Effects of cadmium on ammonia assimilating enzymes and ultrastructures of leaf and nodule in mungbean *Vigna radiata* L. Wilczek

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Received 5 July 1998; revised 24 August 1999.

Mungbean plants (*Vigna radiata*) were grown in earthen pots for three weeks. Cadmium sulphate (CdSO₄.8H₂O) were foliarily applied at the concentrations of 40, 60, 80 and 100 μM at weekly intervals. The activities of ammonia assimilating enzymes, viz., glutamate dehydrogenase, glutamine synthetase and glutamate oxoglutarate aminotransferase were inhibited to variable extents in the root nodules. Of these enzymes, GDH and GOGAT registered maximum decline. The fine structure of chloroplasts in cadmium-treated plants were degenerated similar to the senescing leaves. The principal symptoms of cadmium action were the presence of osmiophilic plastoglobuli and a disorganisation of the lamellar structures, mainly granal stacks. In Cd²⁺-treated nodules, the peribacteroid membranes around the bacteroids seem to degenerate. Probably considerable amount of poly-β-hydroxybutyrate granules inside the bacteroids renders the root nodules ineffective. Uptake of cadmium in different plant parts and Cd²⁺ content in soil increased with the increasing concentrations of cadmium.

Germination and elongation of pea radicles were significantly inhibited when Cd²⁺ was added to the culture solutions. The heavy metals also retarded shoot elongation and leaf development of young seedlings, even at a concentration of 1 μg/ml. In addition to chlorosis and leaf wilting, Cd²⁺ caused severe stem constriction. This observation together with the ultrastructure studies on the vascular system indicate that Cd²⁺ restricts growth through narrowing of vessel pits and deposition of unknown debris which block the water translocation flow. The aim of the present work was to determine the effects of Cd²⁺ on the activity of ammonia assimilating enzymes, viz., GDH, GS and GOGAT in root nodules, leaf and nodule anatomy, Cd²⁺ content in different parts of the mungbean plant and soil. Estimation were done by Atomic Absorption Spectrophotometer.

**Materials and Methods**

*Plant Material*—Seeds of mungbean (*Vigna radiata* L. Wilczek, cultivar B-105) were obtained...
from Oil and Pulse Research Institute, Berhampore, West Bengal. These were sown in pots after surface sterilization with 0.01% mercuric chloride solution for 5 min. When the seedlings were 21 days old, the leaves were sprayed with different concentrations of cadmium sulphate, viz. 40, 60, 80 and 100 μM. One control set, sprayed with distilled water, was maintained throughout the experimental studies. Spraying was done once a week until grain filling was over. The results presented are the averages of three separate experiments.

**Assay of ammonia assimilatory enzymes**—The enzyme activities of glutamate dehydrogenase (GDH), glutamine synthetase (GS) and glutamate oxoglutarate aminotransferase (GOGAT) were estimated in root nodules of mungbean plant at 35 DAS. Tissue extract of freshly harvested nodules were prepared at 4°C in 100 mM Tris-HCl buffer, pH 7.5, containing 1 mM disodium-ethylenediamine tetracate (EDTA), 1% (w/v) polyvinylpolypyrrolidone (PVPP), 1 mM dithiothreitol (DTT), 5 mM cysteine and 1% bovine serum albumin (BSA). Supernatant after cold centrifugation was used for estimating the enzyme activity. The activity of glutamate dehydrogenase, glutamine synthetase and glutamate oxoglutarate amino transferase were assayed according to the methods of Lea and Miflin, Elliott, Sodek and Da Silva respectively. Soluble protein content of tissue extract was estimated by the method of Lowry et al.

**Transmission Electron Microscopy**—For Transmission Electron Microscopy, the second leaf and pink colored nodules were the experimental materials. The nodules were sliced and leaf segments covering an area between the tip and base and halfway between margin and midrib were taken. The sliced segments could facilitate the penetration of the fixative-4% glutaraldehyde in 0.05 M phosphate buffer (pH 7.2). After 4 hours of fixation at room temperature the segments were washed thrice (15 minutes each) with the same buffer and post-fixed for 2 hr in buffered 1% osmium tetroxide. The specimens were dehydrated through a graded ethanol series (30 to 100% ethanol : 10 min for each bath) and left overnight in 100% ethanol. The segments were then washed twice 20 min each with propylene oxide prior to infiltration with a graded Spurr's epoxy resin for 24 hr. The tissues were transferred to fresh resin and embedded at 60°C for 24 hr. Ultrathin sections (500 to 700 A) were cut with a knife in a LKB ultramicrotome (Type 4800 A). A ribbon of 4-10 sections were mounted on uncoated copper grids and stained for 30 min in a saturated solution of uranyl acetate in 50% ethanol followed by 7 min staining in 0.02% lead citrate. The sections were observed in Philips EM 400 Transmission Electron Microscope.

**Cadmium content by Atomic Absorption Spectrophotometer**—The cadmium concentration was determined according to standard method using AA-575 atomic absorption spectrophotometer at wavelength of 228.8 A. The cadmium content was expressed as μg cadmium per g dry weight.

**Results and Discussion**

**Enzymatic changes**—Foliar application of Cd²⁺ showed inhibitory effect on the activity of ammonia assimilating enzymes (GDH, GS and GOGAT). About 80% inhibition (Fig.1A) in GDH activity from control was noticed at the lowest concentration (40 μM). The highest Cd²⁺ level, viz. 100 μM, caused about 89% reduction in the activity of GDH (Fig. 1A). Decreased GDH activity means that there is decline in direct amination of 2-oxoglutarate which ultimately leads to inhibition in glutamate formation.

GS activity was also inhibited by cadmium (Fig. 1B). The minimum concentration tested, 40 μM, caused about 49% reduction in the activity of GS from control. The activity of GS decreased with the increasing concentrations of cadmium sulphate at 100 μM, the highest level of cadmium sulphate tested, the GS activity reduced to 73%. Inhibitory effect of Cd²⁺ on GS activity serves to indicate less incorporation of ammonia and glutamine formation suffers a decline.

Similar to GDH and GS, GOGAT activity also showed markedly decreasing trend proportional to cadmium sulphate concentrations up to 100 μM at which the GOGAT activity showed maximum decline (Fig. 1C). At 40 μM, the lowest dose tested, about 72% inhibition was noticed. At 100 μM, the highest dose tested, the activity was reduced by about 81%. GS and GOGAT mainly constitute the principal route of ammonia assimilation in higher plants. Since these enzymes (GS and GOGAT) are dependent on each other for the provision of substrate, their activities constitute a cycle of glutamate synthase cycle. It appears that this cycle is profoundly affected by Cd²⁺. In this way, reduced activities of ammonia assimilating enzymes (GDH, GS and GOGAT) in cadmium treated nodules indirectly affect the nitrogen status of the plant.
Ultrastructural changes

Leaves—Chloroplasts from higher plants are made of six different compartments (the outer and inner envelope membranes, the intermembrane space, the stroma, thylakoid membranes and the thylakoid lumen). Each of them has its own function and a distinct set of polypeptides.

The ultrastructural study of leaf using Transmission Electron Microscope shows closely packed thylakoids in control chloroplasts (Fig 2,4A). But such packed thylakoids, however, seem to be disorganised to the level of lamellar structure in cadmium-treated leaves (Fig. 3,4B). This lamellar breakdown, revealed by unstacking of grana thylakoids, was followed by the formation of loose and elongated lamellae scattered in the stroma. Presence of electron dense vesicles i.e. osmiophilic plastoglobuli were observed in the cytoplasm of cadmium-treated leaves, which were not detected in control chloroplasts. These osmiophilic plastoglobuli served as reservoir for the sequestration of metal ions in the cytoplasm. Clusters and fine granules of electron dense metal precipitates were also observed throughout the entire side walls of the cadmium-treated leaves.

Drastic reduction in total chlorophyll, chlorophyll-\(a\) and chlorophyll-\(b\) contents was noted in Cd\(^{2+}\)-treated leaves. It also decreased the reducing power of chloroplasts to variable extent\(^{15}\). Chlorophyllase activity showed an increase under cadmium sulphate treatments in mungbean plants\(^{23}\). As compared to control, Cd\(^{2+}\)-treated mungbean plants showed inhibitory effects on sugar (total and reducing), nitrogen, amino acid and nucleic acid (DNA and RNA) contents\(^{25}\).

Therefore, Cd\(^{2+}\) affects the photosynthetic apparatus of the plant and leads to a decrease in the content

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**Table 1—Absorption of cadmium (µg/g dry material) by different parts of mungbean plant (age 56 days) and soil.**

<table>
<thead>
<tr>
<th>Set</th>
<th>Control</th>
<th>Cadmium sulphate, µM</th>
<th>40</th>
<th>60</th>
<th>80</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil (before spray)</td>
<td>ml</td>
<td>4.65 ± 0.03</td>
<td>4.65 ± 0.03</td>
<td>4.65 ± 0.03</td>
<td>4.65 ± 0.03</td>
<td>4.65 ± 0.03</td>
</tr>
<tr>
<td>Soil (after five spray)</td>
<td>ml</td>
<td>4.65 ± 0.03</td>
<td>4.65 ± 0.03</td>
<td>4.65 ± 0.03</td>
<td>4.65 ± 0.03</td>
<td>4.65 ± 0.03</td>
</tr>
<tr>
<td>Leaf</td>
<td>ml</td>
<td>2.48 ± 0.07</td>
<td>2.48 ± 0.07</td>
<td>2.48 ± 0.07</td>
<td>2.48 ± 0.07</td>
<td>2.48 ± 0.07</td>
</tr>
<tr>
<td>Root</td>
<td>ml</td>
<td>3.99 ± 0.10</td>
<td>3.99 ± 0.10</td>
<td>3.99 ± 0.10</td>
<td>3.99 ± 0.10</td>
<td>3.99 ± 0.10</td>
</tr>
<tr>
<td>Nodule</td>
<td>ml</td>
<td>2.55 ± 0.06</td>
<td>2.55 ± 0.06</td>
<td>2.55 ± 0.06</td>
<td>2.55 ± 0.06</td>
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of plastidial pigments and in net photosynthesis together with inhibition of some steps of the photosynthetic electron transport chain.

Maksymiec and Baszynski reported that cadmium induces release of polypeptides from the thylakoid membrane of tomato leaves, which depends on the concentration of the metal and time of incubation. Malik et al. reported that Cd\textsuperscript{2+} causes compositional changes in the thylakoid membrane of wheat leaves which might be responsible for reduced PSII activity and a reduced rate of photosynthesis.

**Nodules:** An important feature of *Rhizobium*-legume symbiosis lies in the presence of PBM (peribacteroid membrane), derived from the root cell plasma membrane, which effectively excludes the bacteroids from the host cell cytoplasm. Loss of PBM, renders the root nodules ineffective.

The ultrastructural study of nodule (age 40 days) by Transmission Electron Microscope (TEM) shows that cadmium-treated nodule was in the late stage of symbiosis during development as opposed to the control nodule, which was in the active stage of symbiosis.

A very distinct and intact peribacteroid membrane that enclosed individual bacteroids or a group of 2-3 together is noted in untreated control nodules (Fig. 5A). These peribacteroid membranes, however, seem to degenerate in cadmium-treated nodules (Fig. 5B). The rhizobia in control nodules contain relatively less amount of electron translucent poly-β-hydroxybutyrate (PHB) granules in comparison with considerable larger amount of PHB granules in Cd\textsuperscript{2+}-treated nodule. Furthermore, in control nodule, host cell was completely filled with bacteroids, while large vacuolization and low cytoplasmic density was found in

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Fig. 2—Transmission electron micrograph from the chloroplast of control mungbean leaf (age 40 days) × 25,000. Lens-shaped chloroplast (CH) was found to be surrounded by a bilayer membrane envelope (E) and contained dense particulate matrix stroma (St) and lamellar grana (G). A distinct cell membrane (CM), mitochondria (M) and endoplasmic reticulum (ER) were also visible.
Therefore, the presence of a prominent peribacteroid membrane (PBM) around the bacteroids (B) and less PHB (poly-β-hydroxybutyrate) granules inside them are distinctive criteria for active and functional stages of nodules as revealed in control ones. Absence of prominent PBM around the bacteroids render the root nodules ineffective as revealed in Cd²⁺-treated nodules.

The highest dose of cadmium sulphate viz. 100 μM reduces nitrogen content by 49%, inhibits the activities of nitrogenase about 78% and nitrate reductase by 64% and also reduces leghemoglobin content by 62%.

Thus cadmium sulphate treatment shows deformative nodule formation, less amount of leghemoglobin, decline in nitrogen and less utilization of combined nitrogen present in the soil through decreased activity of nitrogen assimilatory enzymes. In this way, cadmium treatment hastens the nodule senescence.

**Cadmium absorption**—Absorption of cadmium by different parts of mungbean plant and soil was recorded after 56 days of sowing by atomic absorption spectrophotometer (Table 1). The uptake of cadmium in leaves, roots, nodules and soil increased with increasing concentrations of cadmium. On the contrary, no cadmium was detected in different parts of control plants and also in control soil. The leaves of plants sprayed with 40 μM cadmium sulphate solution, absorbed about 2.40, 3.99, 2.55 and 14.65 μg cadmium per g dry weight of leaves, roots, nodules and soil respectively. As concentration of cadmium sulphate increased, there was a gradual increase in cadmium content in different parts of the plant and soil. At the highest dose of cadmium, viz., 100 μM, tested here,
Fig. 4—Transmission electron micrograph from the chloroplast of control and 100 µM cadmium sulphate treated mungbean leaf (age 40 days) × 24,000.

(A) Chloroplasts from control leaf showing prominent chloroplast envelope (E), grana (G) and stroma (St).

(B) Membranes of the chloroplast envelope (E) was not distinctly visible in cadmium-treated leaf. Loosely arranged grana (G) and stroma (St), osmiophilic plastoglobuli (O) present. Clusters and fine granules of electron dense metal precipitates (MP) were also observed throughout the entire side walls of cadmium-treated leaves.

There was about 17.04, 13.05, 12.00 and 22.66 µg cadmium per g dry weight of leaves, root, nodules and soil respectively. Cadmium content was not detected in soil which is collected before spraying. It may be inferred that foliarly applied cadmium seemed to move from the leaf to the different parts of plant and affecting adversely the plant growth.

Acknowledgement

The author is indebted to Prof. S. Mukherji for supervision. Thanks are due to Prof. P.K. Ray, Dr. V. Sundararaman and Dr. S.K. Gupta of ITRC, Lucknow, for giving permission to use the Transmission Electron Microscopy. Technical assistance received from Dr. L.K.S. Chauhan and his colleagues is acknowledged.

The assistance received from RSIC, Bose Institute, Calcutta, towards using Atomic Absorption Spectrophotometer and the financial assistance from CSIR,
Fig. 5—Transmission electron micrograph from the central tissue of control and 100µM cadmium sulphate treated mungbean nodule (age 40 days) x 15,000.

(A) Bacteroid (B) usually singly or in a group remain enclosed in intact peribacteroid membrane (PBM). Bacteroids (B) contain relatively less amount of poly-β-hydroxybutyrate (PHB) reserves within them and host cell less vacuolated (V).

(B) Bacteroids (B) in a group are present inside a disintegrating peribacteroid membrane (PBM). Bacteroids (B) contain larger amount of poly-β-hydroxybutyrate (PHB) granules and host cell was more vacuolated (V).

New Delhi, are also acknowledged.

References