Some Morphological and Biochemical Changes in Gram Seedlings Under Cadmium Stress

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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 hydropotonically in different concentration of CdCl₂. 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 in 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 results 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 g/cm³ 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 CdCl₂. This study was done in order to contribute towards better understanding of environmental stress adaptation.

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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 µM CdCl₂ in a plant growth chamber in dark at 30 ± 2°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 CdCl₂ ranging from 0-100 µM. They were grown separately in nutrient solution with DW (distilled water), 0, 25, 50, 75, 100 µM and 13%, 15%, 19% and 38% at 25, 50, 75 and 100 µM cadmium chloride respectively. In the shoots, length was reduced about 29%, 18% and 52% reduction in root length at 25, 50, 75 and 100 µM of cadmium chloride compared to control plants. There is about 26%, 27% and 28% reduction in leaf length in leaf, shoot and leaf of gram seedlings under cadmium stress using the method of Rao [22]. This result was corroborating with the findings of Saravanamooorthy 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 traded 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 µM 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 µM cadmium chloride respectively. These results were corroborating with the findings of Saravanamooorthy 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 traded 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].

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

| Concentration of Cadmium Chloride (µM) | Root* | Shoot* | Leaf* |
|---------------------------------------|-------|--------|-------|
| DW(distilled Water)                  |       |        |       |
| 0                                    | 4.64 ± 2.27(101%) | 6.44 ± 2.48(69%) | 0.78 ± 0.47(62%) |
| 25                                   | 4.61 ± 1.79(100%) | 9.35 ± 3.05(100%) | 1.26 ± 0.52(100%) |
| 50                                   | 3.40 ± 1.76(74%) | 8.16 ± 2.85(87%) | 0.73 ± 0.27(58%) |
| 75                                   | 3.28 ± 1.90(71%) | 7.96 ± 4.28(85%) | 0.70 ± 0.35(55%) |
| 100                                  | 3.78 ± 2.29(82%) | 7.58 ± 2.52(81%) | 0.73 ± 0.38(58%) |

*Significant at 1% level

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

| Concentration of Cadmium Chloride (µM) | Fresh Weight (gram) of whole Seedlings* |
|---------------------------------------|----------------------------------------|
| DW                                    | 3.88 ± 0.39(89%)                      |
| 0                                     | 4.38 ± 0.54(100%)                     |
| 25                                    | 4.24 ± 0.53(97%)                      |
| 50                                    | 4.22 ± 0.43(96%)                      |
| 75                                    | 4.00 ± 0.26(91%)                      |
| 100                                   | 3.81 ± 0.17(87%)                      |

*Significant at 1% level
These responses might be responsible for adaptation strategies to resistance of the stress process is accompanied by an important synthesis of soluble protein that protects the vital 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 is caused 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.

**References**

1. Lennette (2004) Water treatment and air purification water treatment. Rotterdamseeweg, Netherlands.  
2. Hawkes SJ (1997) What is a ° Heavy Metal°? J Chem Educ 74: 1374.  
3. Farlex F (2005) Definition environment, the free dictionary. Farlex Inc. Publishing, USA.  
4. Junaid M, Adnan M, Khan N, Khan N (2013) Plant Growth, Biochemical Characteristics and Heavy Metals Contents of Medicago Sativa L., Brassica Juncea (L.) Czern. and Cicer Arietinum L. Fussat J Biol 3: 95-103.  
5. Chehregani A, Malayeri B, Golmohammadi R (2005) Effect of heavy metals on the developmental stages of ovules and embryonic sac in Euphorbia cheirandeni. Pakistan Journal of Biological Sciences 8: 622-625.  
6. Schicker H, Caspi H (1999) Response of antioxidative enzymes to nickel and cadmium stress in hyperaccumulator plants of the genus albus. Plant Physiology 105: 39-44.  
7. Oliver MA (1997) Soil and human health. A review. European Journal of Soil Science 48: 573-592.  
8. Fries W, Fried J, Platzer K, Horak O, Gerzabek MH (2006) Remediation of contaminated agricultural soils near a former PbZn smelter in Austria: batch, pot and field experiments. Environmental Pollution 144: 40-50.  
9. Walker DJ, Clemente R, Roig A, Bernal MP (2003) The effects of soil amendments on heavy metal bioavailability in two contaminated Mediterranean soils. Environmental science and Pollution 122: 303-312.  
10. Kafka Z, Kunas K (1997) Heavy Metals in soils contaminated from different sources. In: Ecological issues and environmental impact assessment. Gulf Publishing Company, Houston, Texas, USA, pp: 175-180.  
11. Di-Toppi SL, Gabrielli R (1999) Response to cadmium in higher plants. Environmental and Experimental Botan 41: 105-130.  
12. Williams CH, David DJ (1973) The effect of superphosphate on the cadmium content of soils and plants. Australian Journal of Soil Research 11: 43-56.  
13. Padmaja K, Prasad DK, Prasad ARK (1990) Inhibition of chlorophyl synthesis in Phaseolus vulgaris seedlings by cadmium acetate. Photosynthetica 24: 399-405.  
14. Sandalio LM, Dalurzo HG, Gomez M, Romero-Puertas MC, Del Rio LA (2001) Cadmium-induced changes in the growth and oxidative metabolism of pea plants. Journal of Experimental Botany 52: 2115-2126.  
15. Shah K, Dubey RS (1998) A 18 kDa cadmium inducible protein complex from rice: its purification and characterization from rice (Oryza sativa L.) seedlings. Journal of Plant Physiology 152: 448-454.  
16. Moya JL, Ros R, Picazo I (1993) Influence of cadmium and nickel on growth, net photosynthesis and carbohydrate distribution in rice plants. Photosynthesis Research 36: 75-80.  
17. FAO (2006) The FAO statistical year books 2005/2006. Syria.  
18. Moore TC (1981) Mineral nutrition in sunflower. Research experiences in plant physiology, A laboratory manual. Springer-Verlag, New York, USA.  
19. Matoh T, Ohta D, Takahashi E (1986) Effect of sodium application on growth of Annarthrus tricolor L. Plant Cell Physiol 27: 187-192.  
20. Bozarsh CS, Mullet JE, Boyer JS (1987) Cell wall proteins at low water potentials. Plant Physiol 85: 261-267.  
21. Lowry OH, Rosenbough NJ, Farr AI, Randall RL (1951) Protein measurement with folin phenol reagent. J Biol Chem 193: 265-275.  
22. Rao CR (1973) The theory of least squares and analysis of variance. Linear statistical inference and its applications. Wiley Eastern Limited, New Delhi, India.  
23. Saravanamuthy MD, Kumari R (2005) Effect cotton yarn dye effluent on physiological and biochemical content of peanuts and green gram. Biochem Cell Archs 5: 113-111.  
24. Srivastava S, Chopra AK, Pathak C (2012) Ferti-irrigational impact of distillery effluent Di-ammonium phosphate on the soil and growth characteristics of Egg plant (solanum melongena L.). Journal of Applied and Natural science 2: 275-293.  
25. Bahmani R, Bhamta MR, Habibi D, Forozesh P, Ahmadvand S (2012) Effect of Cadmium Chloride on Growth Parameters of Different Bean Genotypes (Phaseolus vulgaris L.). ARPN Journal of Agricultural and Biological Science 7: 35-40.  
26. Hirve M, Bafna A (2013) Effect of Cadmium exposures on growth and biochemical parameters of Vigna radiata seedlings. International Journal of Environmental Sciences 4: 312-322.  
27. Haussling M (1988) Ion and water uptake in relation to root development of Norway spruce (Piceaabies (L.) Karst). Journal of Plant Physiology 133: 486-491.  
28. Saha S, Bhattacharjee C (2014) Effect of lead acetate on Bengal gram seeds. International Journal of Clinical and Diagnostic Research 2: 1-10.  
29. Arduini L, Godbold DL, Onnisa A (1995) Influence of copper on root growth and morphology of Pinus pinea L and Pinus pinaster Ait. Seedlings. Tree Physiology 15: 411-415.  
30. Pandey N, Sharma CP (1999) Effect of varying copper levels on safflower. Proceedings of the National Academy of Sciences India Section B Biological 65: 67-73.  
31. Murugalakshikumari R, Ramasubramanian V (2014) Studies on the impact of lead acetate on the growth and biochemical characteristics of Ablemornchus Escentulentus (L.) Medicus. International Journal of Science, Environment and Technology 3: 591-596.  
32. An YJ (2004) Soil ecotoxicity assessment using cadmium sensitive plants. Environ Pollut 127: 21-26.
33. Correa AXR, Rorig LR, Verdineili MA, Cotelle S, Ferrad JF (2006) Cadmium phytoxicity: Quantitative sensitivity relationships between classical endpoints and antioxidative enzyme biomarkers. Sci Total Environ 357: 120-127.

34. He JY, Ren YF, Cheng ZH, Jiang DA (2008) Effects of cadmium stress on seed germination, seedling growth and seed amylase activities in rice (Oryza sativa). Rice Sci 15: 319-325.

35. Ahmad MSA, Hussain M, Ijaz S, Alvi AK (2008) Photosynthetic performance of two mung bean (Vigna radiata (L.) Wilczek) cultivars under lead and copper application. Int J Agric Biol 10: 167-176.

36. Ahmad MSA, Ashraf M, Tabassam Q, Hussain M, Firdous H (2011) Lead (Pb) induced regulation in growth, photosynthesis, and mineral nutrition in maize (Zea mays L.) plants at early growth stages. Biol Trace Elem Res 144: 1229-1239.

37. Bhardwaj P, Chaturvedi AK, Prasad P (2009) Effect of enhanced lead and cadmium in soil on physiological and biochemical attributes of Phaseolus vulgaris L. Nature Sci 7: 63-75.

38. Ashraf MY, Sadig R, Hussain M, Ashraf M, Ahmad MSA (2011) Toxic effect of nickel (Ni) on growth and metabolism in germinating seeds of sunflower (Helianthus annuus L.). Biol Trace Elem Res 143: 1695-1703.

39. Shafi M, Zhang GP, Bakht J, Khan MA, Islam E, et al (2010) Effect of cadmium and salinity stresses on root morphology of wheat. Pak J Bot 42: 2747-2754.

40. Aydinalp C, Marinova S (2009) The effects of heavy metals on seed germination and plant growth on alfalfa plant (Medicago sativa). Bulgarian J Agri Sci 15: 347-350.

41. Oncel I, Kele Y, Ustun AS (2000) Interactive effects of temperature and heavy metal stress on the growth and some biochemical compounds in wheat seedlings. Environ Pollut 107: 315-320.

42. Ahmad M, Akhtar MJ, Zahir ZA, Jamil A (2012) Effect of cadmium on seed germination and seedling growth of four wheat (Triticum aestivum L.) cultivars. Pak J Bot 44: 1569-1574.

43. Xiong Z (1997) Bioaccumulation and physiological effects of excess lead in a roadside pioneer species Sonchus oleraceus L. Environmental Pollution 97: 275-279.

44. Alayat A, Souiki L, Grara N, Djebar MR, Bounedris ZE (2014) Effects of Cadmium on Water Content, Soluble Protein, Proline Changes and Some Antioxidant Enzymes in Wheat (Triticum durum desf.) Leaves. Annual Research & Review in Biology 4: 3835-3847.

45. Halliwell B, Gutteridge JMC (1999) Free Radicals in Biology and Medicine. Oxford University Press, New York, USA.

46. David JC, Grongnet G (2001) Les protéines de stress. Prod Anim 14: 29-40.

47. Côme D (1992) Plants and cold. Herman, Paris.

48. Knights EJ, Acikgoz N, Warkentin T, Beijiga G, Yadav SS, et al. (2007) Area, production and distribution. In: Chickpea Breeding and Management. Cromwell Press, UK, pp: 167-178.