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ROLE OF EXCESS ZINC ON *CICER ARIETINUM* UAR-PUSA 256

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ABSTRACT

Zinc is essential for growth and flowering of plant. The toxic effect of Zinc (Zn) at increasing concentration 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 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 at maximum concentration (100 µM). Radial, hypocotyls length and root length (Tl) and plant height (Tl) were also augmented up to 25 µM of Zn addition and after that a significant reduction were noticed at 75 and 100 µM. The effects of toxicity of Zn on chlorophylls content and antioxidant enzymes activity include CAT, APX and GPX were also investigated. The data showed that the low concentration of Zn (25 µM) 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 of Zn concentration and shoots showed higher activity of antioxidant enzymes than roots. The activity of APX of shoot and root were higher that CAT and GPX.

Key words: *Cicer arietinum*, Tolerance indices, Antioxidant enzymes, Hypocotyls length, Seed germination.

INTRODUCTION

With the development of industries activities, application of waste water and sewage sludge on land, phytoxicity 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

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higher concentrations it can become toxic, thus leading to physiological and morphological disturbances and eventually to decreased yield[3]. It triggers enzymes by incorporating themselves into metal enzymes 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[4]. 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[5]. The protein content was found to be reduced; nitrogen-fixation and nitrate reductase activities were also concluded 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 exhibited that plants exposed to stress conditions including metal stress[6]. ROSs are known to spoil cellular membranes by inducing lipid peroxidation or interruption of electron chain. The activation of lipoxygenase, an enzyme that arouse lipid peroxidation has been reported after cadmium revelation[7]. As a consequence, tissues snubbed by oxidative stress generally contain elevated concentration of APX, GPX and CAT and demonstrate an amplified assembly of ethylene[8].

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.

EXPERIMENTAL

Material and methods

Seeds surface sterilization and treatment process

Seeds of *Cicer arietinum* (Var.- Pusa-256) L. were collected from Seed Cooperative Committee, Varanasi, 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 100 µM 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 ± 2ºC 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 radical length

For germination test ten properly soaked seeds were placed in Petri plates lined with germinating paper in three replicates and germination test was performed after 72 hours in a separate set of experiments. A 2 mm radicle emergence from seed was considered as germinated seed. Measurements of hypocotyls as 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[9].

Tolerance indices (TI) of root length and plant height against each concentration were calculated by following[10] -

$$\mathrm{TI}\,(\%) = \frac{\text{Mean length for metal solution}}{\text{Mean length for control solution}} \times 100$$

Enzyme assay

Different enzymes were assayed in crude extract of root and shoot. For preparation of crude extract, 1.0 g 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 4ºC and supernatant were directly used for assay of CAT, APX and GPX as described by[11-13] 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[1,14].

RESULTS AND DISCUSSION

Germination sssay 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 radical emergence in *Cicer arietinum* seeds. Means of seed germination percentage after 72 hrs are shown in Table 1. Results indicated that seed germination rate had an upward trend up to 25 mM.

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

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 ± 0.82	0.1 ± 0.04	0.05 ± 0.03	100 ± 0.00	100 ± 0.00
10	93.7 ± 2.70	9.9 ± 0.84	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[a]	12.5 ± 0.69[b]	0.2 ± 0.02	0.09 ± 0.02	143.8 ± 3.96[a]	134.3 ± 5.08[a]
50	85.4 ± 2.04	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[a]	0.22 ± 0.02[a]	0.08 ± 0.04	86.1 ± 4.01b[a]	90.8 ± 2.31[a]
100	57.8 ± 4.65[a]	4.5 ± 0.28[bab]	5.6 ± 0.39	0.16 ± 0.02	0.06 ± 0.03	63.0 ± 4.06[a]	59.8 ± 4.78[a]

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

Zn concentration, but at 50-100 mM inhibitory effect were demonstrated compared to their relevant controls. It showed that low concentration (10-25 mM) had significant suppressed the germination ($P < 0.001$). The mean radical and hypocotyl length of *C. arietinum* seedling at 15 days were also augmented significantly ($P < 0.01$) up to 25 mM Zn addition and were the lowest at 100 mM ($P < 0.001$) Zn (Table 1).

The consequence of different Zn concentration 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 50 mM showed a positive response to biomass production ($P < 0.001$). As Zn concentration increased biomass was significantly reduced compared to the control ($P < 0.001$). Root length (Tl) significantly increased ($P < 0.001$) compared to the respective control.

Chlorophyll

The effects of Zn addition on photosynthetic pigment (Chlorophyll a, b) on *Cicer arietinum* plants which gron in 25 mM contained maximum chlorophyll whereas significantly ($P < 0.001$) reduction was observed at 75-100 mM of Zn supply.

Antioxidant enzymes

All antioxidant enzymes were amplified linearly with Zn addition and found upmost at 100 mM 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 antioxidant enzymes activity that roots.

Zn at lower concentration enhanced *Cicer arietinum* seed germination. This is because Zn is a micronutrient and indispensable for plant growth[6]. But at higher quantity it abridged the germination percentage, which is consistent with other researcher's findings[3, 15]. 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[16]. Thus, our result 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 radical 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[17].

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[19].

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[20]. 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[14]. Therefore APX was the enzyme which illustrated maximum activity in *Cicer arietium* 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 part as a micronutrient and this augmented the concentration of antioxidant enzymes in shoots compared to roots.

CONCLUSION

This study showed that a 25 mM of Zn concentration enhanced seed germination augmented radical 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 25 mM Zn concentration, chlorophyll contents and oxidative stress were augmented, which led to diminution in development of *Cicer arietinum* plants. Hence, we recommended that 25 mM Zn may be favorable for plan growth and this concentration of Zn may be recommended for the cultivation of plants.

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