African Journal of Basic & Applied Sciences 1 (5-6): 105-109, 2009
ISSN 2079-2034
© IDOSI Publications, 2009

Morphological and Biochemical Response of *Cicer arietinum* Var.-Pusa-256 Towards an Excess Zinc Concentration

[1]Sudarshana Sharma, [2]Parmanand Sharma, [1]Shankari P. Datta and [1]Varsha Gupta

[1]Department of Biochemistry, Bundelkahnd University Jhansi, India-284128
[2]School of Environmental Sciences, Jawaharlal Nehru University, New Delhi, India-110067

Abstract: The toxic effects of zinc (Zn) at increasing concentrations were studied with special attention being given to the morphological and biochemical response of *Cicer arietinum*. Seeds were grown in different concentration of $ZnSO_4$ (0, 10, 25, 50, 75 and 100µM) for 15 days. In respective to their controls, low concentration (10 and 25µM) of Zn greatly stimulated the seed germination, while it was inhibited at maximum concentration (100µM). Radical, hypocotyls length and root length (TI) and plant height (TI) were also augmented 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 also investigated. The data showed that the low concentration of Zn (25µM) addition induced chlorophyll content and high doses of Zn reduced the chlorophyll synthesis. 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.

Key words: *Cicer arietinum* · Tolerance Indices · Antioxidant enzymes · Hypocotyls Length · Seed Germination

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 [1]. 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 [2].

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 [8]. 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 [2]. Nitrogen metabolism is also affected in diverse ways by an excess of Zn [14]. 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). An extra amount of ROS occur under stressful conditions and over production of these ROS such as superoxide, H_2O_2 and $OH^{\cdot}$ exhibited that plants exposed to stress conditions

Corresponding Author: Dr. Parmanand Sharma, Scientist B, School of Environmental Sciences, Jawaharlal Nehru University, New Delhi, India-110067

including metal stress [9]. 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 [17]. As a consequence, tissues snubbed by oxidative stress generally contain elevated concentration of APX, GPX and CAT and demonstrate an amplified assembly of ethylene [16].

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*. 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.

MATERIAL AND METHODS

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°C 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.

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.

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 as described by [12].

Tolerance indices (TI) of root length and plant height against each concentration were calculated following [5].

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

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 50mM phosphate buffer (pH-7.5). Homogenate were centrifuges for 10 min at 10,000 rpm at 4°C and supernatant were directly used for assay of CAT, APX and GPX as described by [6], [3] [7] respectively.

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).

RESULTS

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* 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

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

		Radical and Hypocotyls length (cm/plant)		Root biomass (g/plant)		Tolerance Index (%)	
Zn treatment (mM)	Germination (%)	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}

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.

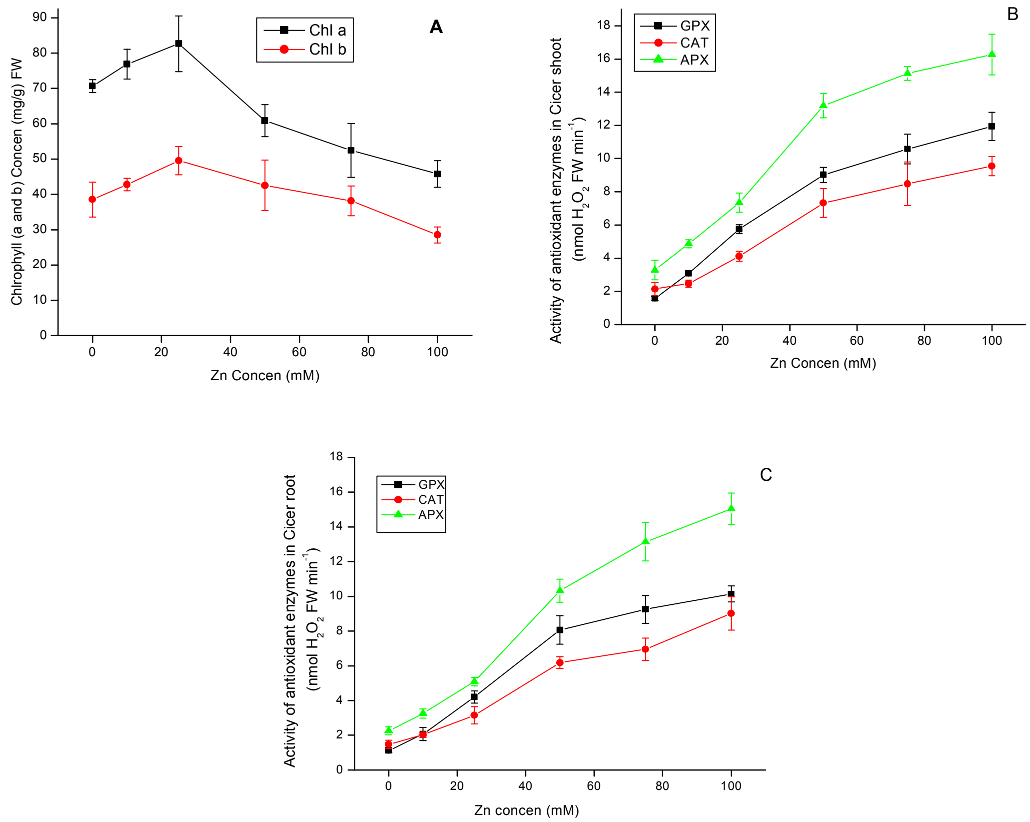

Fig. 1: The effect of Zn toxicity on Chlorophyll contents (A) antioxidant enzymes of shoot (B) and root (C). All the data are mean of three values ±SD.

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).

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 .

Antioxidant Enzymes: The effect of Zn addition in various concentrations on antioxidant enzymes in *Cicer arietinum* shoots and roots are shown in Fig. 1A and 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.

DISCUSSION

Zn at lower concentrations enhanced *Cicer arietinum* seed germination. This is because Zn is a micronutrient and indispensable for plant growth [19]. But at higher quantity it abridged the germination percentage, which is consistent with other researcher's findings [4, 11]. 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 [20]. Thus, our results supported the conclusions of earlier findings [2], which explained that at critical level Zn could behave as toxic metal like other such as Cd and Pb. 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 [19].

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 [18]. Zn subdued photosynthetic CO_2 fixation and Hill activity of isolated spinach chloroplast [10].

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 [15]. 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 [13]. 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.

CONCLUSIONS

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.

ACKNOWLEDGEMENT

The authors like to express their thanks to Vice-Chancellor and Dean Science, Bundelkhand University for giving necessary permission and facilities to carry out this research work.

REFERENCES

1. Ali, G., P.S. Srivastava and M. Iqbal, 1999. Morphogenic and biochemical response of *Bacopa monniera* cultures to Zn toxicity. Plant Sc., 143: 187-193.
2. Ali, G., P.S. Srivastava and M. Iqbal, 2000. Influence of cadmium and Zn on growth and photosynthesis of *Bacopa monniera* cultivated *in vitro*. Biol. Plant, 43: 599-601.
3. Asada, K., 2001. Ascorbate peroxidase: A hydroxide scavenging in plants. Physiol. Plant., 85: 235-241.
4. Ataci, O., G. Agar and P. Battal, 2005. Change in phytohormone contents in chickpea seeds germinating under lead or zinc stress. Biol. Plant, 49: 215-222.
5. Baker, A.J.M., R.D. Reeves and A.S.M. Hajar, 1994. Heavy metals accumulation and tolerance in British population of metallophyte *Thalapsi caerulescens* J. and C. New Phytol., 127: 61-68.
6. Chance, B. and A.C. Mahely, 1959. The assay of catalase and peroxidaseIn: Click, D. (Ed). Method of biochemical analysis, Interscience Publishers. New York, 1: 357-425.
7. Chance, C.J. and C.H. Kao, 1998. H_2O_2 metabolizing enzymes during senescence of rice leaves: Change in enzyme activities in light and darkness. Plant Growth Regulation, 25: 11-15.
8. Collins, J.C., 1981. In Lepp, N.W. (ed.), Effect of Heavy Metal Pollution on Plants, Vol. 1. Applied Science Publishers, London and New Jersey, pp: 145.
9. Galligo, S.M., M.P. Benavides and M.L. Tomoro, 1999. Effect of Cd ions on antioxidant defense system in sunflower cotyledons. Biol. Plant, 42: 49-55.
10. Hampp, R., K. Beulich and H. Ziegler, 1976. Effect of zinc and cadmium on photosynthetic CO2-fixation and Hill activity of isolated spinach chloroplasts, Z. Plant Physiol., 77: 336-344.

11. Herrero, E.M., A. Lopez-Gonzazalvez, M.A. Ruiz, J.A. Lucas-Garcia and C. Barbas, 2003. Uptake and distribution of Zn, Cd, Pb and Cu in *Brassica napus* and *Helianthus annus* grown in contaminated soils. Inter. J. Phytoremed., 5: 153-167.
12. Machlachlan, S. and S. Zalik, 1963. Plastid structure, chlorophyll concentration and free amino acid composition of a chlorophyll mutant on Barley. Can. J. Bot., 41: 1053-1062.
13. Mittova, V., M. Volokita, M. Guy and M. Tal, 2000. Activities of SOD and the ascorbate-glutathione cycle enzymes in subcellular compartments in leaves and roots of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii*. Physiol. Plant, 110: 45-51.
14. Phalsson, A.M.B., 1989. Toxicity of heavy metals (zn, cu, cd, pb) to vascular plants. *Water*, Air, Soil Pollut., 47: 287-319.
15. Rezai, K. and T. Farboodnia, 2008. Manganese toxicity effects on chlorophyll content and antioxidant enzymes in pea plant (*Pisum sativum* L. *c.v. qazvin*). Agri. J., 3: 454-458.
16. Schutzendübel, A. and A. Polle, 2002. Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. J. Exp. Bot., 53: 1351-1365.
17. Smeets, K., A. Cuyoers, A. Lambrechts, B. Semane, P. Hoet, A. Van Laere and J. Vangronsveld, 2005. Induction of oxidative stress and antioxidant mechanism in *Phaseolus vulgaris* after Cd application. Plant Physiol., 43: 437-443.
18. Van Assche, F. and H. Clijsters, 1986. Inhibition of photosynthesis in *Phaseolus vulgaris* by treatment with toxic concentration of zinc; effect on ribulose-1, 5-bisphosphate carboxylase:oxygenase, J. Plant Physiol., 125: 355-360.
19. Wierzbicka, M. and J. Obidzinska, 1998. The effect of lead imbibitions and germination in different plant species. Plant Sc., 137: 155-171.
20. Zeid, I.M., 2001. Responses of *Phaseolus vulgaris* to chromium and cobalt treatments. Biol Plant, 44: 111- 115.