Morphological and Biochemical Response of *Cicer arietinum* L. var. pusa-256 towards an Excess of Zinc Concentration

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Abstract:

The toxic effect of zinc (Zn) at increasing concentration was studied with special attention being given to the morphogenic and biochemical response of *Pisum sativum* L. *Cicer arietinum* plants were grown in different concentration of $ZnSO_4$ (0, 10, 25, 50, 75 and 100 µM) for 15 days. In respective to their controls, low concentration (10 and 25 µM) of Zn greatly stimulated the seed germination, while it was inhibited by highest concentration of Zn (100µM). Radical, hypocotyls length and root length (TI) and plant height (TI) were also increased up to 25 µM of Zn addition and after that a significant reduction were noticed at 75 and 100µM. The effects of toxicity of Zn on chlorophyll content and antioxidant enzymes activity include CAT, APX and GPX were investigated. The data showed that the low concentration of Zn (25µM) addition induced in chlorophyll content and high levels of Zn reduced the chlorophyll synthesis in the leaves of this plant. Maximum and minimum chlorophyll content were observed at 25 and 100 µM of Zn addition respectively. Activities of antioxidant enzymes were indicated close relationship with increase in Zn concentration and shoots showed higher activity of antioxidant enzymes than roots. The activity of APX in shoot and root were higher than CAT and GPX. [Life Science Journal. 2010;7(1): 95 – 98] (ISSN: 1097 – 8135).

Key words: Seed Germination; Hypocotyls Length; *Cicer arietinum* Plant; Antioxidant enzymes.

1. INTRODUCTION

With the development of industries, mining activities, application of waste water and sewage sludge on land, phytotoxicity of the heavy metals pollution has serious implications in soil degradation and it may reduce both the quality and efficiency of plants (Ali et al., 1999). Although certain metals like Cu, Mn, Fe and Zn are crucial for plants and are used as micronutrients, however, at higher concentrations they may reveal strong toxicity. They obstruct plant growth as do the other heavy metals like Cd, Hg, or Pb, which have no function in plant metabolism (Ali et al., 2000).

Zn is a microelement with important physiological functions in plants, however, at higher concentrations it can become toxic, thus leading to physiological and morphological disturbances and, eventually to decreased yield. It triggers enzymes by incorporating themselves into metalloenzymes of the electron transport system. Zn plays a vital role in the cell division, cell expansion, proteins synthesis, and also in carbohydrate, nucleic acid and lipid metabolism (Collins, 1981). As Zn forms stable complexes with DNA and RNA it might also influence DNA and RNA stability. But on the other hand a higher concentration of Zn in the plant tissue seriously affects activity of several enzymes and other fundamental metabolic processes. An excess of Zn also reduced photosynthetic rate as a part of enzymes concerned in the photosynthesis. A toxic concentration of Zn in the plant tissue seriously affects activity of several enzymes and other fundamental metabolic processes. Ali et al., (2000) carried out a brought study and affirmed that an excess of Zn also reduced photosynthetic rate as a part of enzymes concerned in the photosynthesis. Nitrogen metabolism is also affected by diverse ways by an excess of Zn. The protein content is found to be reduced; nitrogen-fixation and nitrate reductase activity was also concealed by Zn toxicity (Phalsson, 1989).

The protein content was found to be reduced; nitrogen-fixation and nitrate reductase activities were also concealed by Zn toxicity. An overindulgence of both essential and toxic heavy metals has been found to be allied with generation of free radicals. Free radicals or ROS are toxic by-products, generated at low levels in non-stressed plant cells in chloroplasts and mitochondria, and also by cytoplasmic, membrane-bound or exocellular enzymes concerned in redox reactions (especially photosynthetic electron transport processes and respiration). Extra amounts of ROS occur under stressful conditions and over production of these ROS such as superoxide, H_2O_2 and OH^* results in the plants exposed to stress conditions including metal stress (Galligo et al., 1999). ROSs are known to spoil cellular membranes by inducing lipid peroxidation or interruption of electron transport chain. The activation of lipoxygenase, an enzyme that arouse lipid peroxidation, has been reported after cadmium revelation (Smeets et al., 2005). As a consequence, tissues snubbed by oxidative stress generally contain elevated concentration of APX, GPX and CAT and demonstrate an increased assembly of ethylene (Schutzendubel et al., 2001).

Hence, the objective of this study was to evaluate the effect of additional supply of Zn in the form of

$ZnSO_4.2H_2O$ on the morphological and biochemical response of *Cicer arietinum* L. Disparity in some stress related parameters such as root, shoot length, plant height, photosynthetic pigments and antioxidant enzymes was also examined in relation to Zn concentrations.

2. MATERIAL AND METHODS:

2.1 Seeds Surface Sterilization and Treatment Process

Seeds of *Cicer arietinum* (Var.-Pusa-256) L. were collected from Seed Cooperative Committee, Jhansi, India and surface sterilized with 1% $HgCl_2$ for 30 min. They were rinsed with tap water followed by double distilled water and allowed to soak in de-ionized water and different concentrations of $ZnSO_4.7H_2O$ solutions for four hours (0, 10, 25, 50, 75 and 100mM solution). For morphological and biochemical studies 25 properly soaked seeds were transferred to plastic boxes, layered with sterilized germinating paper, and kept in incubator at 22 ± 2^0C in three replicates. Paper of boxes was already soaked with different $ZnSO_4.7H_2O$ solutions. Seedlings were harvested after 15 days of treatment, roots and shoots were separated and lengths were measured.

2.2 Seed Germination and Measurement of Hypocotyl and Radicle Length

For germination test ten properly soaked seeds were placed in petriplates lined with germinating paper in three replicates and germination test was performed after 72 hours in a separate set of experiments. A 2mm radicle emergence from seed was considered as germinated seed. Measurements of hypocotyl and radicle length were done with five seedlings from each treatment after 15 days.

2.3 Plant growth parameters and tolerance index

A number of plant growth parameters, viz. root-shoot lengths, root fresh and dry weights, plant height and chlorophyll content in leaves were determined. Chlorophyll a and b in leaves were measured by Machlachlan and Zalik, (1963).

Tolerance indices (TI) of root length and plant height against each concentration were calculated following Baker et al., 1994.

TI (%) = Mean length metal solution/ mean length for control solution X100

2.4 Enzyme assay

Different enzymes were assayed in crude extract of root and shoot. For preparation of crude extract, 1.0 gram of plant material was crushed in chilled mortar and pestle with 5 ml of 50 mM phosphate buffer (pH-7.5). Homogenate were centrifuges for 10 min at 10,000 rpm at 4^0C and supernatant were directly used for assay of CAT, APX, and GPX by Chance and Maehly (1959), Asada (2001) and Chang and Kao (1998) respectively.

2.5 Statistical analysis

All the results were expressed as mean value ±SD for three replications. For each replication we have taken plant material by weight from different boxes. For statistical analyses all the data were subjected to one way ANOVA test using GPIS software (1.13) (Graphpad, California, USA).

3. RESULTS:

3.1 Germination Assay and Morphological Analysis

Seeds were initially exposed to various concentrations of $ZnSO_4.2H_2O$ in order to review the adverse effects of Zn on seed germination and radicle emergence in *Cicer arietinum* L. seeds. Means of seed germination percentage after 72 hours are shown in table-1. Results indicated that seed germination rate had an upward trend up to 25mM Zn concentration, but at 50-100mM inhibitory effects were demonstrated compared to their relevant controls. It showed that low Zn concentrations (10 and 25mM) had significantly stimulatory effect ($p<0.05$) whereas, the higher concentrations (75-100mM) become noxious and significantly suppressed the germination ($p<0.001$). The mean radicle and hypocotyl lengths of *Cicer arietinum* seedlings at 15 days were also augmented significantly ($p<0.01$) up to 25mM Zn addition and were the lowest at 100mM ($p<0.001$) Zn (Table 1).

The consequence of different Zn concentrations on root fresh and dry weight, tolerance index and plant height index were shown in table 1. It was observed that Zn concentration up to 50mM showed a positive response to biomass production ($p<0.001$). As Zn concentration increased (75 and 100mM) biomass was significantly reduced compared to the control ($p<0.001$). Root length (TI) significantly increased ($p<0.001$) up to 50mM and then a significantly declined ($p<0.001$) compared to their respective control. An analogous pattern was observed for plant height (TI).

3.2 Chlorophyll

The effects of Zn addition on photosynthetic pigments (Chlorophyll a, b) are shown in Fig 1A. *Cicer arietinum* plants grown in 25mM of Zn supply, contained maximum chlorophyll content and a statistically significant ($p<0.001$) reduction was observed at 75-100mM of Zn supply

3.3 Antioxidant Enzymes

The effect of Zn addition in various concentrations on antioxidant enzymes in *Cicer arietinum* shoots and roots are shown in Fig. 1A&B. All antioxidant enzymes were amplified linearly with Zn addition and found utmost at 100mM Zn concentration. Rate of APX was highest among three antioxidant enzymes, followed by CAT and GPX at all treatments. Shoots of *Cicer arietinum* plants contained more antioxidants enzymes activity than roots.

Table 1: The effect of Zn addition on seed germination (72 h) and morphology of *Cicer arietinum* seedlings (15 d)

Notation: [a] $p<0.001$; [b] $p<0.01$; [c] $p<0.05$ compared to control within a column. All the data are mean of three values ±SD.

Zn treatment (mM)	Germination (%)	Radical and Hypocotyls length (cm/plant)		Root biomass (g/plant)		Tolerance Index (%)	
		Radical length	Hypocotyls length	Fresh weight	Dry weight	Root length	Plant height
0	88.9±0.78	7.4±0.90	9.3±.82	0.1±0.04	0.05±0.03	100±0.00	100±0.00
10	93.7±2.70	9.9±0.84[c]	11.6±0.79	0.15±0.01	0.07±0.01	134.8±2.72[a]	124.5±4.01[a]
25	96.5±2.69	10.5±0.94[b]	12.5±0.69[b]	0.2±0.02[b]	0.09±0.02	143.8±3.96[a]	134.3±5.08[a]
50	85.4±2.04[cb]	7.6±0.64[cb]	10.8±0.88[ab]	0.24±0.02[ab]	0.1±0.03	103.8±3.07[a]	115.8±4.58[a]
75	76.5±2.39[bac]	6.3±0.61[a]	8.5±1.10[ac]	0.22±0.02[ac]	0.08±0.04	86.1±4.01[ba]	90.8±2.31[a]
100	57.8±4.65[a]	4.6±0.28[bab]	5.6±0.39[c]	0.16±0.02[c]	0.06±0.03	63.0±4.06[a]	59.8±4.78[a]

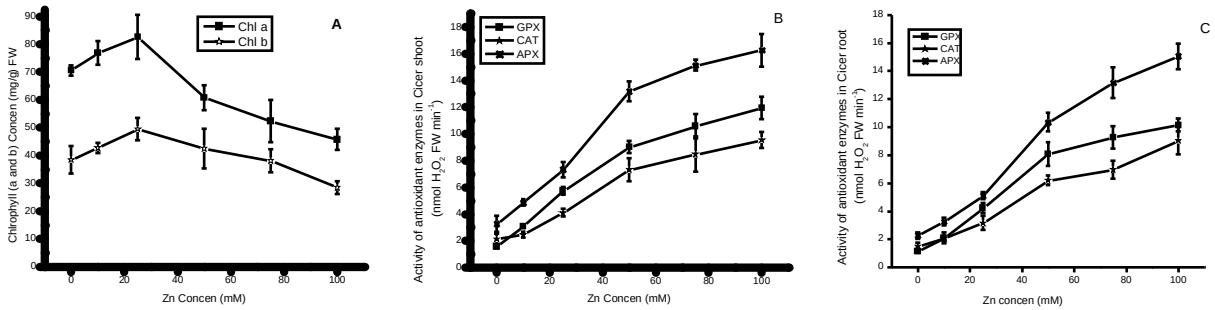

Figure 1: The effect of Zn toxicity on Chlorophyll contents (A) antioxidant enzymes of shoot (B) and root (C). All the data are mean of three values ±SD.

DISCUSSION:

Zn at lower concentrations enhanced *Cicer arietinum* seed germination. This is because Zn is a micronutrient and indispensable for plant growth (Wierzbicka and Obidzinska, 1998). But at higher quantity it abridged the germination percentage, which is consistent with other researcher's findings (Herrero et al., 2003; Ataci et al., 2005). The abridged germination of seeds under Zn stress could be a depressive effect of high concentration of metal on the activity of amylases and on succeeding transfer of sugars to the embryo axes. Zeid (2001). Thus, our results supported the conclusions of Ali et al., 2000, who pragmatic that at critical level Zn could behave as toxic metals such as Cd and Pb. Decline in growth can be due to the obstruction of heavy metals with metabolic processes allied with regular growth of plant (Wierzbicka and Obidzinska, 1998). In the best of our acquaintance it was the first study which deals the Zn toxicity on radicle and hypocotyls length. Declining pattern in growth of plants could be due to the obstruction of metabolic processes which allied with regular growth of plants

Lower Zn concentration increased the chlorophyll content while it explained a diminution at higher values. It was might be due to eagerly gathering of Zn in the leaf, that significantly affects metabolic processes in the chloroplast (Van Assche and Clijsters, 1986). Hampp et al., (1976) observed that Zn subdued photosynthetic CO_2 fixation and Hill activity of isolated spinach chloroplast.

In the present research work activity of antioxidant enzymes increased linearly with Zn supply. Excess of Zn can persuade oxidative stress in plants, which can escort formation of Reactive Oxygen Species. Antioxidant enzymes may alter the H_2O_2 to the H_2O in the plant cells and counteract the toxicity effect of H_2O_2 (Rezai and Farboodnia, 2008). Hence to shield cells against oxidative stress, antioxidant enzymes augmented proportionally, which is also consistent with our results.

APX is the most important peroxidase in H_2O_2 detoxification operating both in cytosol and chloroplasts (Mittova et al., 2000). Therefore, APX was the enzyme which illustrated maximum activity in *Cicer arietinum* shoots and roots. All the antioxidant enzymes studied in this effort explain maximum activity in shoots compared to roots. It might be due to translocation of Zn in aerial parts as a micronutrient and this augmented the concentration of antioxidant enzymes in shoots compared to roots.

This study showed that a 25mM of Zn concentration enhanced seed germination, augmented radicle and hypocotyls lengths, chlorophyll content, fresh weight, as well as tolerance indices. The activities of antioxidant enzymes were also significantly appropriate at this concentration. Below and above 25mM Zn concentration, chlorophyll contents and oxidative stress were augmented,

which led to diminution in development of *Cicer arietinum* plants. Hence we recommended that 25mM Zn may be favorable for plant growth and this concentration of Zn may be recommended for the cultivation of plants.

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