

# Some Morphological and Biochemical Changes in Gram Seedlings Under Cadmium Stress

Shreya Tiwari, Shruti Bajpai and Neerja Srivastava\*

Department of Biochemistry, IBSBT, CSJM University, Kanpur, Uttar Pradesh, India

## Abstract

Heavy metal pollution is a very serious environmental issue throughout the world. These heavy metals do not degrade and accumulate in the environment which is very dangerous to the environmental and human health. Plant responses to heavy metal stress are the combined results of various processes like cellular transport mechanisms and activation of signal transduction pathways, which depend upon type of metal and plant species. In the present study, various morphological and biochemical changes were investigated in the *Cicer arietinum* grown hydroponically in different concentration of  $CdCl_2$ . This study was done in order to contribute towards the better understanding of the mechanism of heavy metal stress adaptation in the gram seedlings. Marked reduction was observed in the length of leaves, shoots and roots as well as fresh weight of gram seedlings at higher concentration of cadmium chloride compared to controls. This reduction in growth indicates metal toxicity. There is increase in protein concentration with increasing concentration of cadmium in gram seedlings. The increase of soluble proteins could result from the activation of genes for synthesis of specific proteins associated with stress that protect the vital set of cellular proteins, and the heat shock proteins which maintains membrane protein and the plant cell structures. It seems that the synthesis of specific proteins is necessary for the hardening.

**Keywords:** Heavy metal stress; Cadmium stress; Stress proteins

## Introduction

Any metallic element that has a relatively high density and is toxic or poisonous even at low concentration is termed as heavy metal [1]. This is a general collective term, which is applied to the group of metals and metalloids with atomic density greater than  $4\text{ g/cm}^3$  or more, greater than water [2]. Various metals like lead (Pb), cadmium (Cd), zinc (Zn), mercury (Hg), arsenic (As), silver (Ag), chromium (Cr), copper (Cu), iron (Fe), and the platinum group elements are placed under heavy metals [3,4].

All heavy metals at high concentration have very strong toxic effects and considered as environmental pollutants [5]. They are very toxic for plants and results in chlorosis, weak plant growth, and yield depression. It can also be accompanied by reduced nutrient uptake, disorders in plant metabolism in leguminous plants, metal toxicity results in reduced ability to fix molecular nitrogen. High levels of heavy metal in soil leads to losses in agricultural yield and also poses hazardous health effects as they enter into the food chain [6]. Although plant growth can be inhibited by metal absorption in heavy metal polluted soils, some plant species are still able to accumulate large amounts of heavy metals without showing any sign of stress. This is very risky for animals and humans [7] as these metals get entered in the food chain [8]. These metals also transmitted through natural ecosystems [9]. All these problems can be solved through phytoremediation which is a process of metal uptake and accumulation by various plants. This process totally depends on the concentration of metals present in soils, solubility sequences and the type of plant species growing on these soils [10].

Cadmium, which is used very widely in industries, is a human carcinogen. It is a highly toxic metal pollutant of the soil. It inhibits root and shoot growth as well as yield production. It also affects nutrient uptake and homeostasis. It is accumulated in agriculturally important crops and enters in the food chain which is hazardous to animal and human health [11]. cadmium content is increased in the soil by the use of sewage sludge, city waste and cadmium containing fertilizers [12]. Cadmium toxicity causes reduction in biomass which could be due to inhibition of chlorophyll synthesis and photosynthesis [13]. There is decreased

uptake of nutrients, inhibition of different enzyme activities, induction of oxidative stress including alterations in the antioxidant defense system enzymes by large amount of cadmium [14]. The reduction of biomass by Cd toxicity could be the direct consequence of the inhibition of chlorophyll synthesis and photosynthesis [13]. Excessive amount of Cd may cause decreased uptake of nutrient elements, inhibition of various enzyme activities, induction of oxidative stress including alterations in enzymes of the antioxidant defense system [14]. According to Shah and Dubey [15] cadmium ranks highest amongst all the metals in terms of damage to plant growth and human health. According to Moya et al. [16] large amount of cadmium in the soil induces many stress symptoms in plants like reduction in root growth, disturbances in mineral nutrition and carbohydrate metabolism, because of which biomass production is drastically reduced.

*Cicer arietinum L.* (Chickpea) is the member of family Fabaceae and is very important leguminous crop in the world. It is a good source of proteins and carbohydrates and ranked first amongst the cold season food legume of the world with about 10,671,503 ha cultivation [17].

In the present work, changes in the length of root, shoot and leaves, fresh weight of seedlings and total protein content were studied in gram seedlings treated with various concentration of  $CdCl_2$ . This study was done in order to contribute towards better understanding of environmental stress adaptation.

\*Corresponding author: Neerja Srivastava, Department of Biochemistry, IBSBT, CSJM University, Kanpur, Uttar Pradesh, India, Tel: +919415154373; E-mail: [neerjas70@gmail.com](mailto:neerjas70@gmail.com)

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## Material and Methods

### Materials

All the chemicals were obtained from Sisco Research Laboratories and Merck (India). Gram seedlings were procured from local village area.

### Methods

**Germination of seeds:** Chick pea (*Cicer arietinum*) seeds were surface sterilized with disinfectant savlon for 15 minutes. Seeds were then thoroughly washed with double distilled water and imbibed in it for six hours. Imbibed seeds were grown hydroponically in plastic trays in nutrient solution at various concentration of cadmium ranging from 0-100  $\mu\text{M}$   $\text{CdCl}_2$  in a plant growth chamber in dark at  $30 \pm 2^\circ\text{C}$  and 80% relative humidity. One more control was set up by growing seeds in distilled water (DW) only, without adding any nutrient. Solution was changed every 48 hours to avoid fungal infection.

**Composition of nutrient solution:** Hoagland nutrient solution was prepared according to Moore [18].

**Cadmium treatment:** Gram seedlings were grown in various concentration of  $\text{CdCl}_2$  ranging from 0-100  $\mu\text{M}$ . They were grown separately in nutrient solution with DW (distilled water), 0, 25, 50, 75, 100  $\mu\text{M}$  of  $\text{CdCl}_2$ .

**Measurement of length:** Seeds were harvested on 10<sup>th</sup> day for measurement of length. They were separated into roots, shoots, and leaves. Their length was then measured in centimeters with the help of a scale.

**Determination of fresh weight:** 10-day old seedling were harvested for determination of fresh weight. Seedlings were blotted dry and their fresh weights were measured [19].

**Isolation of protein:** Protein were isolated according to the method of Bozarth et al. [20] with slight modification. 5 gm of 6-day old gram-seedlings were grounded in 15 ml of homogenizing buffer at  $0^\circ\text{C}$  in a mortar and pestle. The homogenate was then filtered through nylon sieve and centrifuged at  $1000 \times g$  for 5 min at  $4^\circ\text{C}$ . The supernatant was decanted and recentrifuged at  $13000 \times g$  for 5 min. at  $4^\circ\text{C}$ . The resulting supernatant constituted the proteins.

**Quantitative estimation of total protein:** Protein estimation was done by Lowry's method [21].

**Statistical analysis:** One-way analysis of variance (ANOVA) was done to test the significance of physiological and biochemical changes in gram seedlings under cadmium stress using the method of Rao [22].

## Results and Discussion

### Morphological studies

Root, shoot and leaf length was measured in gram seedlings at different concentrations of cadmium chloride on 10th day which is presented in Table 1. It has been observed that length in leaf, shoot and root of gram seedlings decreased with increased concentration of cadmium chloride compared to control plants. There is about 26%, 29%, 18% and 52% reduction in root length at 25, 50, 75 and 100  $\mu\text{M}$  cadmium chloride respectively. In the shoots, length was reduced about 13%, 15%, 19% and 38% at 25, 50, 75 and 100  $\mu\text{M}$  cadmium chloride concentration respectively. There is also decrease in leaf length at higher concentration of cadmium. There is about 42%, 45%, 42% and 35% decrease in length at 25, 50, 75 and 100  $\mu\text{M}$  cadmium chloride

concentrations. These results were corroborating with the findings of Saravanamoorthy and Ranjita Kumari [23] in peanut and green gram, Srivastava et al. [24], in *Solanum melongena*, Bahmani et al. [25] in *Phaseolus vulgaris* L., Hirve and Bafna [26] in *Vigna radiata* L. There was reduction in the root length in treated seedlings in comparison to controls, this reduction in root length could be because of decrease in new cell formation as well as in the cell elongation in the extension region of the root [27,28]. The reduction in leaf size is due to the interaction of Cadmium on the growth and metabolism. Cadmium contamination in the cells causes death of the cells which ultimately reduces the growth of leaves. The reduction in fresh and dry weight of seedlings because of pronounced initiation of shoot and root growth may probably occur due to metal uptake primarily through roots [29]. In nickel treated gram seedlings also same type of results were observed [30,31].

Length of roots, shoots and seedlings are the most sensitive end points and indicates the level of metal toxicity [32-36]. Similar findings have been observed by Bhardwaj et al. [37] while working with green gram. Seedling growth was affected because metal contamination disturbs the plant metabolism due to interactions with enzymes and biochemical reactions take place inside the plant body [38]. Different researchers [39-41] reported adverse effects of Cd on plant growth [42].

Decrease in fresh weight of the whole gram seedlings with increasing concentration of cadmium was observed. There is about 3%, 4%, 9% and 13% decrease at 25, 50, 75 and 100  $\mu\text{M}$  cadmium chloride (Table 2). Bhardwaj et al. [37] reported decline in seedling biomass (Fresh wt. and Dry wt.) proportionately with increasing concentrations of heavy metals. Xiong [43] also reported progressive decline in plant dry weight with increasing concentrations of Pb in soil. Leaf area showed significant decline with increase in concentrations Pb and Cd. For different concentrations of Pb it was 16.87%, 56.27%, 68.62%, 79.78% and for Cd it is 22.96%, 63.47%, and 84.91% respectively.

### Biochemical studies

Increase in protein concentration with increasing concentration of cadmium has been observed in gram seedlings. There is about 9%, 50%, 56% and 54% increase in protein content at 25, 50, 75 and 100

| Concentration of Cadmium Chloride ( $\mu\text{M}$ ) | Root *                 | Shoot*                | Leaf*                 |
|---|------------------------|-----------------------|-----------------------|
| DW(distilled Water)                                 | 4.64 $\pm$ 2.27(101%)  | 6.44 $\pm$ 2.48(69%)  | 0.78 $\pm$ 0.47(62%)  |
| 0   | 4.61 $\pm$ 1.79 (100%) | 9.35 $\pm$ 3.05(100%) | 1.26 $\pm$ 0.52(100%) |
| 25  | 3.40 $\pm$ 1.76 (74%)  | 8.16 $\pm$ 2.85(87%)  | 0.73 $\pm$ 0.27(58%)  |
| 50  | 3.28 $\pm$ 1.90 (71%)  | 7.96 $\pm$ 4.28(85%)  | 0.70 $\pm$ 0.35(55%)  |
| 75  | 3.78 $\pm$ 2.29(82%)   | 7.58 $\pm$ 2.52(81%)  | 0.73 $\pm$ 0.38(58%)  |
| 100   | 2.21 $\pm$ 0.40(48%)   | 5.84 $\pm$ 2.17(62%)  | 0.83 $\pm$ 0.02(65%)  |

\*Significant at 1% level

**Table 1:** Effect of Cadmium on the length (cm) of Root, Shoot and leaf of gram seedlings.

| Concentration of Cadmium Chloride ( $\mu\text{M}$ ) | Fresh Weight (gram) of whole Seedlings* |
|---|---|
| DW  | 3.88 $\pm$ 0.39(89%)                    |
| 0   | 4.38 $\pm$ 0.54(100%)                   |
| 25  | 4.24 $\pm$ 0.53(97%)                    |
| 50  | 4.22 $\pm$ 0.43(96%)                    |
| 75  | 4.00 $\pm$ 0.26(91%)                    |
| 100   | 3.81 $\pm$ 0.17(87%)                    |

\*Significant at 1% level

**Table 2:** Effect of Cadmium on the fresh weight (gram) of gram seedlings.

| Concentration of Cadmium Chloride ( $\mu\text{M}$ ) | Protein conc. ( $\mu\text{g/ml}$ )* |
|---|-------------------------------------|
| DW  | 296.67 $\pm$ 37.53(80%)             |
| 0   | 370.00 $\pm$ 52.00(100%)            |
| 25  | 403.33 $\pm$ 50.80(109%)            |
| 50  | 556.67 $\pm$ 64.10(150%)            |
| 75  | 580.00 $\pm$ 72.00(156%)            |
| 100   | 570.00 $\pm$ 61.60(154%)            |

\*significant at 1% level

**Table 3:** Effect of Cadmium on the protein conc. in the gram seedlings.

$\mu\text{M}$  cadmium chloride concentrations (Table 3). These results were similar to the report of Alayat et al. [44] where increase of protein content in wheat leaves with the increased exposure to the cadmium concentration has been observed. In their work, Cadmium treatment increased soluble protein content which suggested that this increase is due to the increase of antioxidant enzymes and reactive oxygen species. Although expression of certain genes are altered by ROS which also modulates activities of specific defense proteins, but they are extremely harmful to organism in high amounts [45]. Oxidation of proteins, lipids and nucleic acids is done by ROS which leads to changes in cell structure and mutagenesis [46]. Increase of soluble proteins could result from the activation of genes for synthesis of specific proteins associated with stress such as proteins "LEA" that protect the vital set of cellular proteins, and the heat shock proteins which maintains membrane protein and the plant cell structures. The acquisition of resistance to stress process is accompanied by an important synthesis of soluble protein; this is the result of a slower development and storage of molecules in the hyaloplasm or in some organelles (chloroplasts, mitochondria). It seems that the synthesis of specific proteins is necessary for the hardening [47,48].

## Conclusion

It can be concluded from the present study that higher concentration of cadmium is toxic to gram seedlings. While, there is reduction in the length of root, shoot and leaves as well as in the fresh weight of the gram seedlings under cadmium stress, the protein content was increased. These responses might be responsible for adaptation strategies to tolerate cadmium stress. This knowledge will help us to develop the strategies for decreasing the risk of cadmium contamination to crop production.

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