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Research Article
Assessment of Biochemical Parameters in Heavy Metal-stressed Crop of Mung Bean [*Vigna radiata* (L.) R. Wilczek]

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Abstract
Background and Objective: Every year, a significant portion of agricultural yield is hampered due to increasing level of heavy metals and other chemical in the soil leads in low productivity and sometimes no growth at all. This chemical seriously impairs the growth of the plants and affects the overall productivity. The remedy for this problem lies towards microbial degradation of these compounds. The study aimed to elucidate the alterations in the physiological parameters, biochemical parameter and protein profile in stressed seedlings with respect to control. **Materials and Methods:** The present study involved the impact of heavy metals stress (zinc and cadmium) on mung seedlings under *in vitro* conditions. The seeds were of research grade and the same size. The entire study was conducted in sets of three (triplicates) and the data were statistically analyzed, represented the mean of the dataset. **Results:** Data revealed that the water agar seedling symptom test the severity of the effect on the seedlings was in the order as cadmium followed by zinc. During metabolic analysis, initially at low concentration there was an increase and a sharp decrease at higher concentration in the photochemical contents. Protein profiling exhibited a band of 97.4 KDa protein in all the stress induced plant with respect to the control. So this might be the protein that is most probably a part of the stress machinery in the plant. **Conclusion:** As it is rapidly progressing towards the development, the problems associated with it will become increasingly complex in near future. Hence, it is the time to take a precautionary step ahead so that the situation can be handled in future and find a solution for it. This study is initiative to deal with the present problems of the society to have a better and healthy future.

Key words: Biochemical analysis, *Vigna radiata* mung, zinc, cadmium, heavy metals, plant stress

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Plants are restored to many adaptive strategies in response to different abiotic stresses such as high salt, dehydration, cold, heat and excessive osmotic pressure which ultimately affect the plant growth and productivity[1,2]. Against these stresses, plants adapt themselves by different mechanisms including a change in morphological and developmental pattern as well as physiological and biochemical processes[3]. Adaptation to all these stresses associated with metabolic adjustments that lead to the accumulation of several organic solutes like sugars, polyols, betaines and proline[2,4,5].

Heavy metals pollution is widely spread, due to rapid industrialization and current agricultural practices. These pollutants persist in the environment for a longer period, as they are not easily degraded by soil microorganisms and therefore can easily be absorbed by plants[6]. Heavy metals that have been absorbed by the plants results in growth inhibition, increasing senescence which leads to decreasing crop yield. Moreover, heavy metals can be accumulated in the crops and affect human health upon consumption[7], metals can be found in soils because they are present in insecticides, fungicides, sludge and commercial fertilizers[8]. Metals within the soil solution are the only soil fraction directly available for plant uptake[9,10]. Thus, factors which affect the concentration and speciation of metals in the soil solution will affect the bioavailability of metals to plants. There are earlier reports on carbohydrate accumulation during various abiotic stresses in the temperate grasses and cereals from the Gramineae family where long-term carbohydrate storage occurs during reproductive development[11]. Accumulation of sugars in different parts of plants is enhanced in response to the variety of environmental stresses[12-15]. In the case of salt[16,17] and water stress[15], adaptation to these stresses has been attributed to the stress induced increase in carbohydrate level. Hence, to see the effect of water level and ionic changes in growth and metabolic adjustments inside the plants, they are natively grown under dry and salty areas. They selected four different kinds of abiotic stresses like cold, heat, salt (NaCl) and drought (PEG) which affect the water status differentially with respect to each other. Our country's major economy depends on agriculture. Every year, a significant portion of agricultural yield is hampered due to increasing levels of xenobiotics and heavy metals in the soil. The heavy metal source includes various powerhouses, industrial effluents in the agricultural land, etc. Increasing amount of these compounds in the soil results in low productivity and sometimes no growth at all. *Vigna radiata* (*V. radiata*) mung is a plant of the family Fabaceae. It is one of the most widely used pulse crops in India. It has great value as food and is a cheap source of protein for direct human consumption[18]. Study reported the role of Fe-toxicity towards growth and protein profile of *L. Withania somnifera*, resulted in the reduction of growth parameters, even at low concentration of Fe (25 μM) and suggests the relationship between Fe excess[19]. Alterations of protein patterns which will provide a new insight for better understanding of the molecular basis of nutritional stress responses to medicinal plants. Another study evaluated the effect of cadmium toxicity and reported its significant ($p<0.05$) influence on the growth parameters as well as biochemical constituents[20]. This study made an attempt to investigate the effect of this heavy metal stress (zinc and cadmium) on some important physiological and biochemical analysis processes of *V. radiata* (mung beans, variety-virat) closely connected with the mechanisms of adaptability of this crop to the environmental factors.

MATERIALS AND METHODS

Sample source for the study plant: Certified Moong seeds (*V. radiata*) (Virat HY45) purchased from a certified shop in Jaipur. The seeds were of research grade and the same size.

***In vitro* development of plantlets under abiotic stress condition:** Here, MS media was prepared and supplemented with heavy metals.

Aseptic transfer of seeds: The seeds used were first washed with distilled water several times to remove all the dirt particles. Seeds then washed with 70% ethanol as an additional step to reduce contamination. The seeds further washed with 0.1% $HgCl_2$ for 2 min followed by washing with autoclaved water 2-3 times. After that the seeds were ready to be used for water agar seedling symptom test (WASST) experiments.

Water agar seedling symptom test (WASST): The 20 mL of media was dispensed into each culture tubes and autoclaved. After that, the heavy metals added at various concentrations to the media. The media was allowed to cool and solidify; the seeds inoculated in the tubes (1 seed/tube), as well as incubated at the appropriate temperature and light conditions. The symptoms of developed seedlings analyzed under a stressed condition with reference to the control recorded[21-23].

Biochemical analysis of heavy metals stress induced plants: The root shoot length of the plants was recorded and further presented for their biochemical analysis as total protein content[24] and total phenolic content estimation[25]. Total soluble sugar content[26] and total reducing sugar content[27].

Protein lineage of stress induced plants: Isolation of crude protein by trichloroacetic acid (TCA) method[28] and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of protein sample characterized[29].

RESULTS AND DISCUSSION

The use of heavy metal compounds (zinc and cadmium) for induction of abiotic stress on *V. radiata* and to evaluate its effects on plant growth parameters under *in vitro* conditions are a pioneering approach. The result of the study was justified when compared to the outcome of other tests on abiotic stress on *V. radiata.*

Water agar seedling symptom test (WASST): Study of heavy metal effect on root shoot length by WAST, revealed that zinc treated seeds showed a comparative decrease in root shoot length with increasing concentration (10, 100, 300, 500 and 1000 ppm) with respect to the control. The study showed that zinc was toxic to the plants at 1000 ppm concentration. As the concentration of zinc increases, there is a gradual decrease in root length (6 cm in control to 1.3 cm at 1000 ppm) and shoot length (11 cm in control to 3 cm at 1000 ppm). Browning of root tips and root negative geotropism detected at a 100 ppm concentration of zinc (Fig. 1-4). Cadmium treated seeds showed an initial decrease in root-shoot length till 100 ppm (3.1 and 2 cm), whereas, no germination was detected from 300-1000 ppm as compared with the control (6 cm root and 11 cm shoot length), respectively. It was observed that the seeds wilted after 100 ppm concentration. Cadmium is highly toxic to the plants at more than 100 ppm concentration when compared with the effects of zinc as the seeds failed to germinate beyond 100 ppm cadmium (Fig. 2).

A study suggested that the root length of the cadmium chloride seedlings was found to significantly decrease at all concentrations (except 50 μM) compared to untreated seedlings in all varieties of *V. radiata*[20]. Zinc sulphate treated seedlings showed inhibition of growth at all concentrations through inhibition of hypocotyl elongation measuring about 90% observed at the highest concentration (40 mM)[30]. *V. radiata* showed retardations of both root and shoot growth at 400 and 600 μg mL^{-1} of silver nanoparticles but the root growth totally terminated at 1600 μg mL^{-1} [31]. In the case of zinc oxide nanoparticles, the percentage of seed germination was significantly affected by the interaction in mung bean seeds. It was observed in the limited concentration of the plant that there was an increase in plant shoot growth. It can also affect plant growth hormone enhancement[32]. When seeds treated with arsenic and manganese, seed germination percentage and root growth decreased gradually with increase in heavy metals (arsenic and manganese) concentrations[33]. In nickel treated seeds, there was no effect on the root-shoot length until 7 days. After 7 days, there was a reduction with respect to control[34]. The treatment of mung bean seeds with gamma rays (^{60}Cobalt) for 0 and 4 min showed a significant increase in plant height and weight compared to control[35]. Treatment of seedlings with chromium significantly decreased the root and shoot length with an increase in the concentration of Cr. This was due to its high accumulation in roots and the nonexistence of any defined Cr translocation mechanism thereby enhancing the Cr sequestration in the tissue and inhibiting root development[36].

Biochemical analysis of the stress-induced seedlings: Assessment of the phenotypic parameter (root shoot length) of a plant gives an overall idea of the effect of a compound on the plant. A seedling growing under a high-stress condition with good physical length is not proportional to a better yield. Biochemical analysis helps to gain an insight into the plant's phytochemical changes that occur due to the induced stress and hence helps to derive a conclusion on the phenotypes observed under stress. This study section evaluated the changes in the phytochemical parameters (total protein content, total soluble sugar, total reducing sugar and total phenolic content) of the seedlings induced by stress in comparison to the control (Fig. 5a-c).

Total protein content: Evaluation of the level of protein content is important as they are the key players in plant cellular metabolism. Data from the present study revealed that in the case of zinc, the level of the protein content of the seedlings increased at 10 ppm as 0.691 mg g^{-1} followed by decline as 0.612 mg g^{-1} at 100 ppm, 0.519 mg g^{-1} at 300 ppm and remains constant at 500, 1000 ppm as 0.456 mg g^{-1} with increasing concentration as compared to the control (0.685 mg g^{-1}), respectively. In the case of cadmium, the total protein content of seedling increased at first at 10 ppm (0.712mg g^{-1} seedling) then decreased with increase in the heavy metal at 1000 ppm (0.312 mg g^{-1}) as compared to the control (0.685 mg g^{-1}), respectively (Fig. 5a). In a study,

Fig. 1(a-b): Induction of heavy metals stress to the seeds at various concentrations (10, 100, 300, 500 and 1000 ppm) by water agar seedling symptoms test and the phenotypic symptoms observed (a) Cadmium stress and (b) Zinc stress

seedlings treated with cadmium chloride showed a significant decrease in protein content at all concentrations (except 50 µM) of cadmium chloride as compared to untreated seedlings in all studied varieties of *V. radiata* [20]. Chromium treated seedlings showed an increasing amount of protein content with increasing concentration of Cr^{6+}. At a lower concentration (50 µM of Cr^{6+}), a non-significant increase of total soluble observed. A significant increase in protein

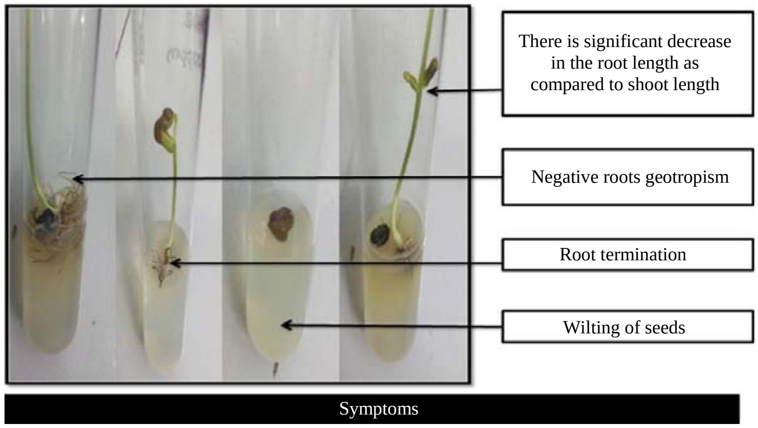

Fig. 2: Symptoms observed due to stress induction(zinc and cadmium) obtained in WASST method

Fig. 3: Recovered *in vitro* propagated seedlings from the WASST method

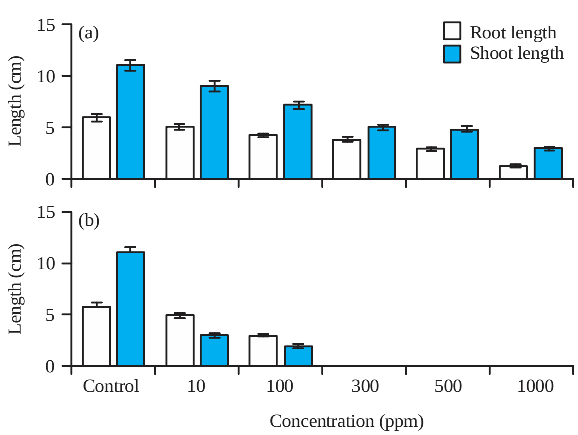

Fig. 4(a-b): Histogram of the root and shoot length (cm) observed in (a) Zinc and (b) Cadmium due to stress compounds for comparative analysis
The dataset conducted in triplicates indicating the mean with percent error bar

content found in seeds treated with 150 and 200 μM Cr^{6+} [30]. It observed that in zinc 500 and 1000 ppm, there is an over-expression of the proteins when compared to the control. It is an indication that these proteins may be part of the cell's stress machinery. In cadmium 500 ppm, there is an over expression of the protein of molecular weight 97.4 kDa. Hence, a protein of 97.4 kDa may most probably be involved in stress machinery or it may be a stress protein as its expression can clearly be seen only in the heavy metal-stressed seedlings with reference to the control.

Total phenolic content: Assessment of phenolic content in the plant is important as it acts as the precursor for the synthesis of many biomolecules. On stress condition, there is a significant decrease in the phenolic contents of the plants. On treatment of seedlings with zinc, there was an initial

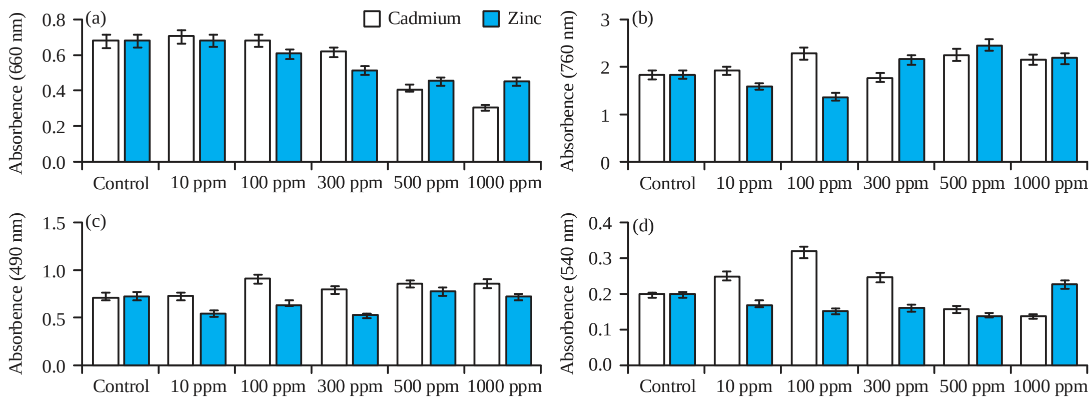

Fig. 5(a-d): Comparative analysis of variations in metabolomic profile of *in vitro* recovered seedlings due to zinc and cadmium stress (a) Total protein content, (b) Total phenolic content, (c) Total soluble sugar content and (d) Total reducing sugar content

The dataset conducted in triplicates indicating the mean with percent error bar

decrease till 100 ppm as 1.392 mg g^{-1} followed by steep increment at 300 ppm (2.185 mg g^{-1}) till highest as 2.5 mg g^{-1} at 500 ppm that further decreased to 2.215 mg g^{-1} at 1000 ppm when compared with the control (1.856 mg g^{-1}). Cadmium treated seedlings showed an increase in the total phenolic content with increasing concentration of heavy metals then, decrease from 300 ppm (1.793 mg g^{-1}) concentration and again increased at 1000 ppm (2.185 mg g^{-1}) as compared with the control (1.856 mg g^{-1}) (Fig. 5b). In a study of seedlings treated with silver nanoparticles, an increase in total phenolic contents at 50 ppm concentrations observed while lead nitrate treated seeds showed a decrease in total phenolic contents at 120 ppm concentrations[37].

Total soluble sugar content: Soluble sugar is an important candidate involved in providing protection of plants from various forms of stress. It protects proteins from stress conditions and also functions as an osmoprotectant. Data from the present study revealed the following treatment of seedlings with zinc showed an increase in the total soluble sugar content with increasing concentration of heavy metal then there was a decrease at 300 ppm (0.529 mg g^{-1}) and further increased as 0.727 mg g^{-1} at 1000 ppm when compared with the control (0.732 mg g^{-1}). Whereas, cadmium treated seedling exhibited increased in the mentioned parameter at 100 ppm (0.915 mg g^{-1}) concentration thereafter decreased to 0.868 mg g^{-1} at 1000 ppm when compared with the control (0.732 mg g^{-1}) (Fig. 5c). Similar reports were proposed in $HgCl_2$ and $ZnSO_4$ treated seeds which showed a significant decrease in total soluble sugar content. At the highest concentration of mercuric chloride, there is 80% decrease in total soluble sugar content. At the highest concentration of zinc, however, there is 11% reduction[30].

Total reducing sugar content: The roles of soluble and insoluble sugar are similar. Sugar reduction serves as an important biomarker. They also protect proteins from stress conditions and functions as an osmoprotectant. The data from the present study revealed the following findings as treatment of seedlings with zinc, there was a decrease in total sugar content at 500 ppm (0.142 mg g^{-1}) concentration. It was followed by an increase at 500 ppm concentration when compared with the control (0.2 mg g^{-1}). Cadmium treated seedlings showed an increase in total sugar content with increase in concentration up to 100 ppm (0.318 mg g^{-1}) then the total sugar content decreased to 0.139 mg g^{-1} at 1000 ppm cadmium as compared with the control as 0.2 mg g^{-1} of *in vitro* recovered seedling (Fig. 5d). According to Gill *et al.* [38], similar results quoted in sorghum seedlings treated with NaCl. It showed a dramatic increase in total reducing sugar content in shoot, root and endosperm whereas, in triticale seeds, there was an increase in the total soluble sugar content 1.6-6.5 fold compared to control[39].

Protein lineage of heavy metal-stressed plants: Protein lineage is an important tool for understanding the physiological status of the plant when exposed to a stress condition. Proteins are the key players of any cell as they are responsible for maintaining normal homoeostasis in the cell. When a plant is exposed to any stress whether abiotic or biotic, it synthesises various stress machinery proteins that are responsible for safeguarding the plants up to a certain range

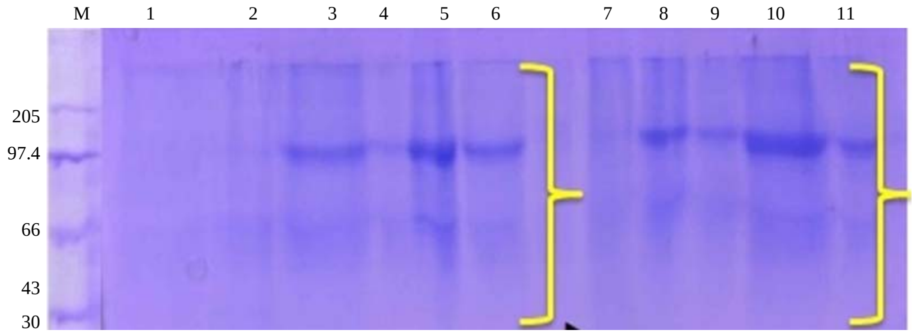

Fig. 6: Protein profiling of the heavy metal stressed *in vitro* recovered seedlings

M: Marker (Merck 29-205 KDa, lane 1control; lane 2-6 zinc treated [lane 2-10 ppm, lane 3-100 ppm, lane 4- 300 ppm, lane 5-500 ppm, lane 6-1000 ppm] and lane 7-11 cadmium treated [lane 7-10 ppm, lane 8-100 ppm, lane 9- 300 ppm, lane 10-500 ppm, lane 11-1000 ppm]

of the stress. Identification of the unique proteins that generated during the stress condition could be beneficial in the engineering of plants with better stress tolerance. In this study, the protein bands of control compared with the test plant (Fig. 6). In a study of salt stress on *Vigna species*, increased protein bands intensity and new induced bands observed. Salinity stress induced two new bands of 45 and 22 kDa in salt tolerant genotypes. Furthermore, band intensity of the salt treated genotypes was higher than that of the control plants[40]. In a study of iron stress in *W. somnifera*, it was found that several new protein bands like 98.14, 38.16, 23.27, 21.25 and 17.49 kDa are newly synthesised and some (87.53 and 66.70 kDa) are more expressed, in leaf samples of treated plants[19]. Water stress deficit in *V. radiata* led to the over-expression of late embryogenesis abundant (LEA) protein, emv2. This protein is speculated to protect the plants in water deficit stress conditions[41].

CONCLUSION

Stress-induced mung seedlings under *in vitro* conditions with heavy metals were studied to know the effects of those compounds on the seedlings and to assess the changes in the biochemical analysis parameter in the plant due to the stress condition. The result obtained from the water agar seedling symptom test showed that the severity of the effect on the seedling physiological parameters was highest in cadmium, followed by zinc. In all the biochemical parameters, there was an initial increase in the level of the phytochemicals at low concentration of the compounds followed by a final decrease in the contents of the phytochemicals at high concentration of the compound. Protein lineage of the stress-induced plant revealed that a band of 97.4 kDa protein was found common in all the stress-induced plant compared with the control.

SIGNIFICANCE STATEMENT

This study signifies the role in identifying the metabolic changes due to abiotic stress (heavy metal) and suggests that the protein is most probably a part of the stress machinery in the plant. The study provided a detail insight of the alterations of the physiological changes synchronized with biochemical parameters and demonstrated that upto 300 ppm concentration of both zinc and cadmium in the seedlings indicated tolerance but beyond this level seedling either fails to germinate or exhibits post germination mortality in *Vigna radiata*. This behavior of the seedlings was noted possibly because of the presence of stress proteins of 97.4 kDa. This research group is also further working on the mitigation of these heavy metals from the soil using microbial consortium.

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