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ULTRASTRUCTURAL CHANGES IN THE ROOT CELLS OF PIGEONPEA [*CAJANUS CAJAN* (L.) MILLSPAUGH] INDUCED BY LOW LEVELS OF CADMIUM IN THE MEDIUM

B. SUJATHA* AND B. PRIYADARSHINI

Department of Botany, Andhra University, Visakhapatnam-530 003

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SUMMARY

The studies on the ultrastructure of the root cells of pigeonpea [*Cajanus cajan* (L.) Millspaugh] exposed to 0.5, 1.0 and 1.5mM Cd (cadmium as $CdCl_2$) for 6 days were made. The electronmicroscopic pictures revealed that Cd increased the vacuolation, damaged the subcellular structures like membranes, organelles and cytosol. Furthermore, electron dense globules of different sizes were noticed indicating that heavy metals like Cd get compartmentalized thus perhaps providing a mechanism to prevent further damages to the root cells.

***Key words*: Cadmium, globules, pigeonpea, ultrastructure, vacuolisation.**

INTRODUCTION

Cadmium belongs to "heavy metals", is a relatively rare having no biological role, and is highly toxic to plants and animals (Alloway 1990). It is well known that Cd is released into the environment by power stations, heating systems, metal-working industries, waste incinerators, urban traffic, cement factories and by-product of phosphate fertilizers (Sanita and Gabbrielli 1999). Cd accumulation in soil and water now poses a major environmental and human health problem (Schützendübel *et al.* 2001). It was reported that Cd is accumulated by many cereals, potatoes, vegetables and fruits and that humans take up at least 70% of the Cd which originates from plant food (Wagner 1993). High levels of Cd disturb many physiological processes in plants (Krupa and Baszynski 1995, Siedlecka 1995). Roots were found to be the main organs for Cd accumulation, however, the concentrations of Cd increased with increasing Cd supply levels (Yang *et al*. 2005, Gusmao Lima *et al*. 2006).

In the present study experiments were carried out to increase our understanding of the cadmium effects on root ultrastructure and accumulation at subcellular levels in the root cells of pigeonpea and the results are presented here. The differential responses of two cultivars are also reported.

MATERIALS AND METHODS

Seeds of pigeonpea (*Cajanus cajan* (L.) Millspaugh) cv. LRG30 and cv. T21 supplied by ICRISAT, Patancheru, India were used in the present study. The seeds of uniform size and free from infection were selected for the experiments. The seeds were surface sterilized with 0.01M sodium hypochlorite for 2 min, washed thoroughly with distilled water and placed separately in trays lined with Whatman No.1 filter papers containing 0, 0.5, 1.0 and 1.5mM $CdCl_2$ (cadmium chloride: $CdCl_2$ $2.5H_20$) as variables. Seedlings raised in distilled water (zero concentration) served as controls. The seeds were allowed to germinate at 30±2°C for 6 days

*Corresponding author, E-mail:sujathaau@yahoo.co.in

under a photoperiod of 12hrs, and at 195µmol $m^{-2}s^{-1}$ PPFD. Then the seedlings were collected for electron microscopic studies.

The terminal 2mm length of the root tips from 6 day old seedlings of pigeonpea at 4°C in dark for 12h were fixed in 3% glutaraldehyde in 0.1M sodium cacodylate buffer (0.2M pH 7.4). The root tips were post fixed in a buffered solution of 1% osmium tetroxide for 1h at 4°C in dark and then washed thoroughly with 0.1M sodium cacodylate buffer. The root tips were transferred to propylene oxide for 20 min, embedded in a mixture of Araldite and toluene (1:3) initially for 1 h at 60°C and latter at room temperature for overnight. All the Araldite A was poured out, Araldite B was added and incubated for 48-72 h at 60°C. The blocks were trimmed and the ultrathin sections (600-700Å) were cut using LKB microtome. These sections were picked on formvar coated copper grids. The sections were then stained with uranyl acetate for 1h followed by lead citrate for 5-7 min, and then washed thoroughly with carbon dioxide free distilled water. Finally, the sections were examined under electron microscope (Philips CM10 at an accelerating voltage of 60KV). The electron microphotographs were taken from the cortex tissue of control and treated pigeonpea root tips.

RESULTS AND DISCUSSION

The root cells of control seedlings of cv. LRG30 and cv. T21 exhibited a well demarkated cell wall, dense cytoplasm rich in organelles especially mitochondria, endoplasmic reticulum, small vacuoles and a prominent nucleus with well developed nucleolus and homogeneously stained nucleoplasm (Fig.1 A and B). Root cells exposed to concentrations of 0.5, 1.0 and 1.5mM of cadmium revealed extensive changes in nucleus with disruption, dilation of nuclear membranes and ill defined nucleolus. Further the mitochondria showed vesicle formation with membrane disruption (Fig. 1C). In addition increased vacuolation with few electron dense globules were observed (Fig. 1D and E). However, some cells showed less dense cytoplasm and detached plasma membrane from the cell walls (Fig. 1F).

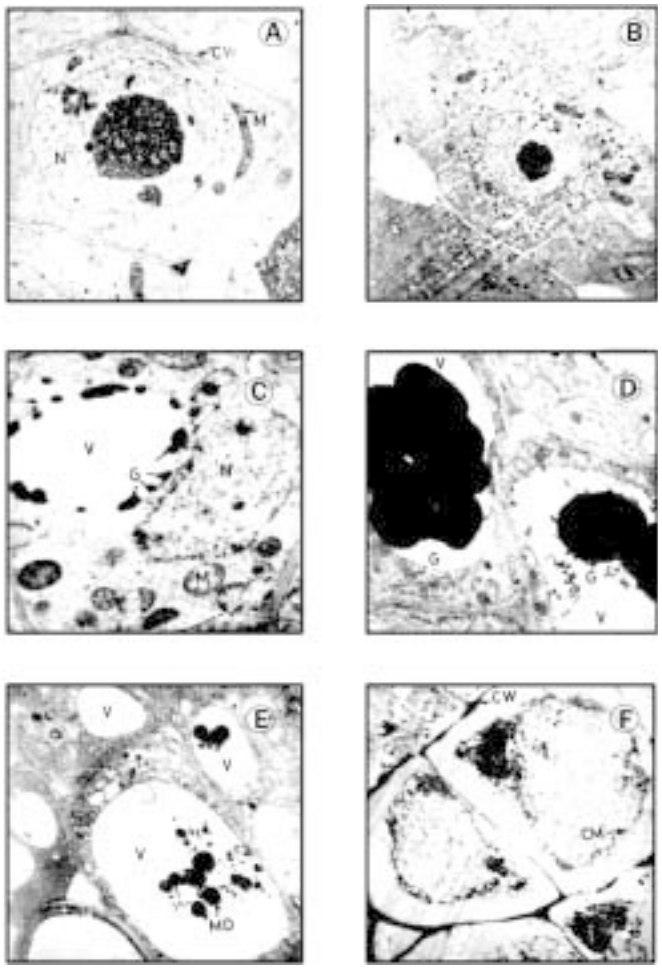

Fig. 1. Electron microphotographs of representative root cortical cells of 6-day-old pigeonpea cv. LRG30 and cv. T21, (A) LRG30: Control root cell showing dense cytoplasm with well distributed cell organelles such as endoplasmic reticulum, mitochondria and a large nucleus with well stained nucleoplasm and with conspicuous nucleolus. The control cells were surrounded by well demarkated and prominent cell walls (x 8,000), (B) T21: Control root cell showing cell wall (CW) nucleus (N) with prominent nuclear membrane (NM) and nucleolus (NU), endoplasmic reticulum (ER) and small vacuoles (V) (x 6,500), (C) T21 Cd 0.5mM: The vacuoles were large and showed peripherally distributed electron dense globules towards inner surface of the tonoplast membrane (x 8,500), (D) T21 Cd 0.5mM: Larger electron dense globules in the cavities of the larger vacuole of cortical cells (x 10,500), (E) LRG30 Cd 1.0mM: Large and prominent vacuoles with scattered metal depositions (x 12,500), (F) LRG30 Cd 1.5mM: Retracted of the plasma membrane from the cell wall and showed disintegration of cell organelles (x 10,500).

Cadmium under the selected concentrations affected the membrane systems to a large extent presumably due to lipid peroxidation (Sandmann and Boger 1980) and also by affecting membrane proteins (De Fillips 1979). This might have resulted in loss of membrane permeability and solute leakage from the seedlings of pigeonpea in both the cultivars. The darkening of cell walls in some of the cells exposed to heavy metals indicates that the cell walls may also play a role in metal partitioning in them (Neumann *et al*. 1997). Depositions of heavy metal in the cell walls were reported in the radish (Lane and Martin 1980). The cell wall probably acts as an important component of the cite of accumulation of toxic heavy metals (Tomsett and Thurman 1988).

Cadmium exposure induces vacuolisation may be considered as a mechanism of heavy metal tolerance. This is evident from the zinc-induced vacuolation in root meristematic cells of *Festuca rubra* L. (Davies *et al*. 1991). Vacuole formation may lead to intracellular compartmentalization by which the heavy metals are sequestered in vacuoles (Thurman and Collins 1983). Further it was also shown that increased tolerance to heavy metals would result from a higher rate of metal transport into the vacuoles (Harmens *et al*. 1993). However, the high proportion of the total amount of metal uptake in the roots of tolerant plants compared with the sensitive ones is a striking feature (Verkleij *et al.*1990). Comparative results were reported for cadmium in populations of *Holcus lanatus* and *Silene vulgaris* (Coughtrey and Matrin 1978, Verkleij *et al*. 1990).

The ultrastructural studies showed significant differences in two cultivars. Low level of cadmium concentrations induced a greater degree of cell vacuolation increasing the compartmentation potential to reduce the cytotoxic effects of the metals. However cv. LRG30 appears relatively less sensitive than the cv. T21.

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