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Effect of Cadmium on Some of the Biochemical and Physiological Processes in Bean Plants

[1]Khadijeh Bavi, [2]Bahman Kholdebarin and [2]Ali Moradshahi
[1]Islamic Azad University-Kazeroon Branch, Iran
[2]Department of Biology, Shiraz University, Shiraz, Iran

Abstract: Effects of different cadmium chloride concentrations (5, 10, 20, 50 and 100 μM) on some of the biochemical and physiological processes including: proline content, Hill reaction, respiration and cadmium partitioning in bean plants (*Phaseolus vulgaris* L. cv. *Khomein*) were investigated. Proline accumulation in shoots increased significantly in the presence of 10, 20 and 50 μM cadmium chloride and was reduced in nutrient solutions containing 100 μM $CdCl_2$. Cadmium did not have any significant effect on proline accumulation in bean roots. In general, proline accumulation can be used as one of the screening tests to evaluate plants stress response to cadmium. The effect of $CdCl_2$ on reduction of 2,6- dichlorophenolindophenol (DCPIP) in the presence of light showed that the rate of DCPIP reduction in bean chloroplasts decreased by an increase in $CdCl_2$ concentrations in reaction mixture. However there was no significant difference in Hill reaction rate in bean plants exposed to low $CdCl_2$ concentration as compared to control. Respiratory O_2 consumption in bean roots was reduced in the presence of different concentrations of $CdCl_2$. Besides, cadmium partitioning showed that 90% of cadmium was retained by the roots and a lesser amount was transferred to the shoots. Restriction of cadmium transport from root to shoot along with its compartmentation in root cell vacuoles can be considered as the mechanism of cadmium tolerance in this plant.

Key words: Bean plants, cadmium effects, biochemical and physiological processes

Introduction

Heavy metal contamination, affects the biosphere in many places worldwide (Meagher, 2000). One of the problems threatening environmental safety today is the contamination of soil, water and agricultural products by heavy metals. Cadmium is a major environmental contaminant (Friberg *et al*., 1971). The toxic cadmium metal is a well known pollutant supplied to the soil by application of phosphate fertilizers, sewage sludge, manure and lime (Greger *et al*., 1993). It is then transferred to the food chain (Wagner, 1993).

Cadmium has a great mobility in soil as compared with other heavy metals (Greger *et al*., 1991) and is easily taken up by roots and is translocated to different plant parts (Cho and Kim, 2003). The uptake of heavy metals by plants is controlled by both soil and plant factors (Costa and Morel, 1993). Cadmium causes perturbations in various plant processes such as photosynthesis, sulfate assimilation and respiration (Balestrasse *et al*., 2003). Plant growth inhibition and alteration in metabolism are mainly due to direct effects of cadmium on various enzymes activities and on growth processes (Chen and Kao, 1995). The selective accumulation of intracellular amino acids often occurs in response to environmental stresses such as drought, flooding, low temperature, or salinity. Higher plants

Corresponding Author: Khadijeh Bavi, Islamic Azad University-Kazeroon Branch, Iran

preferentially accumulate proline under such stress conditions (Antolin and Sanchez-Diaz, 1992). A considerable increase in proline accumulation has been recorded with the increase in concentration of heavy metals in plants (Saradhi and Saradhi, 1991).

Bean plant has been chosen for this study because of its economical importance as a source of protein in human diet. The purpose of this study was to study the effect of excess cadmium uptake by bean plants on: (a) respiration, (b) proline accumulation, (c) cadmium partitioning in shoot and root tissues and (d) Hill reaction activity in chloroplasts.

Materials and Methods

Plant Material and Growth Conditions

Bean seeds (*Phaseolus vulgaris* L. cv. Khomein) were obtained from Agricultural Research Institute in Khomein. The seeds were surface sterilized with 10% sodium hypochlorite for 15 min and then rinsed extensively in distilled water. Seeds were germinated on moist vermiculite. After 10 days individual seedlings were transferred to an aerated full strength nutrient solution. The Hoagland solution pH was adjusted to 5.0 by 0.1N HCl and 0.1N NaOH. After 3 days, Cd was added to the nutrient solution as $CdCl_2$ in a final concentrations of 5, 10, 20, 50 and 100 μM. Plants were kept in growth chamber set at 16/8 h light/dark cycles, 55% RH and day/night temperature of 25/20°C, under 9000 Lux fluorescent lights. Each experiment was repeated three times and three replicates were used for each treatment.

Respiration Studies

One g of root tissue was placed in 3 mL phosphate buffer (0.05 M), pH 5.5 in a Warburg flask and respiration rate was recorded at different time intervals.

Proline Determination

Proline was determined by the method of Bates *et al.* (1973) approximately 0.5 g of plant material was homogenized in 10 mL of 3% aqueous sulphosalicylic acid. The homogenate was centrifuged at 10,000 g for 15 min and the supernatant was used for proline determination. Standard curves were constructed, using known concentrations of proline.

Cadmium Determination

Plants were exposed to different concentrations of Cd for 7 days and were then harvested. Shoots and roots were separated for Cd assay. Shoots and roots were dried at 75°C for 24 h. In order to ash the tissues, 1 g of the dried roots and shoots were placed in a crucible and then ashed in a furnace set at 550°C. The ashes were digested for 4 h in 4 mL concentrated HNO_3 and then another 6 mL portion of HNO_3 was added making the total volume of 10 mL. The material was further digested in 3 mL $HClO_4$ until a clear solution was obtained. The solution was filtered and was made up to 100 mL with distilled water. Atomic absorption spectrophotometry was used for Cd assay.

Hill Reaction

Hill reaction was studied using 100 μM DCPIP (2,6- dichlorphenolindophenol) as an electron acceptor. Light from a projector 1amp (500 W) was used as a source of light for Hill reaction study and the rate of DCPIP reduction was determined spectrophotometrically.

Results

Root tissues of 21-days-old bean plants which had been exposed to different concentrations (0, 5, 10, 20, 50 and 100 μM) of $CdCl_2$ for 7 days, showed a marked decrease in respiration rate. The

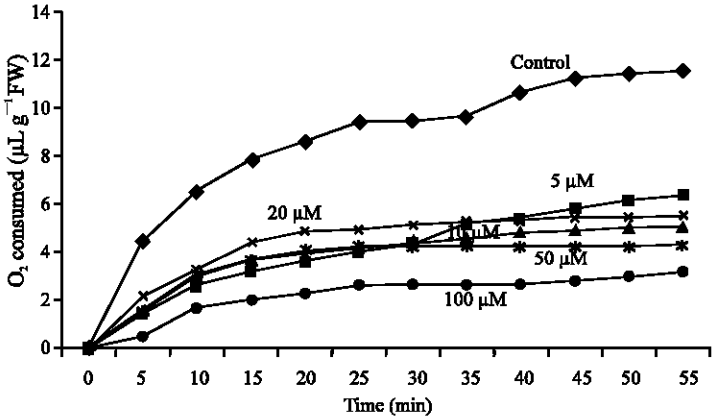

Fig. 1: Effect of $CdCl_2$ on O_2 consumption in roots of *Phaseolus vulgaris* grown on nutrient solution with different Cd concentrations for 7 days

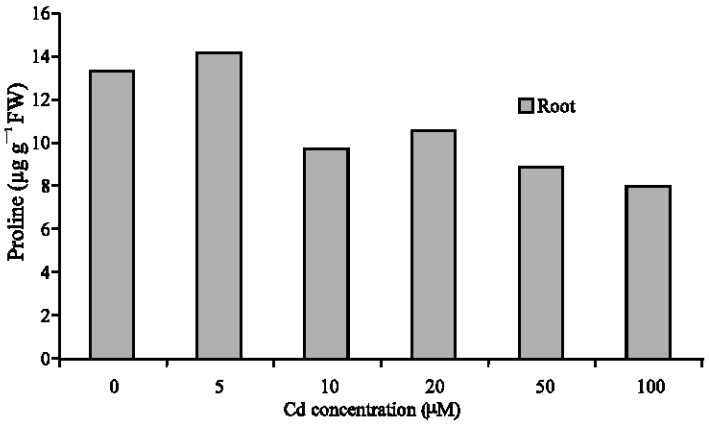

Fig. 2: Proline content of roots of *Phaseolus vulgaris* obtained in nutrient solutions containing $CdCl_2$ (0, 5, 10, 20, 50 and 100 µM) for 7 days. Data are the average of 4 replicates

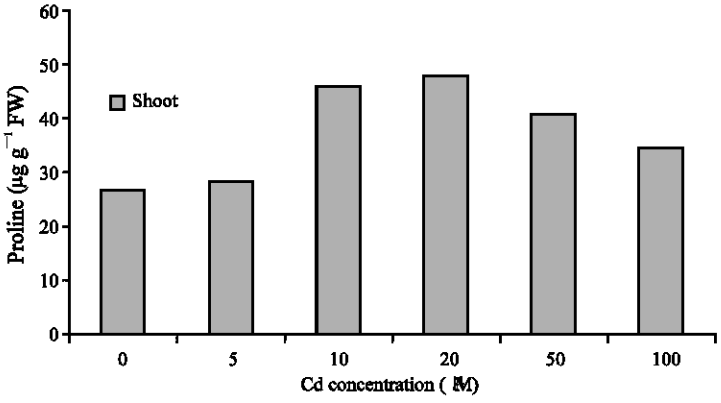

Fig. 3: Proline content of shoots of *Phaseolus vulgaris* obtained in nutrient solutions containing $CdCl_2$ (0, 5, 10, 20, 50 and 100 µM) for 7 days. Data are the average of 4 replicates

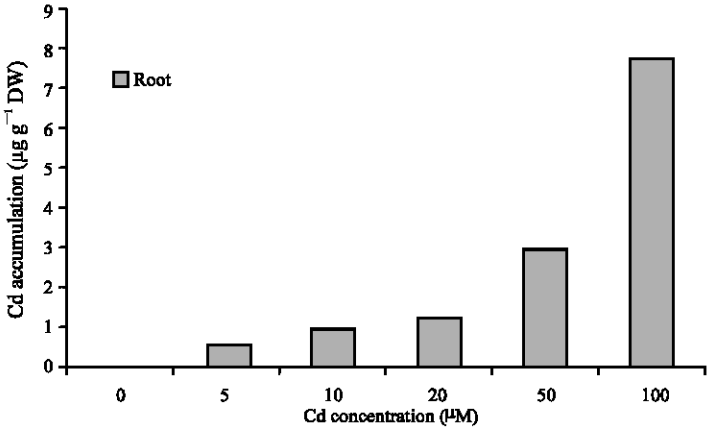

Fig. 4: Cadmium accumulation in roots of 21-days-old bean plants grown in nutrient solutions at different levels of Cd supply. Cadmium was added 7 days before harvest

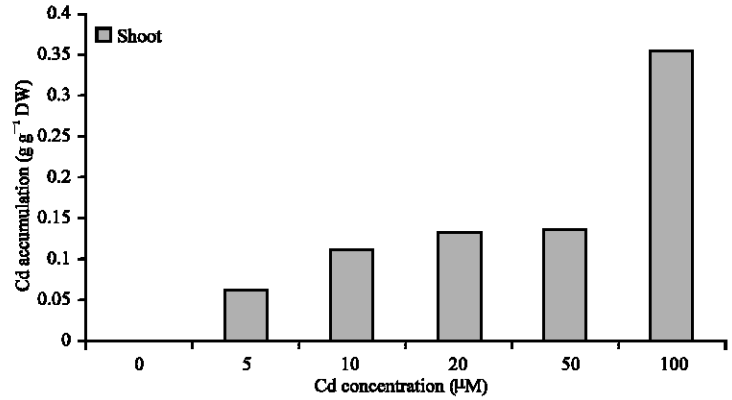

Fig. 5: Cadmium accumulation in shoots of 21-days-old bean plants grown in nutrient solutions at different levels of Cd supply. Cadmium was added 7 days before harvest

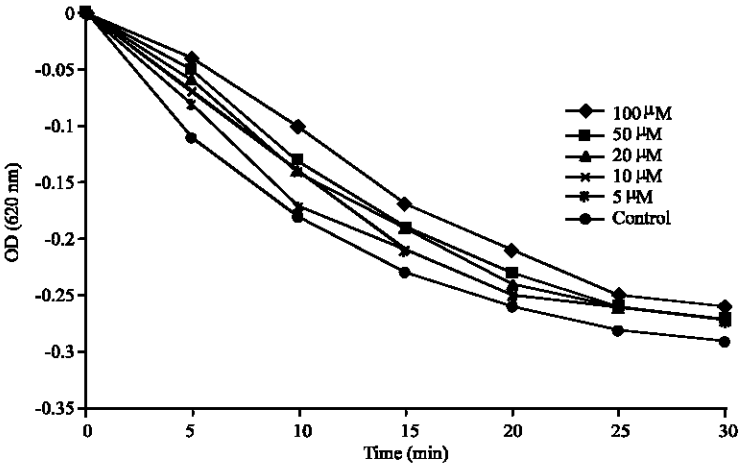

Fig. 6: Hill reaction rate in isolated chloroplasts of bean leaves as affected by different concentrations of Cd in reaction mixture

respiration rate in root segments of plants treated with 5 μM $CdCl_2$ decreased by 45% as compared to control. In plants treated with 50 and 100 μM $CdCl_2$, their roots respiration rate was decreased by 62.7 and 72.3%, respectively (Fig. 1).

In present study, proline content in roots of treated plants was not affected by $CdCl_2$. In the presence of 50 and 100 μM $CdCl_2$ there was a reduction in the amount of proline as compared to control. However the decrease was not significant (Fig. 2).

Cadmium treatment caused an increase in proline content in shoots (Fig. 3). Proline accumulation in bean shoots increased significantly in the presence of 10, 20 and 50 μM $CdCl_2$ and was reduced in plants grown in nutrient solutions containing 100 μM $CdCl_2$.

As shown in Fig. 4 and 5, the Cd content of both shoots and roots increased with increasing concentrations of $CdCl_2$ in the nutrient solution. Low rate of Cd translocation from roots to shoots was observed. Cd accumulation was higher in bean roots as compared to shoots. Both roots and shoots showed a decrease in growth rate with the increase in Cd treatment (data are not shown).

Effect of $CdCl_2$ on photosynthetic reduction of 2,6- dichlorophenolindophenol (DCPIP) showed that DCPIP reduction in bean chloroplasts was reduced by an increase in $CdCl_2$ concentration in reaction mixture. Plants grown in 100 μM $CdCl_2$ solution showed a marked inhibition in DCPIP reduction (Fig. 6). In other cases, although in chloroplasts of Cd-treated bean plants DCPIP reduction was reduced, the difference was not significant as compared to control.

Discussion

Respiratory O_2 consumption in bean roots was reduced in the presence of different concentrations of $CdCl_2$. The reduced root respiration rate could cause a decrease in metabolic dependent processes and thus contributing to reduced growth rate (Greger *et al*., 1991). In agreement with our results, Keck (1978) has reported a decrease in oat respiration rate and ATP production in the presence of 1 mM $CdSO_4$.

Cadmium did not have any significant effect on proline accumulation in bean roots. Cadmium treatment caused an increase in proline content in shoots. In *Mesembryanthemum crystallinum*, copper stressed plants did not show any proline accumulation. This lack of proline accumulation in cu-treated plants was proposed to be either due to inhibitory effects of copper on proline biosynthesis or to a concurrent proline breakdown (Thomas *et al*., 1998).

Proline may be produced at the cost of substances needed for developmental processes (Saradhi and Saradhi, 1991). The enhancement of proline level can be used as an indicator of the species tolerance to copper and cadmium toxicity (Wu *et al*., 1995). Chen and Kao (1995) showed that proline accumulation can be induced in rice roots by treating seedlings with Cd. Proline has been reported to play important roles in: osmoregulation, protecting enzyme denaturation, acting as a source of carbon and nitrogen, stabilizing the machinery of protein cynthesis, regulating the cytosolic acidity and/or scavenging hydroxyl radicals (Saradhi and Saradhi, 1991). An increase in proline level results either from an increased synthesis rate or from a lowered turnover rate of intracellular proline (Wu *et al*., 1995).

Proline accumulation could probably also be related to a decrease in electron transport in plants under stress conditions (Venekamp, 1989). As a result there would be an accumulation of NADH and H^+. An increase in NADH might even affect substrate level phosphorylation besides inhibiting important metabolic reactions that need NAD^+. Proline synthesis from glutamic acid might be an adaptive mechanism to reduce the accumulation of NADH and/or reduce the acidity (Venekamp, 1989; Saradhi and Saradhi, 1991). Since growth is generally thought to be an energy-requiring process, elevated proline level induced by Cd is most likely acting as a way to save energy by inhibiting root growth (Chen and Kao, 1995).

The present study showed that bean plants take up Cd and about 90% of the total cadmium was retained by the roots and a lesser amount was transported to the shoots. It is generally thought that roots act as a barrier, restricting the movement of heavy metals through the soil- plant system (Sameni *et al*., 1987; Weigel and Jager, 1980).

Plants may resist a high levels of heavy metals by limiting the entry of toxic metals into the cells and/or reduce the activity of metals in the cytoplasm by complexing with peptides. Also, excretion of metals out of the root cell may occur (Costa and Morel, 1993).

Hart *et al*. (1998) have reported that reduced movement of Cd to shoots in durum wheat cultivar as compared with the bread wheat cultivar indicated that Cd was retained in the roots, perhaps by a mechanism involving Cd sequestration or its decreased xylem loading. Various mechanisms are suggested for Cd accumulation by plants when exposed to low levels of this metal. These include: metal binding in cell walls, chelatin in the cytosol and transport of free ion into the vacuole followed by sequestration with organic acids and other ligands (Wagner, 1993). Translocation of Cd from root to shoot can be driven by transpiration, as reported for *Brassica juncea* (Chardonneus *et al*., 1998) and Indian mustard (Hart *et al*., 1998).

Harmful effects produced by Cd might be explained by its ability to inactivate enzymes possibly by reacting with SH- groups of proteins (Veselov *et al*., 2003). Cellular sequestration of Cd can have a large effect on the levels of free Cd in the symplast and, thus can potentially influence the movement of Cd throughout the plant. Ionic (Cd^{2+}) cadmium concentrations in the cytosol can be regulated by two processes: (1) Cd^{2+} binding to phytochelatins and (2) compartmentation in vacuole (Hart *et al*., 1998). Vacuolar storage of cadmium plays an important role in the mechanism of cadmium tolerance in *Silene vulgaris* (Chardonneus *et al*., 1998). Cadmium localization was greater in bean roots than shoots, suggesting low mobility of this metal in bean plant. Restriction of cadmium transport from root to shoot along with its compartmentation in root cell vacuoles can be considered as the mechanisms of cadmium tolerance in this plant.

The effect of $CdCl_2$ on reduction of DCPIP in the presence of light showed that the rate of DCPIP reduction in bean chloroplasts decreased by an increase in $CdCl_2$ concentrations in reaction mixture. Excised leaves of silver maple when exposed to various concentrations of Cd^{2+} exhibited reduced net photosynthetic rates (Lamoreaux and Chaney, 1978). Strong inhibitory effects of Cd on both photosynthesis and transpiration has also been suggested to be due to stomatal closure by cadmium treatment (Bazzaz *et al*., 1974). In isolated chloroplasts, cadmium treatment inhibited photosystem II activities, whereas those of photosystem I appeared to be insensitive to Cd treatment. Cadmium inhibits net photosynthesis by increasing both stomatal and mesophyll resistance to carbon dioxide uptake (Lamoreax and Chaney, 1978). Cadmium has been shown to inhibit photosynthetic electron transport in isolated chloroplasts. This inhibition seems to be at the oxygen- evolving site of photosystem II (Bazynski *et al*., 1980).

Baszynzki *et al*. (1980) reported that tomato plants grown in nutrient solution containing cadmium exhibited reduced rate of photosynthesis and also reduced amount of both chlorophyll and accessory pigments. Maize plants treated with high Cd concentrations showed toxicity symptoms such as leaf bleaching, ultrastructural alterations of chloroplasts and lowering of photosynthetic activity (Rascio *et al*., 1993). Cadmium, markedly decreased ferredoxin dependent $NADP^+$ photoreduction, while it had no effect on electron transport from 2,6- dichlorophenolindophenol, to methyl viologen, indicating that cadmium interfered with electron transport on reducing side of photosystem I (Siedlecka and Baszynski, 1993).

In this study isolated chloroplasts of 21 days old bean plants grown in nutrient solution containing different concentrations of cadmium did not show a significant change in the rate of Hill reaction. This indicates that during the 7 days of treatment any cadmium ions taken up by plants may have been localized in root cell vacuoles thus have been kept away from photosynthetic sites, protecting photosynthesis from the harmful effects of cadmium.

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