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PROTEIN CONTENT AND AMYLASE ACTIVITY IN GERMINATING SEEDS OF PIGEONPEA UNDER LEAD STRESS

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The effect of lead on the percent seed germination, amylase activity and protein content were investigated in 2-8 day old pigeonpea seedlings (cv. LRG38 and cv. ICPL85063). The percent seed germination, amylase activity and total protein content declined progressively with increasing concentrations of lead. From these observations it could be emphasized that the cultivar differed in response to lead treatment.

Keywords : Amylase; Germinating seeds; Non-essential; Protein; Stress.

Introduction

Plant productivity is severely affected by abiotic stress factors which include salinity, drought, high and low temperature and heavy metals[1]. Plants growing in metal contaminated environments would inevitably accumulate trace and abundant metal ions. Certain trace metals are important for metabolism of plants while a number of them are not essential imposing stress on the metabolic functions *i.e.* As, Cd, Cr, Hg, Sb, Sn, Cu[2]. Further lead is most wide spread pollutant and is absorbed and accumulated to a greater extent than any other heavy metal[3]. Pb toxicity inhibits germination of seeds and retards growth of seedlings. Pb decreases germination percent, germination index and root/shoot length[4]. Inhibition of germination and retardation of plant growth are commonly observed due to lead toxicity[5-10]. Negative effects of lead toxicity on seed germination and seedling growth of some tree species were examined[11-12]. Lead toxicity in many plants is known to be associated with inhibition of growth, changes in enzyme activities and also inhibition of root elongation[13-14]. Toxicity may result from the binding of metals of sulphydryl groups in proteins, leading to inhibition of activity or disruption of structure, or from displacement of an essential element, resulting in deficiency effects[15]. Increased protease activity leads to reduction in protein content and to a rising amount of the low molecular products of their decacy; carbohydrate metabolism is altered[16]. Pb toxicity lowers the protein content of tissues and causes significant alterations in lipid composition[17-18].

Material and Methods

Two cultivars of pigeonpea *(Cajanus cajan* (L.) Millspaugh) seeds namely LRG38 (a medium duration) and ICPL85063, (a short duration) supplied from ICRISAT, Patancheru and LAM, Guntur, India were used for the experiment. Seeds of uniform size and free from infections were selected and were surface sterilized with 0.001M sodium hypochlorite for 2 min. The seeds were thoroughly washed with distilled water then spread over plastic trays (approximately 45 seeds per tray) lined with two layered Whatman No. I filter paper containing 0, 0.02, 0.04 and 0.06 mM concentrations of lead (chloride salt of lead ($PbCl_2$)). The seedlings raised in distilled water (zero concentration) served as controls. The seeds of the two cultivars were allowed to germinate at 30±2°C for 8-days under a photoperiod of 12 h and at 195 μ mol $m^{-2} s^{-1}$ PPFD. The seedlings were washed in 10 mM $CaCl_2$ to remove lead accumulation on their surface. Then the analyses were made in different parts of the seedling *viz.* root, shoot and cotyledons were collected at 2, 4, 6, and 8-days seedling growth for biochemical analysis.

Percent Seed Germination : The seeds were allowed to germinate at 30±2°C in different concentrations of lead for 24 h, the seeds with 0.4 cm radical emergence were considered as germinated. The germination data was recorded and expressed as percent seed germination.

Amylase : Amylase activity was estimated by the method of Filner and Varner[19] as followed by Kapoor and Sachar[20].

Total proteins : Total protein content was estimated by the method of Lowry *et al.*[21].

Results and Discussion

Lead treatment significantly decreased percent seed germination as compared to control. A gradual decline in percent seed germination of both the cultivars of pigeonpea decreased with increasing concentrations of Pb (Fig.1A). Among the two cultivars ICPL85063 exhibited greater

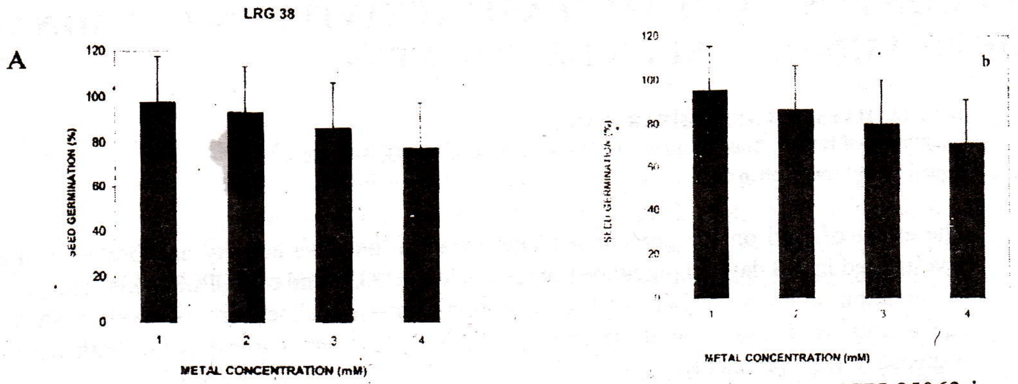

Fig.1. A. Percent seed germination of two cultivars of pigeonpea, cv. LRG38 and cv. ICPL85063 in response to Pb stress. 1. 0 mM control; 2. 0.02 mM; 3. 0.04 mM; 4. 0.06 mM.

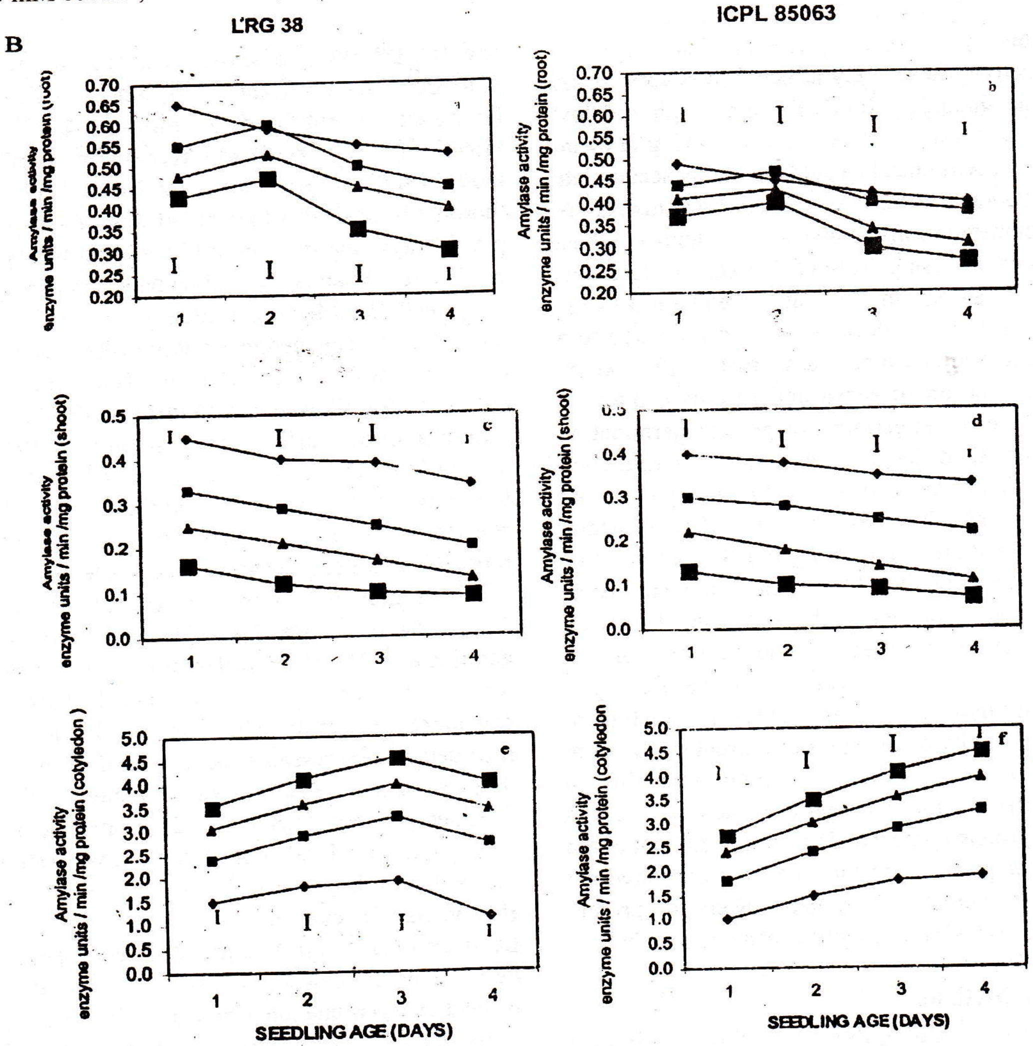

Fig.1. B. Amylase activity of two cultivars of pigeonpea, cv. LRG38 and cv. ICPL85063 in response to Pb stress. 1. 2 days; 2. 4 days; 3. 6 days; 4. 8 days.
Control : u-u; 0.02 mM : n-n; 0.04 mM : s-s : 0.06 mM : n-n

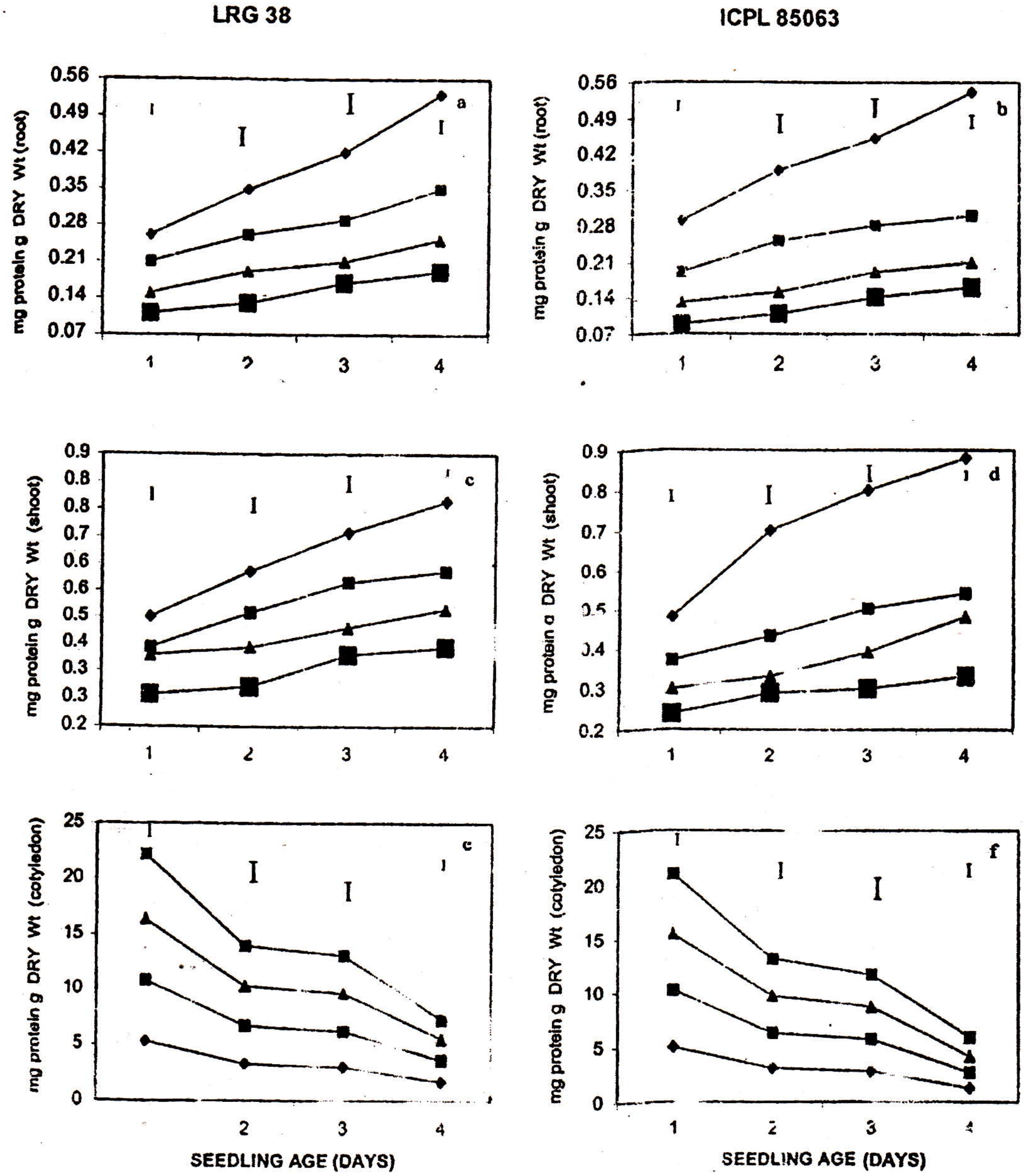

Fig.2. Protein content of two cultivars of pigeonpea, cv. LRG38 and cv. ICPL85063 in response to Pb stess. 1. 2 days; 2. 4 days; 3. 6 days; 4. 8 days.
Control : u-u; 0.02 mM : n-n; 0.04 mM : s-s : 0.06 mM : n-n

reduction in percent germination than the cv. LRG38 recorded. Seed germination and seedling growth inhibition by heavy metals has also been reported by many other workers[5,12,22,23].

The pigeonpea seedlings greminated and grown in different concentrations of lead showed an initial rise in the level of amylase activity in roots. The amylase activity in roots decreased with increasing concentrations of externally supplied lead. The amylase activity in shoots of the lead treated germinating seeds of pigeonpea showed a continuous decrease from 2-8 days of seedling growth. In addition, the amylase activity in shoots also decreases with increasing concentrations of lead used in the study. The amylase activity in cotyledons in the two pigeonpea cultivars increased with increasing seedling age and decreased with increasing concentrations of externally supplied lead (Fig. 1B). Amylase plays an important role in seed germination[24-25]. It was observed that the amylase activity increased in soybean under lead[26] and in *Cicer* under Pb, Cd, Ni, Zn and Hg treatments[27]. This was followed by associated increase in the accumulation of sugars with corresponding decrease of starch content under stress conditions in various plant species[28-29]. However, at higher concentration of metal ions and with increasing seedling age, amylase activity was decreased to considerable extent. This may probably due to the binding of metal ions with the SH-groups of the enzyme proteins and decreased synthesis and transport hormones from the embryonic axes to the cotyledons under the influence of heavy metals[30].

The protein content in the root and shoot during seedling growth of pigeonpea cultivars showed a continuous increase associated with a concomitant decrease in their respective cotyledons. Though the protein content in the root and shoot of the lead treated seedlings exhibited a trend similar to that of the controls with age their values were always lower than their controls. The protein content in the cotyledons of the lead treated pigeonpea seedlings showed more retention with inreasing lead concentrations (Fig.2). This is in accordance with the findings of Salgare and Acharekar[31] who reported that growth performance, as well as pigment, carbohydrate and protein content showed a decreasing trend with increase in the level of industrial pollution. Decreased levels of protein content in heavy metal exposed tissues have been reported by many workers[32-33]. Relatively strong affinities of heavy metal ions for side chain ligands of protein indicate that enzyme and other functional proteins are one of the primary targets of metal toxicity[34]. It was demonstrated that protein content in maize[35] and *Pisum*[36] decreased in reponse to lead and cadmium treatments, respectively. The lowered protein content of the pigeonpea root and shoot of the treatments may also be due to breakdown of proteins response of lead stress[37]. The relations of proteins in the cotyledons of the treated pigeonpea seedlings is due to the decreased hydrolysis and transport of amino acid products from the cotyledons to the growing seedling axes under heavy metal stress. This has led to the decreased protein content in the seedling axes of the lead treated pigeonpea seedlings resulting in decreased gowth[38].

The percent seed germination, enzyme activity and protein content were relatively more affected in cv. ICPL85063 than the cv. LRG38. Further studies are needed to specify the parameters associated with lead exclusion and lead detoxification capacity of plants as well as those biochemical parameters associated with tolerance to lead in plants. Such studies will enlighten the mechanism of the genetic and biochemical basis of lead tolerance in crops and based on biotechnological tools it should be possible to produce plants with enhnced lead tolerance.

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