) treatment were imposed in cv. Pusa Vijay (NPJ-93) as cadmium nitrate [Cd $(NO_3)_2$] in plant tissue culture medium. The negative effect of different cadmium nitrate concentration on seed germination, seedling growth parameters and antioxidant enzymes activity of Indian mustard cv. Pusa Vijay (NPJ-93) after 15 days of incubation was observed. Results showed that cadmium adversely affected the seed germination and change in antioxidant enzyme activities. The effective concentration of cadmium caused 6.0% inhibition of seed germination. The root and shoot growth of mustard seedlings were also negatively affected with increasing concentrations of cadmium nitrate. Cadmium treated plants showed increased activities of antioxidant enzymes like APX, CAT, SOD and GR.

Keywords: Brassica junicea, heavy metal, cadmium, ascorbate peroxidase, catalase, superoxide dismutase, glutathione reductase.

INTRODUCTION

Heavy metals reside in the atmosphere as a natural component or as a result of human activities like burning of fossil fuel, automobiles etc. Soil, water and air contained different levels and types of heavy metals which causes pollution after crossing certain threshold level. Heavy metals are frequently released into the atmosphere by environmental factors like weathering of rocks, volcanoes, industrials effluents, mining, combustion of fossil fuels etc. (De Abreu *et al*. 1998) [6]. At present time, agricultural soil contamination by heavy metals is also observed due to industrialization. Therefore, heavy metal contamination is found to be a risk factor for primary as well as secondary consumers like humans (Zeller and Feller, 1999) [33]. Cadmium (Cd), Lead (Pb), Zinc (Zn) and copper (Cu) are among the most harmful heavy metals found in agricultural soil (Förstner *et al*. 1995) [13]. These metals can easily be absorbed by plant roots and prove to be toxic for plants itself affecting their growth and development by inhibiting their biochemical processes like enzyme activity, photosynthetic activity, protein penetration etc. (Arun *et al*. 2005) [1].

In the recent year, cadmium contamination gained a reasonable attention to environmentalist because of its discharge, mobility and the small concentration necessary to manifest its effect on human health (Jackson & Alloway 1990). Mostly cadmium is accumulated in the cultivated soil which is near to urban and industrial area because of automobiles and industrial waste product (Di Toppi and Gabrielli, 1999) [7]. In spite of taking regulatory measures in many countries to check on Cd input in agricultural soil, it continues to be one of the most serious global environmental hazards in the developing countries (Yang *et al*., 2000) [31].

In plants, cadmium toxicity results in reduce biomass because of inhibition of chlorophyll synthesis and reduced photosynthesis (Padmaja *et al.*, 1990) [27]. High level of cadmium input in plants may cause decrease uptake of nutrient elements, induction of oxidative stress like alteration in antioxidative enzyme activities (Sandalio *et al.*, 2001). Minute concentration of Cd exhibit toxic effects on those plants that do not accumulate this heavy metal. Therefore, it is difficult to study the mechanism of Cd translocation in plants. So mustard as being the hyperaccumulator of cadmium is found to be the best choice to study the metal and mineral nutrient uptake as well as to study phytoremediation.

Cadmium is considered as the essential micronutrient for plants as a co-factor (Eskew *et al.*, 1983), however beyond threshold concentration this metal become toxic for plants. The most common symptoms of cadmium toxicity in plants include generation of reactive oxygen species, necrosis, chlorosis, wilting, abnormal photosynthetic machinery etc (Madhava Rao *et al.*, 2000) [19]. Production of reactive oxygen species (ROS) is very common response of any plants to heavy metal toxicity. In response to reactive oxygen species plants start synthesizing some antioxidative enzymes and non-enzymatic low molecular weight antioxidants. Ascorbate peroxidase (APX, EC 1.11.1.11) plays an important role in decomposition of H_2O_2 that is generated within chloroplast as well in cytoplasm. This enzyme is found in stroma, microbodies, cytosol and mitochondria and acted as an electron donor for H_2O_2 decomposition (Elavarthi *et al.*, 2010; Yoshimura *et al.*, 2000) [10]. Superoxide dismutase (SOD) is a very important anti-oxidative enzyme that catalyzes conversion of superoxide anion (O^{2-}) to H_2O_2 and O_2. Catalase (CAT) scavenges H_2O_2 by converting it into O_2 and H_2O. Glutathione transferase (GR) catalyzes the conjugation of endogenous/exogenous electrophilic substrates to reduced glutathione (GSH). The resulting compounds are transported to vacuoles for further processing or degradation (Marrs *et al.*, 1997) [20]. Therefore, GST is found to be play an important role in detoxification of harmful byproducts of lipid and protein peroxidation. It is also been reported that GST may be involved in plant resistivity against diverse environmental stresses (Davis and Swanson 2001, Sudhakar *et al*. 2001) [30, 5]. Proline is the most widespread metabolic product of plant tissues under biotic and abiotic stress conditions (Hartzendorf and Rolletschek 2001; Matysik *et al*. 2002) [21]. The aim of this present study is to find out the effect of cadmium toxicity on seed germination plant growth parameters and antioxidant enzyme activities in Indian mustard cv. Pusa Vijay (NPJ-93) which is one of the high yielding Indian variety.

MATERIALS AND METHODS

Seeds of high yielding Indian mustard cv. Pusa Vijay (NPJ-93) were collected from Indian Agricultural Research Institute, Pusa, India (IARI) and were used as experimental material. Cadmium as Cadmium nitrate [Cd $(NO_3)_2$] was used as heavy metal source. Mustard seeds were surface sterilized with 70% alcohol for 45 sec followed by 0.1% aqueous solution of $HgCl_2$ for 5 min. The seeds were then thoroughly washed three times with autoclaved distilled water. 30-40 seeds were placed in glass culture dishes containing 20ml of MS basal medium. Seeds were arranged in such a way that each seedling did not touch each other after germination.

Mustard seeds were subjected with 6 different concentration of cadmium nitrate [Cd $(NO_3)_2$] that is 0µM, 200µM, 400µM, 600µM, 800µM, 1000µM. All the 6 different concentrations of cadmium were given in replicates of three. Seedlings were sub-cultured every week on fresh medium for the proper supply of nutrient medium. Mustard seeds were set under a photoperiod of 12 hr, and 22/18 °C day/night temperature. The mustard seedlings were harvested after 15 days and the percentage germination, root and shoot length, root and shoot dry and wet weight and activities of antioxidant enzymes were recorded.

ENZYME ASSAYS

Leaf samples from both control and treated seedlings were homogenized with 50mM phosphate buffer (pH 7) containing 100mM KCl, 1mM Ascorbic acid (AsA), 5mM ß-mercaptoethanol and 10% (w/v) glycerol in pre-chilled pestle and mortar. The final extract was then centrifuged in a fixed angle rotor at 20,000 g for 20 minutes in fixed angle rotor and supernatant was used for enzyme assay.

ASCORBATE PEROXIDASE (APX)

Ascorbate peroxidases found to be play an important role in decomposition of H_2O_2, generated both in the chloroplasts as well as in cytosol. Enzyme ascorbate peroxidase was extracted in 50mM phosphate buffer (pH 7). Enzyme activity was measured by using the methods describe by Nakno and Asada (1981). 200mg dry leaves were homogenized in a mortar and pestle with 4ml of ice-chilled extraction buffer (100mM potassium phosphate buffer, pH 7 and 0.1 mM EDTA). The homogenate was then filtered through muslin cloth and centrifuged in fixed angle rotor at 14000 rpm for 15 minutes at 4°C. The supernatant obtained was used as crude extract to determine enzyme activity.

The reaction mixture consisted of crude enzyme extract and 50mM sodium phosphate buffer, pH 7 (0.2mM EDTA, 0.5 mM ascorbic acid, 50 mg BSA). APX enzyme activity was started immediately after addition of H_2O_2 at final concentration of 0.1 mM. Oxidation of ascorbic acid started after 2 min of reaction initiation and was detected by absorbance at 290 nm. The difference in absorbance was divided by the ascorbate molar extinction coefficient (2.8 $mM^{-1}.cm^{-1}$) and the enzyme activity expressed as µmol of H_2O_2 min^{-1} mg^{-1} protein, taking into consideration that 1.0 mol of ascorbate is required for the reduction of 1.0 mol of H_2O_2 (McKersie *et al.*, 1994). Activity was calculated from the change in absorbance at 265 nm for 1 min using an extinction coefficient of 14 mM^{-1} cm^{-1}.

CATALASE (CAT)

Catalase is a heme protein that catalyzes the decomposition of H_2O_2 into H_2O and O_2. Activity of enzyme catalase was analyzed spectrophotometrically by monitoring the decrease in absorbance of H_2O_2 at 240 nm as described by Noctor and Foyer (1998). The enzyme assay solution contained 50mM phosphate buffer saline and 10mM H_2O_2. The reaction was started immediately after addition of enzyme aliquot to the reaction mixture. The change in absorbance was followed 2 min after starting the reaction. The decrease in absorbance (by decomposition of H_2O_2) was read at 240 nm for 1 min.

SUPEROXIDE DISMUTASE (SOD)

SODs are copper/zinc containing metalloproteins that catalyze the dismutation reaction of two superoxide free radicals into O_2 and H_2O_2. This enzyme is considered as the first line of defense against damage caused by the ROS. Activity of this enzyme was determined by using the protocol described by El-Shabrawi *et al.* (2010) [11]. Reaction mixture contained enzyme extract, xanthine oxidase (0.1 units), K-P buffer (50mM), NBT (2.24mM), CAT (0.1 units) and xanthine (2.36 mM). Catalase was also added to avoid the H_2O_2 mediated inactivation of CuZn-SOD during the reaction. The change in absorbance was observed at 560nm.

GLUTATHIONE REDUCTASE (GR)

Glutathione reductase is a NADPH-dependent enzyme that catalyzes the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH). This enzyme helps in maintaining the ratio of GSH/GSSG as part of the ascorbate-glutathione cycle, thus playing an important role in cell metabolism. GR enzyme activity was calculated by using the protocol described by Hasanuzzamen *et al.* (2011). The reaction mixture contained enzyme solution (final volume upto 1ml), 0.1 M K-P buffer (pH 7), 1mM GSSG, 1 mM EDTA and 0.2 mM NADPH. The reaction was initiated with oxidized glutathione and the decrease in absorbance was observed at 340 nm for 1 min.

STATISTICAL ANALYSIS

The experiments were conducted using three replicate i.e. all the assays were repeated three times under same conditions. Analysis of quantitative variables was done by one-way ANOVA test. Newman-Keuls correlation was used to find out the interaction between two variables. p value <0.05 were considered as statistically significant. Variance was used to determine the significance of regression models.

RESULTS EFFECT OF CD ON SEED GERMINATION AND SEEDLINGS GROWTH

The percentage of seed germination reflects the effect of living environment on plant seeds growth. Table 1 represents the effect of different concentration of Cd on seed germination, shoot length, root length, relative water content, fresh and dry weight of seedlings. Results obtained from present study confirmed that as we increased the concentration of Cd in tissue culture media, percentage of seed germination decreases significantly. 1000µM Cd reduces the seed germination by 10%. No reduction of seed germination was observed at concentration up to 200 µM. Chugh and Sawhney (1996) [4] showed that administration of heavy metal affect seed germination in *Pisum sativum* by affecting water absorption, transport and lowering water stress tolerance. Concentration above 200 µM of Cd affects the shoot and root growth as well but by a different extent. Negative effect of higher concentration of Cd is more on root growth as compare to shoot growth. High cadmium concentration resulted in the browning of the shoots that might be because of the deposition of suberin (Punz and Sieghardt, 1993) [28]. Reduction in relative water content was found to be 8.9% at concentration 1000 µM of Cd when compare with control seedlings.

Table 1

Cd concentration	% Seed germination	Root length (cm)	Shoot length (cm)	Fresh weight (mg)	Dry weight (mg)	Antioxidant enzyme activity			
						APX	CAT	SOD	GR
						µmol/m/mg FW			
0 µM	100	5.67	7.88	354mg	35.10mg	0.0097	0.0025	0.035	0.0078
200 µM	100	4.04	5.91	325mg	24.33mg	0.0106	0.0027	0.038	0.0085
400 µM	97.8	2.05	4.41	285mg	18.52mg	0.0116	0.0031	0.041	0.0093
600 µM	95.7	0.98	3.30	244mg	13.41mg	0.0256	0.0040	0.053	0.0120
800 µM	93.5	0.42	2.14	215mg	8.73mg	0.0358	0.0052	0.068	0.0156
1000 µM	89.6	0.10	1.32	188mg	4.21mg	0.0590	0.0067	0.095	0.0218

Different letters represent significant differences between means at $P<0.05$

ACTIVITY OF ANTIOXIDANT ENZYMES

The antioxidative enzyme's activity like Ascorbate peroxidase, Catalase, Superoxide dismutase and Glutathione reductase in leaves of mustard seedlings after heavy metal treatment increased significantly in a concentration dependent manner. The highest cadmium level (1000 µM) caused maximum increase in enzyme activity i.e 35% in CAT, 83% in SOD, 65% in APX and 74% in GR as compare to control treatment.

DISCUSSION

Plant growth parameters like root and shoot growth, dry and wet weight, percentage seed germination and chlorophyll content shown to be very sensitive to heavy metals in some plants (Arun *et al.*, 2005) [1]. Inhibitory effect of cadmium on mustard has been studied by many authors (Heidari *et al*., 2011; Jiang *et al*., 2004; Chen *et al*., 2014). In the present study, it is clearly illustrated that plant growth and development was affected negatively by Cd toxicity and root and shoot biomass decreases significantly at 400 µM of Cd in plant tissue culture media.

Cadmium accumulation in both shoot and root increases with increasing cadmium concentration. Cadmium affects root growth more than shoot growth because of the fact that roots are the first plant part to receive cadmium ion from tissue culture media/soil via apoplastic transport resulting in higher cadmium accumulation there (Drążkiewicz *et al.*, 2003). Exposure of mustard plants to high cadmium toxicity caused many-fold raise in SOD and other enzymes activity in root and shoots. Activity of antioxidative enzymes increases under heavy metal stress as antioxidative enzymes plays an important role of physical barrier and avoids entry of toxic metals inside cell (Díaz *et al*. 2001). Mustard seedlings exposed to high Cd concentration results in a significant increase in CAT activity. Since heavy metals, including Cd, found to be induce peroxidation of membrane lipids it is found that, plants subjected to heavy metal stress, catalase enzyme involved in the

removal of toxic products of lipid peroxidation (Baccouch *et al*. 1998) [2]. Marrs and Walbot (1997) [20] described that catalase may participate in the transport of phytochelatin-metal complexes to vacuole. Higher antioxidative enzyme activities in mustard plant after cadmium toxicity indicating that the increased activity might reflect a damage response to stress factors, which was also observed in the report of Mittal and Dubey (1991) [23], who observed that high antioxidative ability were part of a damage response to salinity in rice.

In conclusion, present research work showed that Cd at a concentration of 200 µM did not affect the studied parameters of mustard seedlings. Thus below a particular level, this metal seem to be non-toxic to mustard cv. Pusa Vijay (NPJ-93). Above a threshold level, cadmium cause the inhibition of antioxidative enzyme activities. Our study suggest that induction of APX, CAT, SOD and GR activities plays an important role in the response of Mustard seedlings to cadmium toxicity.

REFERENCES:

1. Arun KS, Cervantes C, Loza-Tavera H, Avudainayagam S. Chromium toxicity in plants. Environ. Int,2005:31:739-753.
2. Baccouch S, Chaoui A, El Ferjani E. Nickel-induced oxidative damage and antioxidant responses in Zea mays shoots. *Plant Physiology and Biochemistry*,1998:*36*(9):689-694.
3. Chen X, Wang J, Shi Y, Zhao MQ, Chi GY. Effects of cadmium on growth and photosynthetic activities in pakchoi and mustard. *Botanical studies*, 2011, *52*(1).
4. Chugh LX, Sawhney SK. Effect of Cd on seed germination. Amylase and rate of respiration of germinating pea seeds. Environ. Poll.,1996:92:1-5.
5. Davis DG, Swanson HR. Activity of stress-related enzymes in the perennial weed leafy spurge (*Euphorbia esula* L.). - Environ. exp. Bot.,2001:46:95-108.
6. De Abreu CA, De Abreu MF, De Andrade JC. Distribution of lead in the soil profile evaluated by DTPA and Mehlich-3 solutions. Bragantia.,1998:57:185-192.
7. Di Toppi SL, Gabrielli R. Response to cadmium in higher plants. Environ. Exp. Bot.,1999:41:105-130.
8. Dıaz J, Bernaĺ A, Pomar F, Merino F. Induction of shikimate dehydrogenase and peroxidase in pepper (Capsicum annuum L.) seedlings in response to copper stress and its relation to lignification. *Plant Science*,2001:*161*(1):179-188.
9. Drążkiewicz M, Baszyński T. Growth parameters and photosynthetic pigments in leaf segments of Zea mays exposed to cadmium, as related to protection mechanisms. *Journal of Plant Physiology*, 2005:*162*(9):10131021.
10. Elavarthi S, Martin B. Spectrophotometric assays for antioxidant enzymes in plants. In *Plant Stress Tolerance*. Humana Press., 2010, 273-280.
11. El-Shabrawi H, Kumar B, Kaul T, Reddy MK, Singla-Pareek SL, Sopory SK. Redox homeostasis, antioxidant defense, and methylglyoxal detoxification as markers for salt tolerance in Pokkali rice. Protoplasma,2010:245:85-96.
12. Eskew DL, Welch RM, Cary EE. Nickel: an essential micronutrient for legumes and possibly all higher plants. – Science,1983:222:621-623.
13. Förstner U. In: Metal speciation and contamination of soil. (AE Herbert, CP Huang, GW Bailey, AR Bowers). London Lewis., 1995, 1-33.
14. Hartzendorf T, Rolletschek H. Effect of NaCl-salinity on amino acid and carbohydrate contents of *Phragmites australis*. - Aquat. Bot.,2001:69:195-208.
15. Hasanuzzaman M, Hossain MA, Fujita M. Nitric oxide modulates antioxidant defense and the methylglyoxal detoxification system and reduces salinity-induced damage of wheat seedlings. Plant Biotechnology Reports,2011:5:353-365.
16. Heidari M, Sarani S. Effects of lead and cadmium on seed germination, seedling growth and antioxidant enzymes activities of mustard (Sinapis arvensis L.). *ARPN Journal of Agricultural and Biological Science*,2011:*6*(1):44-47.

17. Jackson AP, Alloway BJ. The bioavailability of Cd to lettuces and cabbages from soil previously amended with sewage sludge. Environ Lett,1990:37:238–254.
18. Jiang XJ, Luo YM, Liu Q, Liu SL, Zhao QG. Effects of cadmium on nutrient uptake and translocation by Indian Mustard. *Environmental Geochemistry and Health*,2004:*26*(2):319-324.
19. Madhava Rao KV, Sresty TVS. Antioxidative parameters in the seedlings of pigeonpea (*Cajanus cajan* (L.) Millspaugh) in response to Zn and Ni stresses. - Plant Sci.,2000:157:113-128.
20. Marrs KA, Walbot V. Expression and RNA splicing of the maize glutathione S-transferase Bronze2 gene is regulated by cadmium and other stresses. *Plant physiology*,1997:*113*(1):93-102.
21. Matysik J, Alia Bhalu B, Mohanty P. Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. - Curr. Sci.,2002:82:525-532.
22. McKersie BD, Leshem YY. Stress cropping in cultivated plant. Kluwer Academic Pulishes, Dordrecht., 1994.
23. Mittal R, Dubey RS. Influence of salinity on ribonuclease activity and status of nucleic acids in rice seedlings differing in salt tolerance. *Plant Physiology and Biochemistry-New Delhi*,1991:*18*:57-57.
24. Nakano Y, Asada K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidses in spinach chloroplasts. Plant Cell Physiol., 1981:22(5):867-880.
25. Noctor G, Foyer CH. Ascorbate and glutathione: keeping active oxygen under control. Annu. Rev. Plant Physiol. Plant Mol. Biol., 1998:49:249-279.
26. Noctor G, Foyer CH. Ascorbate and glutathione: keeping active oxygen under control. Annu. Rev. Plant Physiol. Plant Mol. Biol.,1998:49:249-279.
27. Padmaja K, Prasad DDK, Prasad ARK. Inhibition of chlorophyll synthesis in *Phaseolus vulgaris* Seedlings by cadmium acetate. Photosynthetica.,1990:24:399-405.
28. Punz WF, Sieghardt H. The response of roots of herbaceous plant species to heavy metals. Environ. Exp. Bot.,1993:33:85-98.
29. Sandalio LM, Dalurzo HC, Gomez M, Romero-Puertas MC, Del Rio LA. Cadmium-induced changes in the growth and oxidative metabolism of pea plants. J Exp Bot.,2001:52:2115-2126.
30. Sudhakar C, Lakshmi A, Giridarakumar S. Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. – Plant Sci.,2001:161:613-619.
31. Yang YY, Jung JY, Song WY, Suh HS, Lee Y. Identification of rice varieties with high tolerance or sensitivity to lead and characterization of the mechanism of tolerance. Plant Physiol.,2000:124:1019-1026.
32. Yoshimura K, Yabuta Y, Ishikawa T, Shigeoka S. Expression of spinach ascorbate peroxidase isoenzymes in response to oxidative stresses. Plant Physiol,2000:123:223–234.
33. Zeller S, Feller U. Long-distance transport of cobalt and nickel in maturing wheat. European J. Agron.,1999:10:91-98.