

# The effects of heavy metal on growth and mitochondrial changes in the *Cajanus cajan* (L.) Millspaugh

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## Abstract

This study was carried out to determine the effects of heavy metal (Pb and Cd) on growth, relative growth index, tolerance index and ultrastructural alterations of mitochondria in pigeonpea cultivars. The results showed that enhanced concentrations of Pb and Cd decreased the growth, relative growth index, tolerance index of pigeonpea cultivars. Further the ultrastructural studies revealed alterations in the mitochondrial structures in both the cultivars in response to heavy metals.

**Keywords:** Cadmium, *Cajanus cajan*, heavy metals and mitochondria ultrastructural alterations.

## INTRODUCTION

Industrial chimney gases and exhausts from traffic cause air pollution in terms of heavy metal, which can also accumulate in soil, resulting in rapid uptake by plants. Rain, irrigation water, rich in heavy metals and agricultural chemicals are the most important sources of combination. Moreover, chimney gases resulting from metallurgical procedures, dust and particles scattered round, and the waste filtered from garbage mix in the deep and surface water and subsequently increase the heavy metal contents of soil [1]. Many studies report that heavy metals such as Cd, Pb, Zn and Hg at toxic levels inhibit growth and seed germination, causing ultrastructural changes. Other negatively affected characteristics include germination percentage, germination index, and root and shoot lengths and root and shoot dry matter rates [2,3].

Most of the studies on the effect of heavy metal have been carried out on ultrastructural studies and the information is not available on mitochondrial changes of crop plants. Hence the study deals with the different growth data parameters and mitochondrial changes under Pb and Cd stresses in cultivars of pigeonpea.

## MATERIALS AND METHODS

The seeds of uniform size and free from infection were selected for the experiments. Seeds of two cultivars of pigeonpea (*Cajanus cajan* (L.) Millspaugh) viz. T21 and LRG30 supplied by ICRISAT, Patancheru, Andhra Pradesh, India were used in the present study. 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 1.5 mM lead (lead acetate:  $(\text{CH}_3\text{COO})_2\text{Pb} \cdot 3\text{H}_2\text{O}$ ) and

$\text{CdCl}_2$  (cadmium chloride:  $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ ). Seeds germinated and seedlings grown in distilled water (zero concentration) served as controls. The others exposed to different concentrations of Pb and Cd was termed as treatments. In each tray 50 seeds were kept for germination maintaining the equal distance between each seed. Each treatment has three replications. The trays were kept in growth chamber and were exposed to light of  $195 \mu\text{mol m}^{-2} \text{s}^{-1}$  and at a temperature of  $30 \pm 2^\circ\text{C}$ . Analysis was carried out on seedlings at 4 intervals i.e. 2, 4, 6, 8 days after germination. For every 2 days filter papers were changed and fresh solution were added to prevent fungal growth. The seedlings were separated into seedling axes and cotyledons before each experimental analysis.

### Length

The length of the seedling axes was measured using a scale and expressed in cm.

### Fresh weight

The seedlings were separated into seedling axes and cotyledons and their fresh weights were determined separately.

### Dry weight

The seedling axes and cotyledons were kept in a hot air oven maintained at  $80^\circ\text{C}$  for 48 h, by which time constant dry weights were obtained.

### Relative growth index (%)

Relative growth index of the seedling axes was calculated based on dry weights by following the method [4].

### Tolerance index (%)

Tolerance index (TI) of each concentration against each of the metals exposed to 6-day old pigeonpea seedlings was calculated by following the method [5, 6].

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### Seedling viability

The seedling viability as affected by different concentrations of Pb and Cd treated 6-day old pigeonpea seedlings were determined using the modified version of TTC (2,3,5-triphenyl tetrazolium chloride) seed viability test [7].

### Transmission electron microscopy

Root tip of 6-day old pigeonpea seedlings of control and treatments were fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (0.2 M pH 7.4). The root tips were post fixed in a buffered solution of 1% osmium tetroxide for 1 h at 4°C in dark and then washed thoroughly with 0.1 M sodium cacodylate buffer. The root tips were transferred to propylene oxide for 20 min, embedded in a mixture of Araldite A and toluene (1:3) initially for 1 h at 60°C and later 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 1 h 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 60 kV). The electron microphotographs were taken from the cortex tissue of control and treated pigeonpea root tips.

## RESULTS AND DISCUSSION

A continuous elongation of the seedling axes length was observed in control seedlings of 2-8 days of germination in both the cultivars of pigeonpea. Though the elongation of the seedling axes of the treatments showed a trend similar to that of controls, they always registered lower values when compared to their respective controls in response to Pb and Cd (Fig-1A).

The fresh weight in seedling axes of the control seedlings of both the cultivars of pigeonpea increased with increasing age of the seedlings. The cotyledons showed a continuous decrease of fresh weight with increasing age. Though the fresh weight of seedling axes of treatments showed a trend similar to the control, the values always remained low in both the cultivars of pigeonpea. The fresh weight of the cotyledons in the treated seedlings also showed a continuous decrease with increasing age with Pb and Cd concentration (Fig-1B).

The seedling axes of controls showed a gradual increase in dry weight with increasing age of the germinating seeds. A continuous decline in the dry weight was noted in the cotyledons of both the cultivars of pigeonpea. The reduction of seedling axes dry weight was showed in contrast to their controls. The retention of dry weight in cotyledons also increased with increasing concentration of Pb and Cd (Fig-1C).

Relative growth indices of pigeonpea seedlings which indicate seedling vigour showed a decline with increasing concentrations of Pb and Cd in both the cultivars of pigeonpea (Fig-2D).

The tolerance index of 6-day old seedling axes of pigeonpea

cultivars decreased with increasing concentrations of Pb and Cd used (Fig-2E).

Tetrazolium reduction test was conducted to assess seedling viability of 6-day old pigeonpea seedlings in response to Pb and Cd treatments. The quantitative values which were expressed as the absorbance of formazan formed for the Pb and Cd treated seedlings of two pigeonpea cultivars registered lower values when compared to their respective controls (Fig-2F).

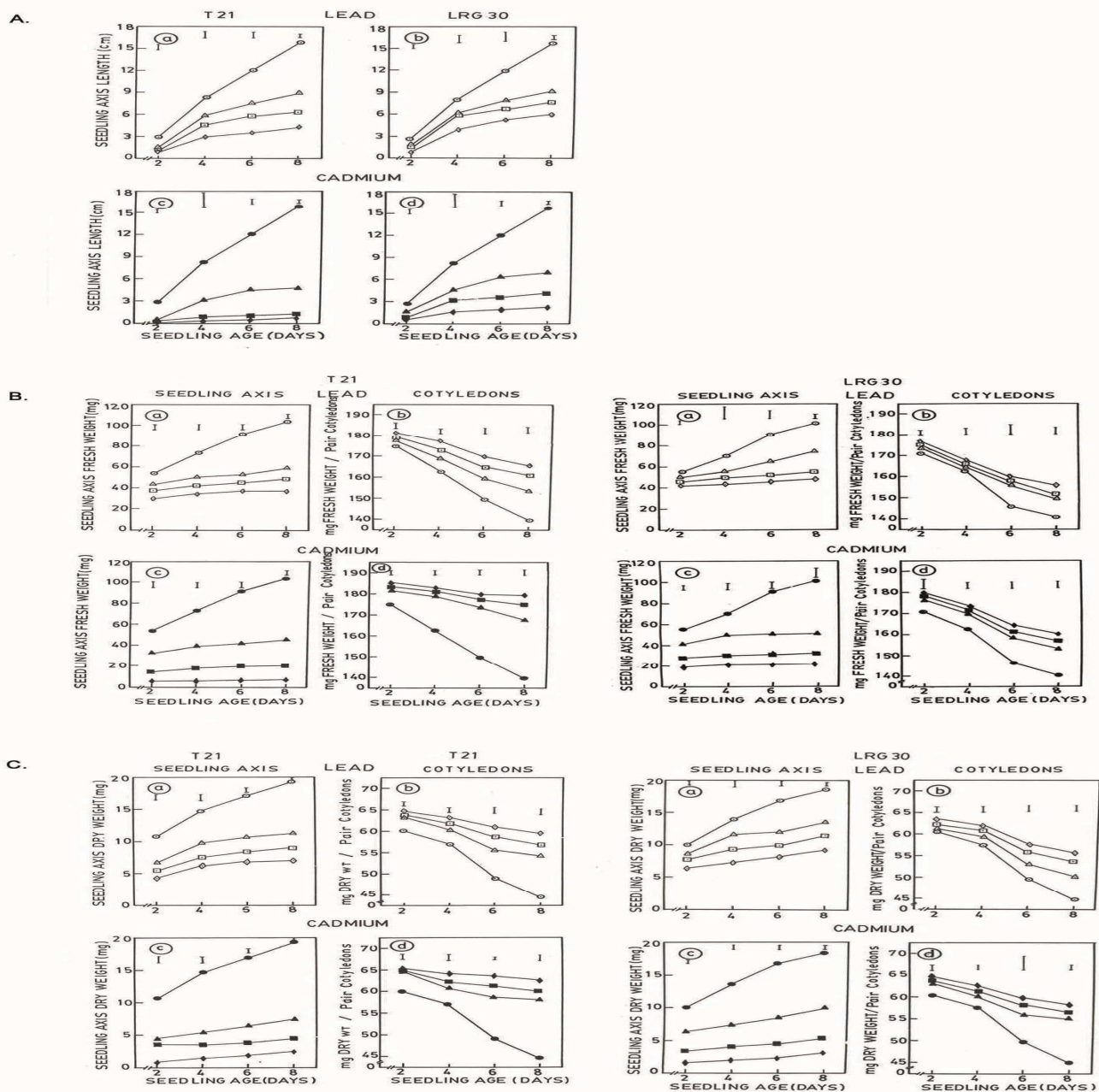
The ultrastructure of root cortical cells of the control seedlings of T21 and LRG30 exhibit a well demarcated cell wall, dense cytoplasm rich in organelles especially mitochondria, endoplasmic reticulum, small vacuoles and prominent nucleus with well developed nucleolus and homogeneously stained nucleoplasm, mitochondria with well developed cristae (Fig-3a, b). However by increasing heavy metal concentration the mitochondrial morphology is altered. The cortical cells of T21 and LRG30 under the influence of 1.0 mM Pb exhibited abnormally shaped mitochondria with internal vesicle and endoplasmic reticulum (Fig-3c, d). The cortical cells of T21 and LRG30 exposed to 0.5 mM Cd exhibited considerable damage to the mitochondria by the disintegration of cristae (Fig-3e, f). Further in control pigeonpea seedlings exhibited mitochondria with well developed cristae and ribosomes, many of which found attached to well formed endoplasmic reticulum.

Seedling axes length was decreased with increasing concentrations of Pb and Cd in both the cultivars of pigeonpea when compared to their respective controls. Root length was affected more than the shoot length in both the cultivars of pigeonpea in response to Pb and Cd treatments. Root growth, its elongation and extension are essential for plants for scavenging water and mineral nutrients [8] and for seedling establishment [9].

The rapid reduction in seedling axes length resulted in gradual reduction in fresh weight and dry weight of the Pb and Cd treated pigeonpea seedlings. This may be due to the interference of heavy metals with mobilization and hydrolysis of stored reserves in the cotyledons as well as interference with the transport of soluble products formed to the growing seedling axes [10,11]. Metal tolerant and metal sensitive plants can be distinguished by their growth performance in the presence of heavy metals [12]. The two pigeonpea cultivars exhibited differences in relative growth index values. The cv.T21 recorded lower values of relative growth index compared to the cv.LRG30. Further the two pigeonpea cultivars selected for the present study exhibited different tolerant index values. Among the two cultivars studied LRG30 recorded higher tolerance index values than T21. The relative growth index can be linked to the mechanism of tolerance [13-17,4].

Tetrazolium test differentiates the living and dead tissues by the presence or absence of a red substance, known as formazan. The test is based on the reduction of the tetrazolium to a red coloured substance, formazan by the dehydrogenase enzymes of respiration [18-20]. The tetrazolium test denoted by the reduction of tetrazolium to formazan by the seedlings of two pigeonpea cultivars showed a progressive loss of red colouration with increasing Pb and Cd concentrations. This may be associated with damage to mitochondria and mitochondrial membrane as is evident from the ultrastructural studies. Direct relationship between germination and

tetrazolium test were shown by many other workers [21-24].



**Fig 1:** A. Seedling axes length during seedling growth of pigeonpea cv.T21 and cv.LRG30 in response to Pb and Cd stresses (Vertical lines represent S.E.).

Lead : a and b

Control : O - O; 0.5: mM Δ-Δ; 1.0: mM □-□; 1.5: mM ◇-◇

Cadmium : c and d

Control : ●-●; 0.5: mM ▲-▲; 1.0: mM ■-■; 1.5: mM ◆-◆

B. Fresh weight of seedlings of pigeonpea cv.T21 and cv.LRG30 in response to Pb and Cd stresses (Vertical lines represent S.E.).

Seedling axis : a and c

Cotyledon : b and d

Lead : a and b

Control : O - O; 0.5: mM Δ-Δ; 1.0: mM □-□; 1.5: mM ◇-◇

Cadmium : c and d

Control : ●-●; 0.5: mM ▲-▲; 1.0: mM ■-■; 1.5: mM ◆-◆

C. Dry weight of seedlings of pigeonpea cv.T21 and cv.LRG30 in response to Pb and Cd stresses (Vertical lines represent S.E.).

Seedling axis : a and c

Cotyledon : b and d

Lead : a and b

Control : O - O; 0.5: mM Δ-Δ; 1.0: mM □-□; 1.5: mM ◇-◇

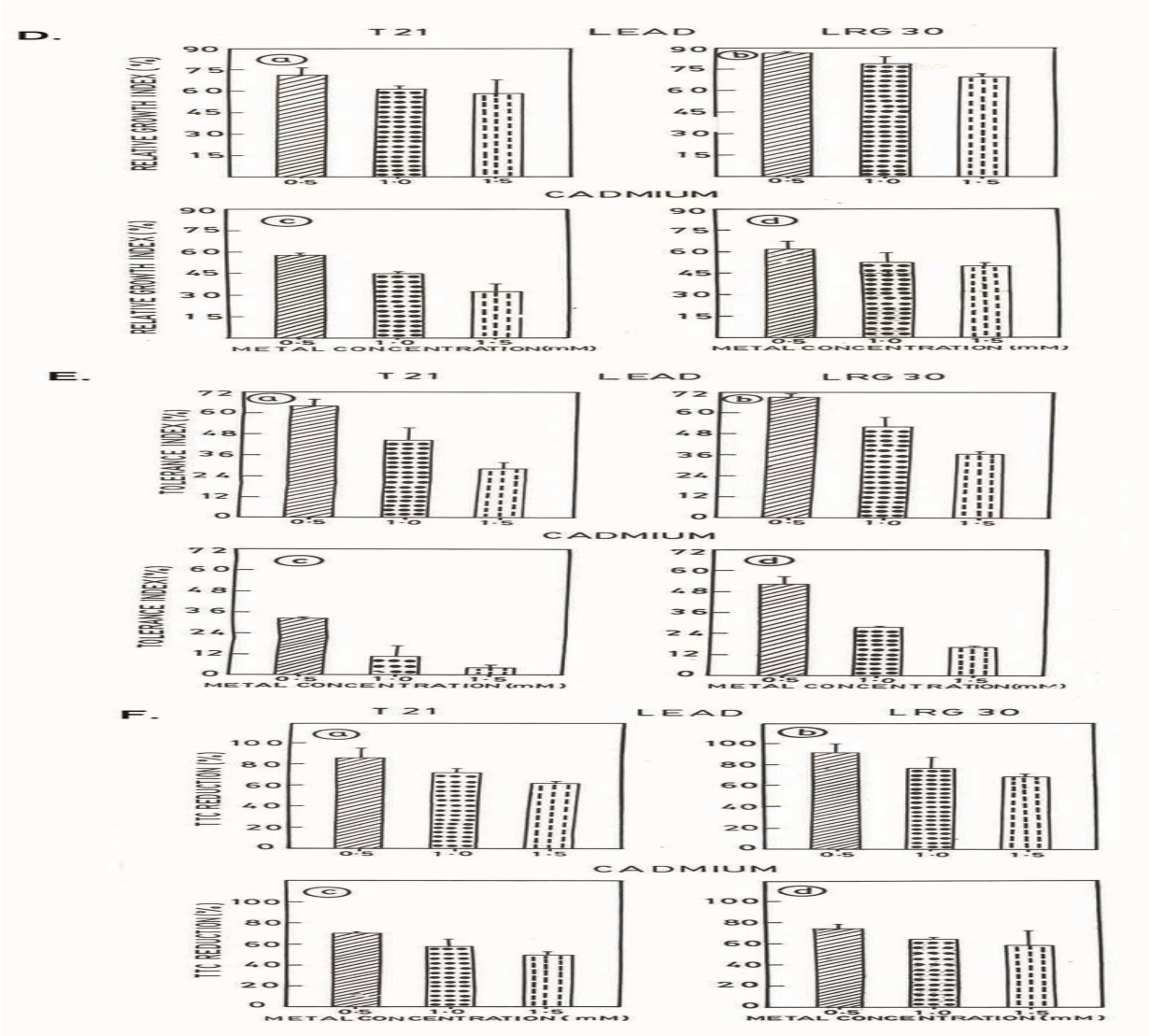
Cadmium : c and d

Control : ●-●; 0.5: mM ▲-▲; 1.0: mM ■-■; 1.5: mM ◆-◆



The mitochondria play, generally, an important role in redox buffering within plant cells has been recently described for Arabidopsis plants. In response to hydrogen peroxide, mitochondria were found to maintain the original redox status better than the cytosol, thus indicating that mitochondria have a higher capacity to buffer redox changes than the cytoplasm [25]. During such conditions an increase of ROS could lead to damage of mitochondrial membrane components resulting in the release of cytochrome C, which then could trigger caspase-dependent cell

death [26,27]. A significantly higher number of condensed mitochondria with the electron-dense matrix and swollen cristae were observed in soybean [28]. The higher number of condensed mitochondria in soybean cultivar after chilling probably lowered ROS production and together with the higher level of phenolic compounds, protected cell structures against oxidative damage [29]. Vacuolation and enlarged cristae were noted by Ishikawa [30], in mitochondria of chilled mung bean cells.



**Fig 2:** D. Relative growth index (% of control) of 6-day old pigeonpea cv.T21 and cv.LRG30 in response to Pb and Cd stresses (Vertical lines represent S.E.).

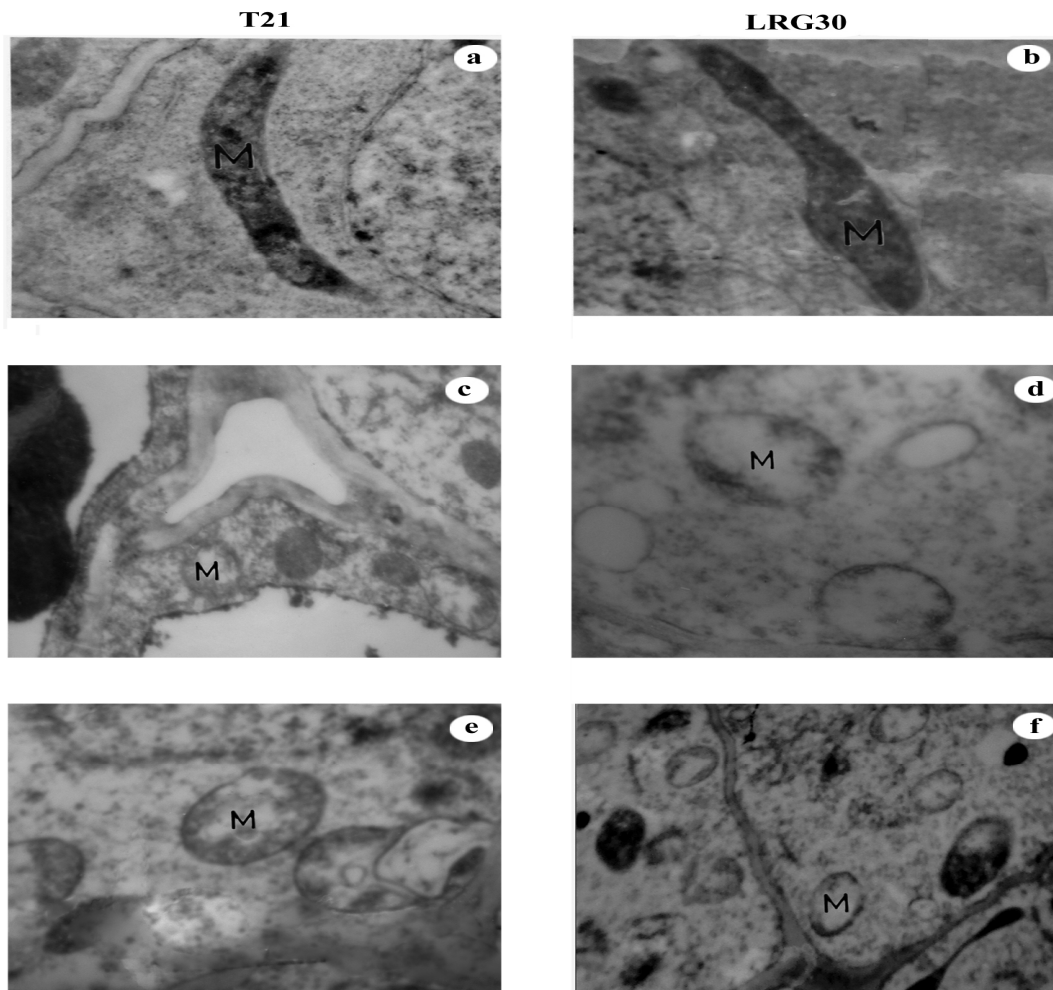
Lead : a and b  
 Cadmium : c and d  
 0.5 mM : ; 1.0 mM ; 1.5 mM

E. Tolerance index (% of control) of 6-day old pigeonpea cv.T21 and cv.LRG30 in response to Pb and Cd stresses (Vertical lines represent S.E.).

Lead : a and b  
 Cadmium : c and d  
 0.5 mM : ; 1.0 mM ; 1.5 mM

F. Tetrazolium reduction (% of control) of 6-day old pigeonpea cv.T21 and cv.LRG30 in response to Pb and Cd stresses (Vertical lines represent S.E.).

Lead : a and b  
 Cadmium : c and d  
 0.5 mM ; 1.0 mM ; 1.5 mM



**Fig 3:** Electron microphotographs of representative root cortical cells 6-day old pigeonpea cv.T21 and cv.LRG30; a and b:Mitochondria (M) with well developed cristae (T21, control, x10,500) and (LRG30, control, x10,500); c :Obvious decrease in mitochondrial cristae with internal vesicle (T21, 1.0 mM Pb, x8,500).; d : The interior of the mitochondria is greatly altered and appears to have been damaged (LRG30, 1.0 mM Pb, x15,500); e : Electron dense granules localized on the surface of membranes in mitochondria (T21, 0.5 mM Cd, x12,500); f : Smaller mitochondria (LRG30, 0.5 mM Cd, x15,500).

## CONCLUSION

The pigeonpea cultivars T21 exhibited lower values of TTC reduction activity than LRG30 with increasing heavy metal concentrations, indicating its low viability and increased susceptibility to Pb and Cd treatments. Further biochemical analyses should be made to resolve this problem.

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