Research Paper Engineering

Effects of Heavy Metals in Pigeonpea Cultivars on Stomatal Resistance, Total Chlorophyll and $^{14}Co_2$ Fixations

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ABSTRACT

Biochemical and structural alterations in two cultivars of pigeonpea (Cajanus cajan (L.) Millspaugh) T21 and LRG30 in response 0, 0.5, 1.0 and 1.5 mM lead and cadmium on the SEM studies of stomatal apparatus, total chlorophyll and 14CO2 fixation were studied. Lead and cadmium caused significant reductions in photosynthetic activity and some stomata were severely affected. Lead and cadmium ions affected both the cultivars. However the effect was more in cadmium treated seedlings than in lead treated ones.

Keywords : $^{14}CO_2$ fixation, Heavy metal stress, Stomatal resistance, Total chlorophyll content.

INTRODUCTION

Essential and non-essential heavy metals are known to cause curtailing of nearly all growth parameters [1] and productivity [2, 3] of plants, with varying degrees of severity. They also cause some structural [4] and ultrastructural alterations in the vegetative organs of plants [5]. Heavy-metal-induced decreases in cell wall elasticity have also been recorded [6]. The physiological effects of heavy metals on plants include: (i) perturbation of stomatal functions leading to changes in water relations and rates of gas exchange [7, 5]; (ii) reduction of photosynthetic pigments and activity [8, 9] and replacing the central atom of chlorophyll (Mg) to produce photosynthetically inactive HM-chlorophylls [10], thus diminishing the photosynthetic activity [11]; and (iii) disruption of the integrity of cellular membranes [12, 13]. Since heavy-metal-polluted soils and irrigation water usually contain several in mixtures [14], plants are seldom exposed in nature to the impact of a single heavy metal.

The present study was undertaken to analyze the impact of structural alterations induced by Pb and Cd (as a non-essential heavy metals) on growth criteria, photosynthetic pigments and stomatal apparatus of Cajanus cajan (L.).

MATERIALS AND METHODS

Seeds of pigeonpea (Cajanus cajan (L.) Millspaugh) cv.T21 and cv.LRG30 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 0.01 M 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: $(CH_3COO)_2Pb\ 3H_2O$) and $CdCl_2$ (cadmium chloride: $CdCl_2\ 2.5H_2O$). Seedlings raised in distilled water (zero concentration) served as controls. The seeds were allowed to germinate at 30 ± 2°C for 6-days under a photoperiod of 12 h, and at 195 µmol $m^{-2}s^{-1}$ PPFD and washed in 10 mM $CaCl_2$ to remove Pb and Cd accumulated on their surface. Then the seedlings were collected for scanning electron microscopic studies and biochemical analysis.

Scanning electron microscopy

To study the leaf surfaces of the primary leaves from the 6-day old pigeonpea seedlings of treated and controls were fixed in 2.5% glutaraldehyde in 0.025 M phosphate buffer, dehydrated with alcohol series and then subjected to critical point drying in solid carbon dioxide. Ten mm^2 of the dried specimens were coated with gold palladium and examined on scanning electron microscope (JEOLJSM-T330A)

Total chlorophyll content

Total chlorophyll content was estimated using the method of [15].

Photosynthetic rate ($^{14}CO_2$ fixation)

Photosynthetic rate of shoots of the control and treated seedlings of two pigeonpea cultivars was determined by feeding with $NaHCO_3$ and the incorporation of $^{14}CO_2$ was measured following the method described by Jones and Osmond [16], by using the Automatic liquid scintillation system model LSS-341 ECIL, India.

RESULTS

The scanning electron microscopic studies of stomata were carried out on the primary leaves of 6-day old seedlings of pigeonpea both in controls and treatments. The stomata of the primary leaves of the control seedlings were fully grown with the well developed borders and ledges (Fig-1a, b). The seedlings of cv.T21 the stomata were defective and abnormal stomata exposed to 1.0 mM Pb and 0.5mM Cd exhibited small and undeveloped stomata with narrow stomatal pore (Fig-1c, e). The stomata of the primary leaves of LRG30 treated with 1.0 mM Pb exhibited partially opened stomata and 0.5 mM Cd exposed primary leaves always remained undeveloped and exhibited closure (Fig-1d, f).

Total chlorophyll content of the shoots of the seedlings of two pigeonpea cultivars decreased with increasing concentration of lead and cadmium ions and registered lower values when compared to their respective controls (Fig.2A).

The incorporation of $^{14}CO_2$ by the seedlings of two pigeonpea cultivars decreased with increasing external concentrations of lead and cadmium. Lower incorporation of $^{14}CO_2$ was registered in seedlings grown in cadmium treatment in both the cultivars of pigeonpea (Table-1).

DISCUSSION

The scanning electron microscopic studies of the stomata were carried out on primary leaves of pigeonpea cultivars. The primary leaves of treated seedlings exhibited abnormal and at some times completely collapsed stomata. Primary leaves of the Pb and Cd treated seedlings of pigeonpea exhibited two types of defective stomata, one type always stayed open and the other always closed, in addition to certain un-

developed stomata. This indicates that stomata were severly affected by Pb and Cd. The stomata close after direct addition of heavy metals to leaves [17, 18]. The defective stomata probably might have lost a functional closing mechanism and were unable to regulate the exchange of water vapour and CO_2. Hagemayer et al. and Schlegel et al. [19, 17] found that Cd decreased the rate of transpiration in Fagus sylvatica and Picea abies.

Heavy metal stress by Cu and Cd not only affects the growth criteria of Sorghum bicolor reported previously [8], but it also had an adverse effect on its anatomical structure, especially when the plant was subjected to both metals simultaneously. The limitations of stomata on photosynthesis are well-documented [20]. The combination of reduction in the photosynthetic pigments and diminished efficiency of the stomatal apparatus would lead to a marked reduction in the photosynthetic activity. Kasim, [21] observed decrease in stomatal frequency induced by heavy metals in Sorghum bicolor. Limitation in water supply (leading to stomatal closure) and stomatal frequency might impinge on gas exchange and also the rate of photosynthetic electron transport [20].

Cadmium treatment decreased total chlorophyll content in tomato [22], barley [23], Triticum aestivum [24] and Cajanus cajan [25, 26]. The reduction in chlorophyll content under lead and cadmium treatments may be due to their impact on synthesis [23] by affecting S-aminolevulinic acid dehydratase and porphobilinogen deaminase leading to accumulation of intermediates of chlorophyll synthesis such as ALA and porphyris [27-29]. The rapid destruction of chlorophyll pigments by the free radical formation in response to oxidative and heavy metal stress was also reported by Kunert [30] and Shaaltiel [31]. The decrease in chlorophyll content under lead and cadmium treatments were more in cv. T21 than in cv. LRG 30, indicating that the latter was more tolerant than the former. Wainwright and Woolhouse [32] also observed a greater loss of chlorophyll in leaf segments of susceptible ecotypes than in tolerant ecotypes of Agrostis tenuis.

The incorporation of $^{14}CO_2$ by the seedlings of two pigeonpea cultivars decreased with increasing external concentrations of lead and cadmium. Lead treatment of spinach [33, 34], Glycine max, Heliantlius annuus, Zea mays L. [35-38], Lobolly pine, autumn olive [39] and Plantanus occidentalis [40] and cadmium treatment of Cajanus cajan, Triticum aestivum [41, 25, 42] exhibited reduced rates of photosynthesis.

CONCLUSION

It might be concluded that exposure of pigeonpea seedlings to toxic levels of Pb and Cd, triggers a number of closely interrelated structural and functional events in the stressed plants.

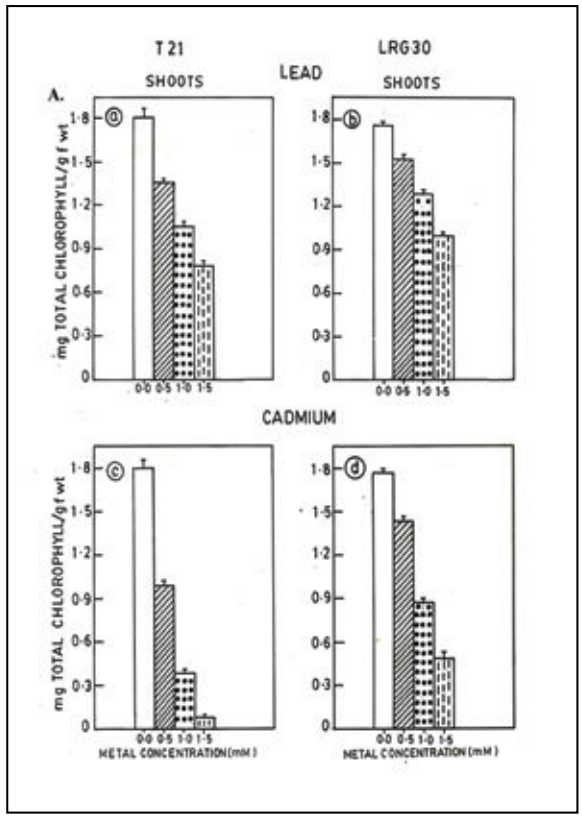

Fig-2

Table - 1. Effect of lead and Cadmium on Photosynthetic Rate $^{14}CO_2$ fixed (cpm/h/cm^2×10^2)

Photosynthetic Rate $^{14}CO_2$ fixed (cpm/h/cm^2×10^2) Lead	
T21	LRG30
140±0.02	141±0.01
101±0.02	125±0.04
72±0.03	110±0.02
50±0.02	100±0.03
Cadmium	
141±0.01	135±0.02
45±0.02	115±0.03
15±0.02	95±0.02
5±0.01	60±0.01

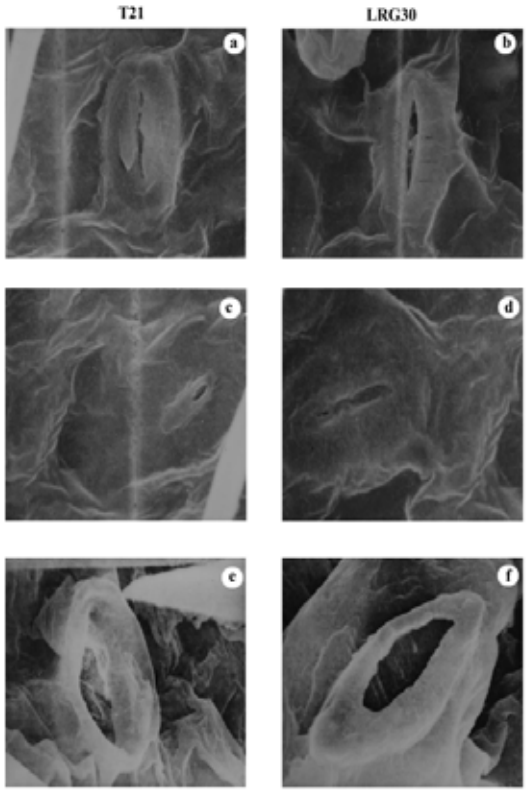

Fig-1

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