The seedlings of *Cicer arietinum*, grown in sand culture, were supplied with 0, 50, 100 or 150 µM of cadmium in the form of cadmium chloride at 5, 15 or 25 day stage and assessed for specific characteristics, 10 days after each treatment. It was a general observation in all the samplings that length, fresh and dry mass of both root and shoot and activity of carbonic anhydrase in leaf decreased, whereas proline content both in leaf and root increased. The intensity of the damage was proportionate with the concentration of the metal but was prominent at the earliest stage of growth (i.e. 5 days). Values for all the observed parameters increased as the growth of the seedling progressed.

Key Words: Carbonic anhydrase, chickpea, proline

Introduction

Heavy metal belongs to major pollutants that are accumulated in the environment. However, the accumulation in the soil has become a worldwide problem leading to the loss of agricultural productivity. Cadmium (Cd) is a toxic trace pollutant for humans, animals and plants, which enters the environment mainly from industrial processes and phosphate fertilizers and then is transferred to the food chain (1). Cd is strongly phytotoxic and causes growth inhibition and even plant death. Cd produces alteration in various physiological processes that include growth retardation, inhibition of enzymes, and altered stomatal action (2-3). Photosynthesis is also sensitive to Cd, chlorophyll being one of the targets (4), as well as the enzyme involved in CO₂ fixation (5).

In this work, the effect of growing chickpea plants with cadmium chloride (CdCl₂) on growth and carbonic anhydrase (CA) activity was studied in order to know the possible involvement of Cd in growth. Attempts were also made to determine the stage at which supply of metal may be highly injurious.

Materials and Methods

The seeds of chickpea (*Cicer arietinum* L.) cv. Uday were obtained from National Seed Corporation Ltd., New Delhi. The healthy seeds were surface sterilized with 0.01% mercuric chloride (HgCl₂) followed by repeated washing with double distilled water (DDW). The surface sterilized seeds were inoculated with specific *Rhizobium*. The seeds were sown in plastic cups (5 cm diameter) filled with acid washed sand. The plants were supplied with (20 cm³) Hoagland nutrient solution daily. The composition of the nutrient solution was macronutrients [m mol/L]: 2.0 Ca(NO₃)₂, 1.5 KNO₃, 1.0 Mg(NO₃)₂, 1.0 KH₂PO₄, 0.5 MgSO₄ and micronutrients [m mol/L element]: 2.5 Fe (EDTA), 1.0 MnSO₄, 0.2 CuSO₄, 0.4 ZnSO₄, 0.2 MoO₃, 0.25 H₃BO₃. The seedlings of *Cicer arietinum*, grown in sand culture, were supplied with 0, 50, 100, 150 µM of Cd, in the form of CdCl₂, through the nutrient solution at 5, 15 or 25 days after sowing (DAS) and assessed for the length, fresh and dry mass of root and shoot, leaf CA activity and proline content in leaves and roots at 10 days after each treatment. The CA activity was measured by the method of Dwivedi and Randhawa (6) and proline content by adopting the method of Bates et al. (7). The data was statistically analyzed using SPSS package.

Results

The length of root and the fresh and dry mass of root and shoot increased as the growth progressed (Figures 1-2). However, the supply of the Cd decreased the values of
all the above parameters. Moreover, it was observed that the damage was most prominent in the plants that received the Cd at the earliest stage of growth (i.e. 5 days).

The activity of CA increased as the growth progressed from 5 to 25 days (Figure 2c). The supply of Cd decreased the values of CA, in proportion to the concentration of metal. The presence of Cd at the maximum concentration (150 µM) was the most inhibitory. The time of application of Cd also affected the CA activity, in order of 5 > 10 > 15.

The proline content in the roots and leaves (Figure 3) increased as the plant growth progressed. The levels

Figure 1. Effect of cadmium on the length (a), fresh (b) and dry mass (c) of root in *Cicer arietinum*.

Figure 2. Effect of cadmium on the fresh (a) and dry mass (b) of shoot and CA activity (c) in *Cicer arietinum*. 
were further elevated in the presence of Cd, and the increase in the level due to Cd was more prominent in the root as compared to leaves.

Discussion

The present study reveals that the presence of Cd had an adverse effect on root growth, length, and fresh and dry mass (Figures 1-2). In most of the environmental conditions, Cd first enters the roots, and consequently they are likely to experience Cd damage first (8). Cd easily penetrates the root through the cortical tissue and reaches the xylem through an apoplastic and/or a symplastic pathway (9), complexed by several ligands such as organic acids and/or phytochelatins (9). Normally, Cd ions are retained in the roots and only small amounts are transported to the shoots (10). The inhibitory effect of Cd ions on root elongation is mediated through altered cell growth. Cd in cells gets associated with cell walls and middle lamella and increases the cross-linking between the cell wall components, resulting in the inhibition of the cell expansion growth (11). Moreover, Cd also alters the water relation in plants, causing a physiological drought (2) and causes metabolic dysfunctions such as production of reactive oxygen species (12), photosynthesis (13-14), and nutrient uptake (15). These and other such altered processes lead to the decrease in the length and fresh and dry mass of the plants subjected to Cd stress (Figures 1-2).

The presence of Cd in the growing medium caused a dramatic decrease in the CA activity in proportion to the concentration of metal as compared to that of control (Figure 2c). This may be the consequence of a general inhibitory effect of Cd on enzyme activities and alteration of their structure. Moreover, Cd causes the stomatal closure (11), therefore, decreasing CO₂ concentration, which is the requisite factor in regulation of CA activity (16). Therefore, the activity of CA in Cd-supplied plants was lower than that of the control. The earlier observation made by Siedlecka et al. (17) also favors our results.

The Cd stress also resulted in an increase in the level of proline both in the leaves and root of chickpea (Figure 3). The accumulation of free proline in response to heavy metal exposure is widespread among plants (8,18-19). The accumulation of proline is a gene-regulated process which is the consequence of the overexpression of the genes involved in its biosynthesis and depression of those responsible for its degradation, in the plants under stress (20). The increase in the level of proline in the present study seems to be mediated through water stress or a physiological drought generated by Cd (2). However, Kastori et al. (21) suggested that the accumulation of proline in metal-exposed plants is directly due to metal uptake, rather than to water deficit stress. It is possible that the functional significance of proline accumulation under heavy metal stress might include water balance maintenance (19), scavenging of hydroxyl radicals (22), or metal chelation (23).

The exposure of plants to Cd at the earliest stage of growth (5-day stage) resulted in a severe inhibition of all the above-mentioned parameters compared to those supplemented at the latter stages (10 or 15 days) of growth. The reason may be the interference of Cd in the metabolism of the seedlings at the juvenile stage, when the plants are in the most active stage of metabolism.
Conclusion

It may be concluded from the above study that the treatment of chickpea (*Cicer arietinum*) by cadmium (50, 100 or 150 µM) at different stage of growth (5, 10, 15 DAS) resulted in reduced growth and CA activity in the leaf, whereas the proline content both in leaf and root increased. The highest concentration and earliest day (5 DAS) of treatment with cadmium is more injurious.

References