Toxic effects of cadmium on growth parameters of *Vigna unguiculata* (L.) Walp.

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Abstract

Background: This study was designed to examine the effect of cadmium(Cd) stress in cow pea plants by analysing various growth parameters. **Methods**: The seedlings of *Vigna unguiculata* (L.) Walp. were subjected to the treatment of different levels of cadmium chloride (CdCl2) solution (25, 50, 75,100 and 125mM) along with an untreated control. The expression of Cd stress in diverse morphological parameters such as root length, shoot length, number of leaves, leaf area, fresh weight, dry weight and tolerance index percentage was evaluated. **Findings**: The reduction in plant growth of *V. unguiculata* seedlings exposed to Cd treatment was recorded. All the evaluated plant growth characteristics are adversely influenced by the application of Cd. The high concentrations severely affect the plants resulting in ultimate death of plants. The study helped to fix the concentration of 50 mM of Cd which can cause an average range of heavy metal toxicity and that can be reduced using appropriate control measures.

Keywords: Cadmium, Growth parameters, Heavy metal stress.

1. Introduction

Plants have to cope with a variety of abiotic stresses during their life. Abiotic stresses include salinity, drought, high or low temperature, pH, and heavy metal toxicities. The effects of metal and metalloid toxicity are increasing worldwide, mainly due to anthropogenic causes. Metals and metalloids are a natural part of our planet. But, the soil contamination due to their high concentration can result in toxic effects to many of its life forms[1]. A heavy metal is defined as any element exhibiting high density and that exerts its harmful effects even when available in trace amounts. The present agricultural practice with extreme use of agrochemicals like pesticide and fertilizers result in contamination of agricultural soils [2]. Although some metals are essential micronutrients responsible for many regular processes in plants, their excess level can cause harmful effects by directly influencing the growth, senescence and energy synthesis processes [3].

Among heavy metals, cadmium(Cd) is a non-essential and toxic metal,rapidly taken up by roots and accumulated in various plant tissues [4]. Cd stress in plants causes many negative impacts in plant cells and produces reactive oxygen species which induces oxidative stress and ultimately effects various growth parametrs in plants [5,6]. Cow pea is a nutrient rich vegetable and it has a vital role in human diet. Depending on its importance, it is essential to investigate is responses under heavy metal induced oxidative stress. Henceforth, this study was done in the purpose of evaluating heavy metal toxicity of *V. unguiculata* under various concentrations of Cd. This study was designed to evaluate the effect of various concentrations of Cd on growth parameters of *V. unguiculata* seedlings. This study will be helpful to find out the Cd tolerability levels of *V. unguiculata* and appropriate control measures for alleviating the toxic effects.

2. Materials and methods

2.1 Plant material and experimental setup

Vigna unguiculata (L.) Walp. also known as black-eyed pea is one of the important leguminous vegetable. Seeds of Anaswara variety were collected from Regional Agricultural Research Station (RARS) of Kerala Agricultural University, Pattambi, Kerala, India. The experiment was carried out at Botanical garden, KAHM Unity Women's College, Manjeri, Malappuram, Kerala from January-May under natural conditions. Malappuram district located at 75 ^{0}E - 77 ^{0}E longitude and 10 ^{0}N - 12 ^{0}N latitude. The pot experiment was conducted under the green house conditions for 5 months. Cow pea was selected as experimental crop. Before sowing, the seeds were thoroughly washed with water and 6-8 seeds were shown in plastic grow bags containing equal amount of soil, weighing about 1 kg. Each bag was filled with potting mixture (soil + cow dung and coir pith in 1:1:1 ratio).

2.2 Cadmium treatment

After germination, the seedlings were thinned and those with best growth performance were retained (3 plants per pot). Irrigation was done regularly using tap water. On 10th day of germination, the soil was treated with cadmium chloride ($CdCl_2$) solution (200 ml/kg soil) of different concentrations namely 25, 50, 75, 100 and 125 mM along with an untreated control. The experiment was conducted in triplicates. On the second day, toxic limits of cadmium were found in plants treated with 75, 100 and

125 mM/kg. In the same day, all treated plants including control were separated out from the pots for analysis.

2.3 Determination of growth parameters

The shoot length was measured with the help of scale in cm in the day fixed (2nd day). The shoot length was measured from the point where the root and shoot joints to top of the shoot. The root length was measured from the point where the root and shoot joints to the end of the root. Number of leaves per plant was counted for each concentration including control. Total number of leaves for each concentration was added. Each leaf from the plant excluding the petiole was cut and placed on the graph paper to draw the leaf shape. The leaf area was found by counting the all squares in each leaf diagram for each concentration.

After harvesting the seedlings, the fresh weight was recorded using electronic weighing balance and samples were dried in an oven at 60^{0}C for 24 hours to record dry weight and tolerance index percentage was calculated.

$$TI = \frac{observed\ value\ of\ root\ length\ in\ solution\ with\ metal}{observed\ value\ of\ root\ length\ in\ solution\ without\ metal}$$

$$TI = \frac{observed\ value\ of\ root\ length\ in\ solution\ with\ metal}{observed\ value\ of\ root\ length\ in\ solution\ without\ metal} \times 100$$

3. **Results**

3.1 Shoot length

Shoot length of Cd (25, 50, 75, 100 and 125 mM) treated cow pea plants were significantly decreased by Cd treatment compared with the untreated plants. Compared to control plants, maximum decrease of shoot length was clearly visible in the plants treated with 125 mM (21%) as compared to other treatments of Cd. However, the percentage of decrease in shoot length of plants treated with 25 mM was less (4%) than other stress treatments. Moreover, shoot length of the plants subjected to 50, 75 and 100 mM cadmium stress also decreased as compared to control cow pea plants (6, 10 and 15% respectively) (Table 1; Plate 1).

3.2 Root length

The root length was also decreased with increasing Cd concentration in cow pea plants and maximum decrease (42%) was observed when subjected to 125 mM $CdCl_2$ as compared to control plants. Likewise, the declines in root length were found by 10, 20 and 30%, respectively in cow pea plants subjected to 50, 75 and 100 mM Cd stress. However, there was no significant variation in cow pea plants treated with 25 mM $CdCl_2$ as compared to untreated plants (Table 1; Plate 1).

3.3 Number of leaves per plants

Number of leaves per plant was counted to analyze the morphological variations of different Cd treatments in cow pea seedlings. In the case of cow pea plants subjected to 100 and 125 mM Cd, the number of leaves per plant was highly reduced (45 and 55% in 100 and 125 mM Cd concentrations, respectively) as compared to control plants. Likewise, 18% reduction was noticed on the number of leaves per cow pea plant after subjected to 25 and 50 mM $CdCl_2$. Cow pea plants treated with 75 mM Cd showed 27% reduction in the number of leaves per plant (Table 1; Plate 1).

3.4 Leaf area

With increase in the Cd stress concentration (25 - 125 mM) in the soil induced a decline in the leaf area of cow pea plants and the deleterious effect of Cd became more severe with increasing Cd level. The reduction in leaf area was less in cow pea plants subjected to 25 and 50 mM Cd stress (11 and 19% respectively) as compared to control plants and it was maximum in plants subjected to 100 and 125 mM Cd (40 and 53% respectively) as compared to control plants. Moreover, cow pea plants treated with 75 mM Cd showed 28% reduction in leaf area as compared to control plants (Table 1; Plate 1).

3.5 Fresh weight

Cow pea plants subjected to 25 mM Cd stress showed only negligible reduction in fresh weight as compared to control plants. Likewise, fresh weight of cow pea plants subjected to 75, 100 and 125 mM cadmium was highly decreased (15, 18 and 21%, respectively) as compared to untreated plants. About 8% reduction in fresh weight of cow pea plants were noticed upon exposure to 50 mM cadmium stress (Table 1).

3.6 Dry weight

Dry weight of Cd stressed cow pea plants (25, 50, 75, 100 and 125 mM) were decreased by Cd treatments compared with the control plants. Compared to control plants, maximum decrease of dry weight was recorded in the plants treated with 75, 100 and 125 mM (50-55%) as compared to other treatments of Cd. Whereas, 50 mM Cd induced reduction in dry weight was less (20%) as compared to untreated cow pea plants. Moreover, 35% reduction in dry weight was recorded when subjected to 50 mM heavy metal stress in cow pea plants (Table 1).

3.7 Tolerance index (TI)

There were significant differences in tolerance index (TI) in cow pea plants exposed to different Cd concentrations. Compared with the control, the TI changed little when cow pea plants were treated with 25 mM Cd, while TI was decreased obviously after 50, 75, 100 and 125 mM Cd treatments (10, 20, 30 and 41% respectively) as compared to untreated cow pea plants (Table 1).

Table 1: Shoot length (cm), root length (cm), number of leaves, leaf area (cm^2), fresh weight (g), dry weight (g) and tolerance index (%) of cow pea plants subjected to different concentrations of $CdCl_2$ (0, 25, 50, 75, 100 and 125 mM).

Treatments	Shoot length (cm)	Root length (cm)	Number of leaves	Leaf area (cm^2)	Fresh weight (g)	Dry weight (g)	Tolerance index (%)
Control	30.30	9.96	11	33.20	16.70	1.76	100
25 mM	28.83	9.76	9	29.50	16.25	1.15	97.99
50 mM	28.40	8.96	9	26.60	15.31	1.41	89.97
75 mM	27.43	7.93	8	23.80	14.21	0.90	79.54
100 mM	25.83	6.93	6	19.80	13.58	0.83	69.50
125 mM	23.73	5.80	5	15.40	13.21	0.79	58.17

Plate 1: Effects of different concentrations of $CdCl_2$ (0, 25, 50, 75, 100 and 125 mM) in cow pea seedlings.

4. **Discussion**

The results showed that the plant growth characteristics were adversely influenced by application of Cd in 10 days old cow pea seedlings. Leaf morphological characteristics are among those traits that are very sensitive stress condition particularly to heavy metals toxicity is known to reduce leaf chlorophyll index, leaf area expansion, greenness, and many other leaf metabolic processes [7]. Higher levels of Cd reduced leaf area in cow pea plants probably due to restriction of cell division and cell expansion. The reduction in major nutrient element such as potassium and nitrate could also result in reduced leaf area due to heavy metal application [8]. Moreover, sensitivity of various enzymes in chlorophyll biosynthesis process is probably involved in reduction of reduced leaf greenness under heavy metal toxicity [7].

In the present study, the application of heavy metals has been shown to reduce many plant growth parameters including shoot length, root length, leaf area, number of leaves, fresh weight, dry weight and tolerance index when compared to control plants. Cadmium is the major heavy metal with toxic effect on many biological systems. The Cd toxicity can have adverse effects on membranes function, enzyme activity that generally resulting harmful oxidative stress [9]. Even a single application or limited amounts of Cdm can cause considerable growth reduction. Plants can resist the heavy metal stress through the synthesis of various enzymatic antioxidants, non-enzymatic antioxidants, osmolytes and chelating agents. Since Cd can accumulate in plants and enter human body through food chain, causing persistent poisoning and endangering human health, it is of essential to find out appropriate control measures for alleviating these toxic effects. In this study, by the treatment of different levels of $CdCl_2$, an optimum concentration of cadmium which can cause an average range of heavy metal toxicity before killing the plant is observed and that toxicity can be mitigated through appropriate control measures.

5. Conclusion

By evaluating these parameters in 10 days old seedlings, It was observed that cadmium stress severely affect the plant growth and thus affects the biomass which can lead to plant death. The higher concentrations of cadmium lead to significant alterations in the normal plant growth thereby causing chlorosis, leaf rolls, necrosis and stunting as visible symptoms. The seedling treated with 125 mM showed extreme leaf curling, chlorosis, wilting and overall deformities of the plant and ultimately the death of plant. It helped to fix concentration of 50 mM of cadmium which can cause an average range of heavy metal toxicity and that can be reduced using appropriate control measures.

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7. **References**

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