Research Article

CHANGES IN LIPID AND PHOSPHOLIPID CONTENT OF *CAJANUS CAJAN* SEEDLINGS IN RESPONSE TO CADMIUM TOXICITY

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ABSTRACT

Seedlings of pigeonpea (*Cajanus cajan* L. Mill.) grown on treated with different cadmium (Cd) concentrations representing 0, 0.02, 0.04 and 0.06 mM were used in three pigeonpea cultivars, LRG30, LRG41 and ICPL85063 on membrane permeability, lipid and phospholipid contents were studied. The consequent loss of membrane permeability to nonelectrolytes were expressed more in cv.LRG41 and ICPL85063 in response to Cd treatment indicating heavy metal sensitivity of LRG41 and ICPL85063. Decreased lipid and phospholipid contents were more in cv.LRG41 and ICPL85063 in response to Cd treatment indicating heavy metal sensitivity of LRG41 and ICPL85063.

INTRODUCTION

Various heavy metal ions such as Pb^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}, and Ni^{2+} when present at an elevated levels in the environment may eventually find their way into the plants by aerial deposition on foliage or through uptake by roots from the soil (Wettlaufer *et al.*, 1991). The physiological basis of metal ion uptake depends on the plants environment in which they are growing. At low external metal concentrations active absorption dominates, but at high metal concentrations damage to cell walls and to membrane integrity were likely to occur, and therefore under these circumstances passive flow of metals into the plants becomes clearly dominant (Fernandes and Henriques, 1991; Harmens *et al.*, 1993). However the concentration of heavy metal ions in plants depends on the genetic makeup and physiological status of the plant and also on the chemical properties of metal ions especially valance, ionic radius and capacity to form organic complexes (Malik *et al.*, 1992; Arduini *et al.*, 1994).

The evidence for metal ion-induced changes in membrane structure also comes from the studies of quantifying ion leakage from roots or cells exposed to a variety of metal ions (Woolhouse, 1983; De Vos *et al.*, 1991). Cd enhances solute leakage from leaf discs of *Phaseolus vulgaris* (Fuhrer, 1982).

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Copper exposure leads to increased permeability of a variety of tissues including roots of *Triticum aestivum* (Jensen and Adalsteinsson, 1989), as well as cells of *Enteromorpha compressa* (Reed and Moffat, 1983). The metal-induced change in membrane properties not only effect K^+ and H^+ extrusion, the functioning of membrane carriers and ion channels, but also affects the permeability of membranes to water. The water permeability decreased and nonelectrolyte permeability increased in *Quercus rubra* root cortex cells treated with Al and maize root segments treated with Ni, Co and Zn (Cocucci and Morgutti, 1986; Barceló and Poschenrieder, 1990).

In higher plants, Cd negatively affects both plant growth and development, resulting in stunting and eventually plant death. Adverse effects of cadmium on plant growth have been widely investigated and reported. Most commonly, cadmium adversely affects germination and growth (Greger and Ogren, 1991; Quadir and Iqbal, 1991), functionality of membranes by inducing changes in lipid composition (Quariti *et al.*, 1997), enzymatic activities associated with membranes such as H^+ATPase (Fodor *et al.*, 1995) and more importantly enzymes involved in metabolism (De Filippis and Ziegler, 1993). Many toxic effects of heavy metal ions are due to Ca^{2+} replacement at essential sites of cell membranes which may affect membrane function (Breckle and Kahle, 1992). Fodor *et al.* (1995) reported that the interaction between Cd and the membrane constituents such as lipids and/or proteins may result in the alteration of membrane fluidity, which in turn affects the activities of membrane bound enzymes. Kennedy and Gonsalves (1989) demonstrated that supply of cations, Zn^{2+}, Cd^{2+}, Hg^{2+}, Cu^{2+}, Pb^{2+} to maize roots causes inhibition of

ATPase activity and also affects the related processes such as H^+ ion efflux and transmembrane and transroot potentials. Inhibition of H^+-ATPase activity of *Zea mays* (Suhayda and Haug, 1986; Tu and Brouillete, 1987), *Beta vulgaris* (Lindberg and Wingstrand, 1985), *Pisum sativum* (Matsumoto and Yamaya, 1986), *Hordeum vulgare* (Matsumoto, 1988) has also been reported in the presence of Cd, Zn, Mn and Al.

Lipids are the important constituents of biological membranes which are involved in structural and vital biochemical functions. They also perform several other cellular functions (Kates and Marshall, 1975). Changes in lipid composition in response to heavy metal stress have been demonstrated in many plants. In addition to lipid composition, heavy metals also affect the levels of fatty acids and various phospholipid constituents of membranes (Svennignsson and Lilijenberg, 1986; Norberg and Lilijenberg, 1991). Therefore the current study was undertaken to analyze the changes in the membrane permeability, lipid and phospholipid contents in response to Cd in three cultivars of pigeonpea, an important pulse crop of India.

MATERIALS AND METHODS

Plant material and growth conditions

Seeds of three cultivars of pigeonpea (*Cajanus cajan* (L.) Millspaugh) namely LRG30 (Long duration, 180-300 days), LRG41 (Medium duration, 150-180 days), and ICPL85063 (Short duration, 100-150 days) obtained from ICRISAT, Patancheru and LAM, Guntur, Andhra Pradesh, India were used for the present investigation. These varieties are grown around the Visakhapatnam and its surrounding villages.

The seeds of healthy and uniform size were selected and surface sterilized with 0.001 M mercuric chloride for 2 min, washed thoroughly with glass-distilled water and then soaked in distilled water for 2 h. The soaked seeds were then spread over plastic trays (approximately 50 seeds per tray) lined with two-layered whatman No.1 filter paper containing different concentrations of cadmium. Cadmium as cadmium chloride: $CdCl_2$ H_2O was used in three concentrations of metal representing 0.02, 0.04 and 0.06 mM for cadmium. These concentrations were selected on the basis of preliminary experiments in which the concentrations less than 0.02 mM for cadmium. The seeds raised in distilled water served as controls. Twenty five ml of each test solution was added separately to each tray and the filter papers were replaced on every alternate day during the study period. The seeds of the three cultivars were allowed to germinate at 30 ± 2°C for 8 days under a photoperiod of 12 h and at a photosynthetic photon flux density (PPFD) of 195 $\mu mol\ m^{-2}s^{-1}$. The analyses were made in different parts of the seedling viz. root, shoot and cotyledons separated prior to start of each experiment. Five replicates were used for each treatment.

Leaching

For leaching experiments, fifteen seedlings (without separating into individual parts) were taken from each treatment at four growth stages of three pigeonpea cultivars. These seedlings were rinsed in distilled water, thoroughly blotted and incubated for 24 h in glass distilled water at room temperature. The eluate was then collected and filtered through Whatman No.1 filter paper. The control and treated seedlings including cotyledons were placed in 100 ml beakers and 50 ml distilled water was added. The beakers were placed in room temperature for 24 h. The eluate was then collected and made up to 50 ml for analyses (Simon and Rajaharam, 1972). The leachates collected were analysed for sugars, total organic acids and amino acids.

Sugars: The sugar content in the leachates was determined according to the method of Dubois *et al*. (1956). To 1 ml of the eluate, 1 ml of 5% (w/v) phenol was added and then 5 ml of concentrated sulphuric acid was added in such a way that it hits the surface of the liquid directly. The mixture was shaken immediately and cooled at room temperature. After 30 min, absorbance was read at 490 nm in a Schimadzu (UV-240) spectrophotometer. Glucose was used for the preparation of standard curve.

Total organic acids: Total organic acid content of the leachates were determined according to the method of Ting and Dugger (1968). Ten ml aliquots of the eluate were titrated against 0.01 N NaOH using phenolphthalein as indicator and the results were expressed as milliequivalents of acid leached per seedling.

Amino acids: Amino acid content in the leachates was estimated according to the method of Moore and Stein (1948) and the results were expressed as µg amino acid leached per seedling.

Total lipids

Extraction and estimation: Total lipid extraction was carried out according to the method of Bligh and Dyer (1959). One g of different parts of seedlings were washed and macerated with 14.4 ml boiling mixture of chloroform : methanol : water (1:2:0.6 V/V/V). The contents were filtered using muslin cloth. The filtrates were transferred into stoppered test tubes and the residues were reextracted with 8.0 ml of hot methanol and again transferred to stoppered tubes. To these tubes, 12 ml each of chloroform was added. After keeping the samples over night at 0°C in deep freeze, 11.2 ml of water was added to each tube, shaken well and centrifuged for phase separation. The final ratio of chloroform: methanol: water in each tube was 2:2:1.8 (V/V/V). The aqueous phases were removed with suction. The lipid phases were washed thrice with methanol: water (2:1.8 V/V) mixture. The contents were centrifuged in a refrigerated centrifuge at 3000 xg for 10 min in order to remove the water soluble compounds. The lipid extracts were evaporated to dryness.

The dried lipid residues were taken into chloroform and transferred to pre-weighed bottle and evaporated to dryness under nitrogen. The bottles were kept in a vaccum dessicator over potassium hydroxide under reduced pressure for several hours and weighed again. The difference between the weights was taken as the weight of the total lipid present in each sample. The total lipid of each sample was dissolved in chloroform, made upto 5 ml and was preserved at 0°C in tightly stoppered standard flask until further analysis. The total lipid content was expressed as mg per gram dry weight.

Total phospholipids

The total phospholipid content was determined as the phosphorus content of the phospholipids by the procedure out lined by Bartlett (1959). The lipid samples were taken into test tubes marked with 10 ml. The organic solvent was removed by passing compressed air into the test tubes. One ml of 60% perchloric acid was added to each tube and digested at 170-

180°C on a heating mantle. Digestion was continued until the samples were clear. After digestion, to each tube 4.5 ml of 0.44% ammonium molybdate reagent was added, followed by 0.2 ml of 1-amino-2-naphthol-4-sulphonic acid reagent. The contents of the tubes were shaken well and heated over a boiling water bath for 10 min. After cooling, the volume was adjusted to 10.0 ml with glass distilled water. The colour intensity of each sample was measured at 660 nm using Schimadzu (UV 240) Spectrophotometer. Aliquots of KH_2PO_4 solution containing 2 µg phosphorus/ml were used as standards. Blanks were run simultaneously. For computing the total phospholipid content, the lipid phosphorus was multiplied by factor 2.5. The results were expressed as µmole phosphorus per organ and µmole phosphorus per g dry weight.

Preparation of reagents

Preparation of ammonium molybdate reagent: 4.4 gm of ammonium molybdate mixed with 14.0 ml of concentrated sulphuric acid and the final volume was made up to one litre with distilled water.

Preparation of 1-amino-2-naphthol-4-sulphonic acid reagent: 0.5 g of 1-amino-2-naphthol-4-sulphonic acid, 6.0 g sodium sulphite and 30.0 g sodium bisulphite were added and the volume was made up to 250 ml with distilled water.

RESULTS

Membrane permeability

The changes in the permeability of membranes to nonelectrolytes were studied in response to Cd treatments by estimating the different components of the leachates collected from the three pigeonpea cultivars at four stages of seedling growth. The studies on the leachates were carried out on whole seedlings without separating them into their individual organs.

Sugars: The sugar content in the leachates of the Cd treated germinating seeds of pigeonpea increased steadily with increasing seedling growth. In addition, the increasing concentrations of externally supplied Cd also led to a steep increase in the leaching of sugars from the three pigeonpea cultivars. At 0.02 mM Cd concentration exhibited a slow and gradual increase and 0.04 and 0.06 mM Cd concentrations exhibited a rapid increase in the leaching of sugars with increasing age of the seedlings (Fig.1).
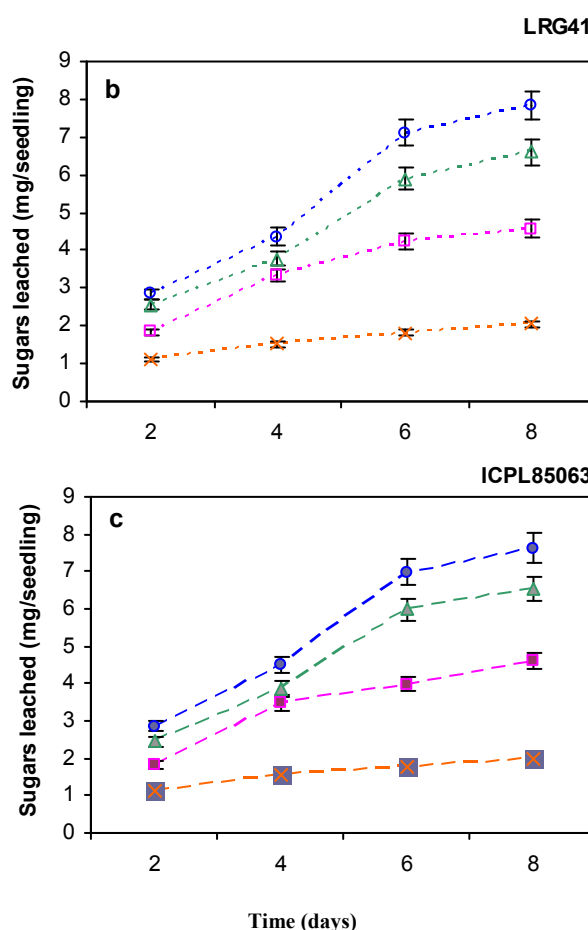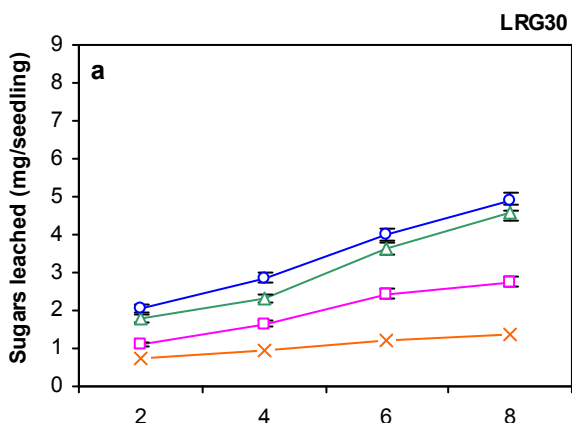
Fig 1 Leaching of sugar content of whole seedlings of the three pigeonpea cultivars LRG30, LRG41 and ICPL85063 in response to cadmium stress (Vertical lines represent S.E.).

At all stages of seedling growth the leaching of sugars from the Cd treated pigeonpea cultivars registered higher values than their respective controls. The amount of sugars leached from the 6-day old pigeonpea seedlings germinated and grown in 0.02, 0.04 and 0.06 mM Cd concentrations showed an increase of 1.97, 2.99 and 3.26 folds in LRG30; 2.34, 3.25 and 3.93 folds in LRG41 and 2.26, 3.40 and 3.97 folds in ICPL85063 respectively over their appropriate controls. Among the three pigeonpea cultivars, the higher levels of sugars were noted in the leachates collected from the seedlings of LRG41 and ICPL85063 in response to Cd treatment.

Total oraganic acids: The total organic acid content of the leachates increased gradually with increasing age of the seedling. The total organic acids leached out from the Cd treated germinating seeds exhibited an increasing tendency with increasing concentrations of externally supplied Cd and registered higher values when compared to their controls (Fig.2). The amount of the total organic acids leached out from the 6-day old pigeonpea seedlings germinated and grown in 0.02, 0.04 and 0.06 mM Cd concentrations showed an increase of 4.65, 6.48 and 7.50 folds in LRG30; 4.22, 5.11 and 6.56 folds in LRG41 and 4.21, 5.14 and 6.46 folds in ICPL85063 respectively over their controls. Among the three pigeonpea cultivars, LRG30 registered higher values of the total organic

acids in the leachates compared to LRG41 and ICPL85063 in response to Cd treatment.

LRG30
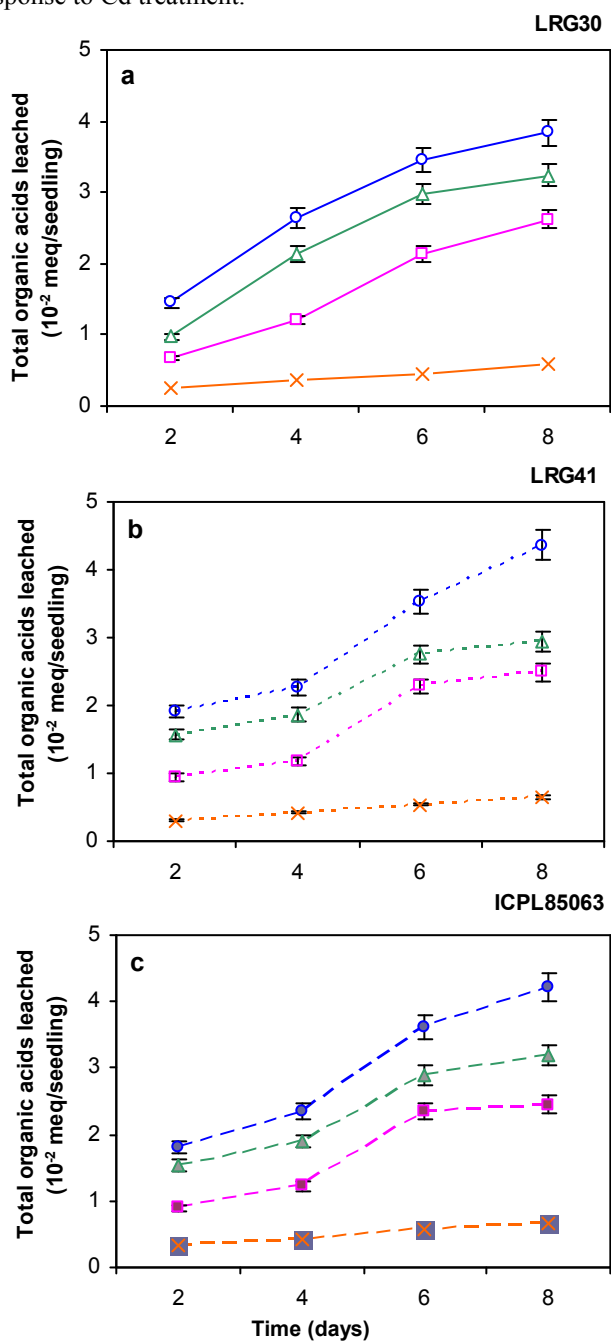

LRG41

ICPL85063

Fig 2 Leaching of total organic acids of whole seedlings of the three pigeonpea cultivars LRG30, LRG41 and ICPL85063 in response to cadmium stress (Vertical lines represent S.E.).
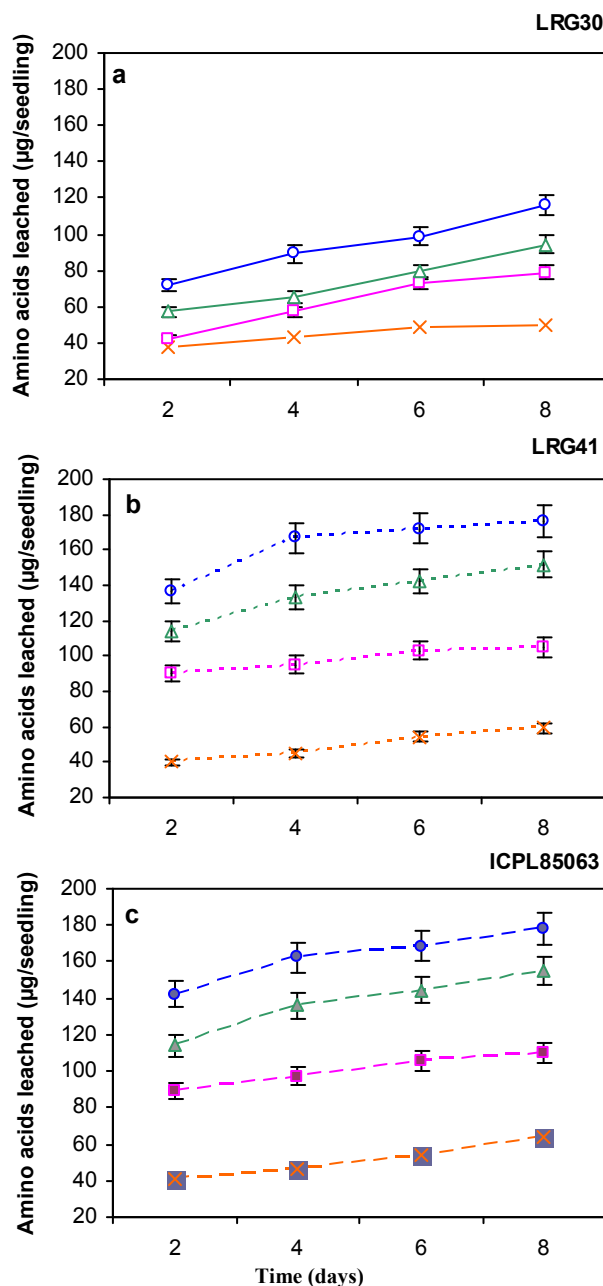
their appropriate controls. The leachates collected from the pigeonpea cultivar, LRG30 exhibited lower levels of amino acids when compared to LRG41 and ICPL85063 in response to Cd treatment.

LRG30

LRG41

ICPL85063

Fig 3 Leaching of amino acids of whole seedlings of the three pigeonpea cultivars LRG30, LRG41 and ICPL85063 in response to cadmium stress (Vertical lines represent S.E.).
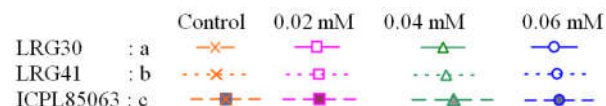
		Control	0.02 mM	0.04 mM	0.06 mM
LRG30	: a				
LRG41	: b				
ICPL85063	: c				

Amino acids: The amino acid content in the leachates of the three pigeonpea cultivars showed a gradual increase with increasing age of the seedling. The increasing concentrations of externally supplied Cd resulted in the increased leaching of amino acids from the seedlings of three pigeonpea cultivars and registered higher values when compared to their controls (Fig.3). The amino acid content in the leachates of 6-day old pigeonpea seedlings germinated and grown in 0.02, 0.04 and 0.06 mM Cd concentrations showed an increase of 1.51, 1.65 and 2.04 folds in LRG30; 1.90, 2.63 and 3.18 folds in LRG41 and 1.96, 2.67 and 3.12 folds in ICPL85063 respectively over
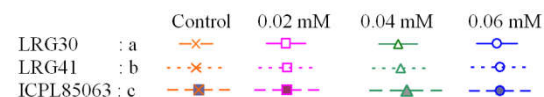
Total lipids

The total lipid content of the roots of the three pigeonpea cultivars increased slightly with increasing age of the seedlings in all the Cd treatments. However, the lipid content of the roots decreased with increasing concentrations of externally supplied Cd and registered lower values when compared to their controls (Fig.4a, b, c).
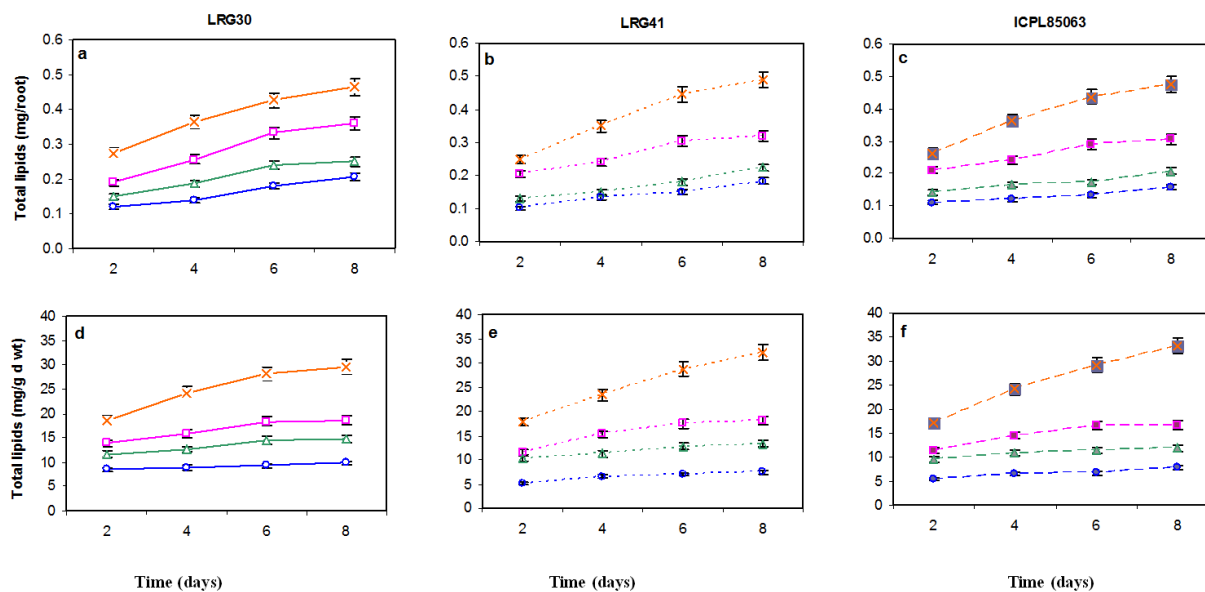
Fig 4 Total lipid content of roots of seedlings of the three pigeonpea cultivars LRG30, LRG41 and ICPL85063 in response to cadmium stress (Vertical lines represent S.E.).
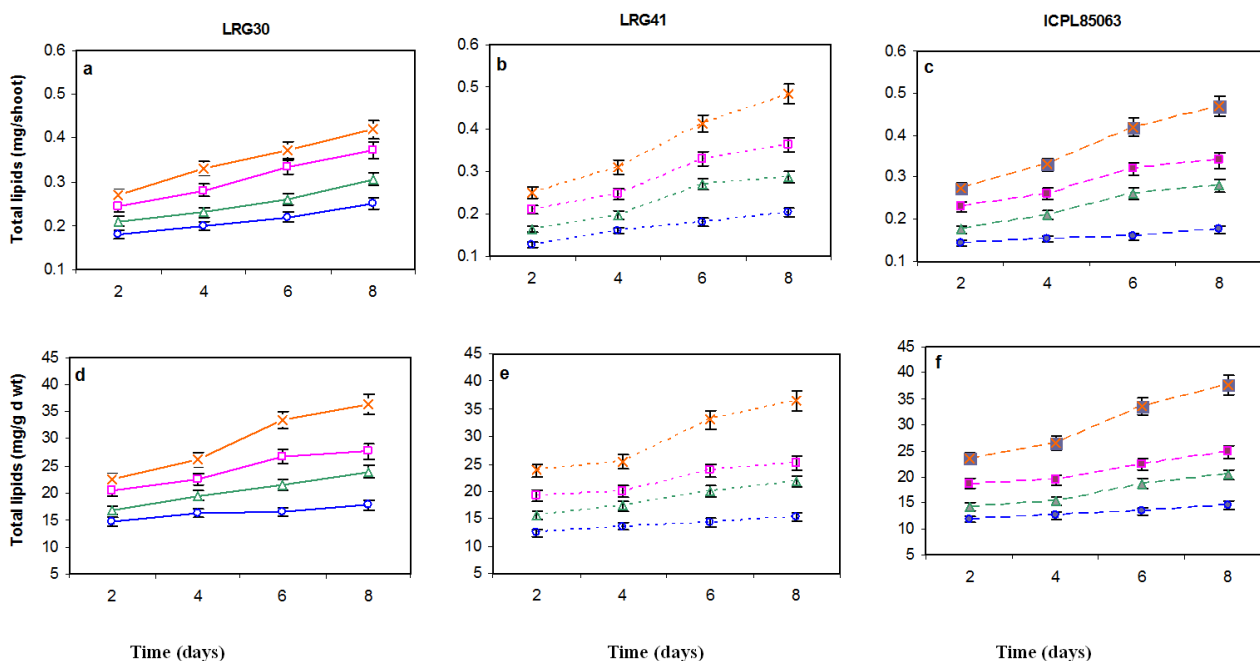

Fig 5 Total lipid content of shoots of seedlings of the three pigeonpea cultivars LRG30, LRG41 and ICPL85063 in response to cadmium stress (Vertical lines represent S.E.).

The total lipid content of the shoots of the three pigeonpea cultivars exhibited a trend similar to that observed for roots both with increasing seedling age and with increasing concentrations of externally supplied Cd (Fig.5a, b, c). On the other hand, the total lipid content of the cotyledons of the three pigeonpea cultivars decreased with increasing age of the seedling as well as with increasing concentrations of externally supplied Cd (Fig.6a, b, c). The total lipid content of the cotyledons of the Cd treated germinating seeds exhibited lower values when compared to their controls.

The per cent decrease in the total lipid content of the roots of the 6-day old pigeonpea seedlings germinated and grown in 0.02, 0.04 and 0.06 mM Cd concentrations were 23.26, 44.19 and 58.14% in LRG30; 33.34, 60 and 66.67% in LRG41 and 34.10, 61.37 and 70.46% in ICPL85063 when compared to their controls. The total lipid content of the shoots of the 6-day old pigeonpea seedlings grown in the respective Cd concentrations showed a decrease of 10.82, 29.73 and 40.55% in LRG30; 19.52, 34.15 and 56.10% in LRG41 and 23.81, 38.10 and 61.91% in ICPL85063 with respect to their controls.
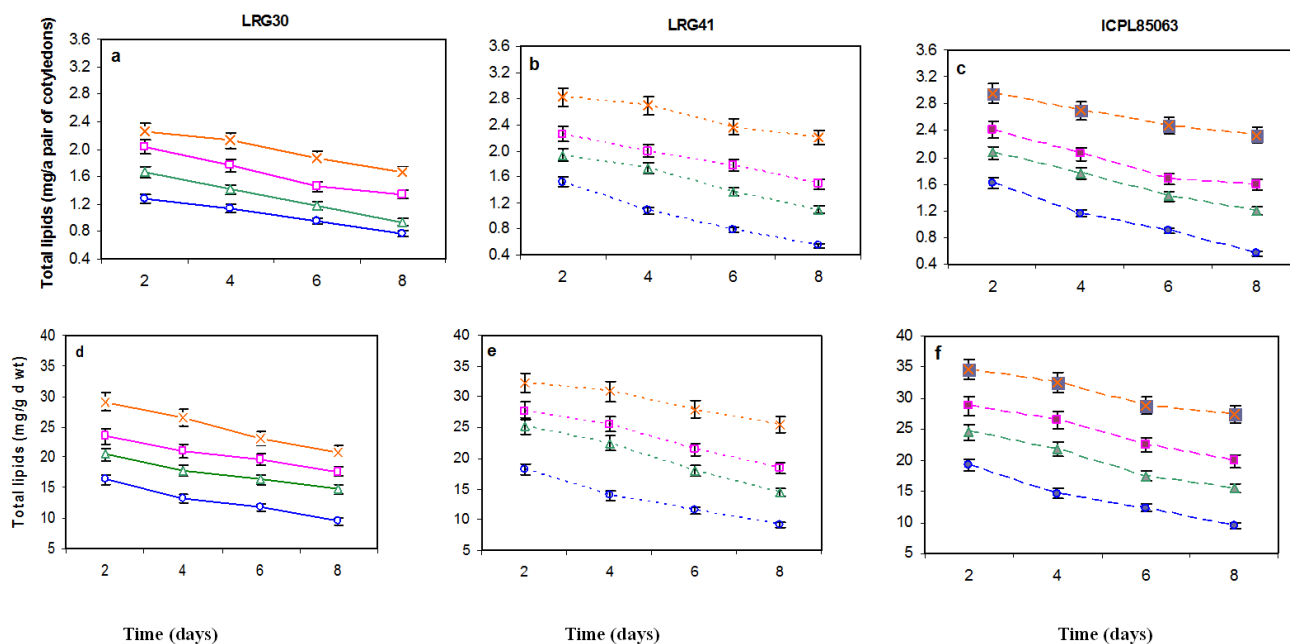
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Fig 6 Total lipid content of cotyledons of seedlings of the three pigeonpea cultivars LRG30, LRG41 and ICPL85063 in response to cadmium stress (Vertical lines represent S.E.).
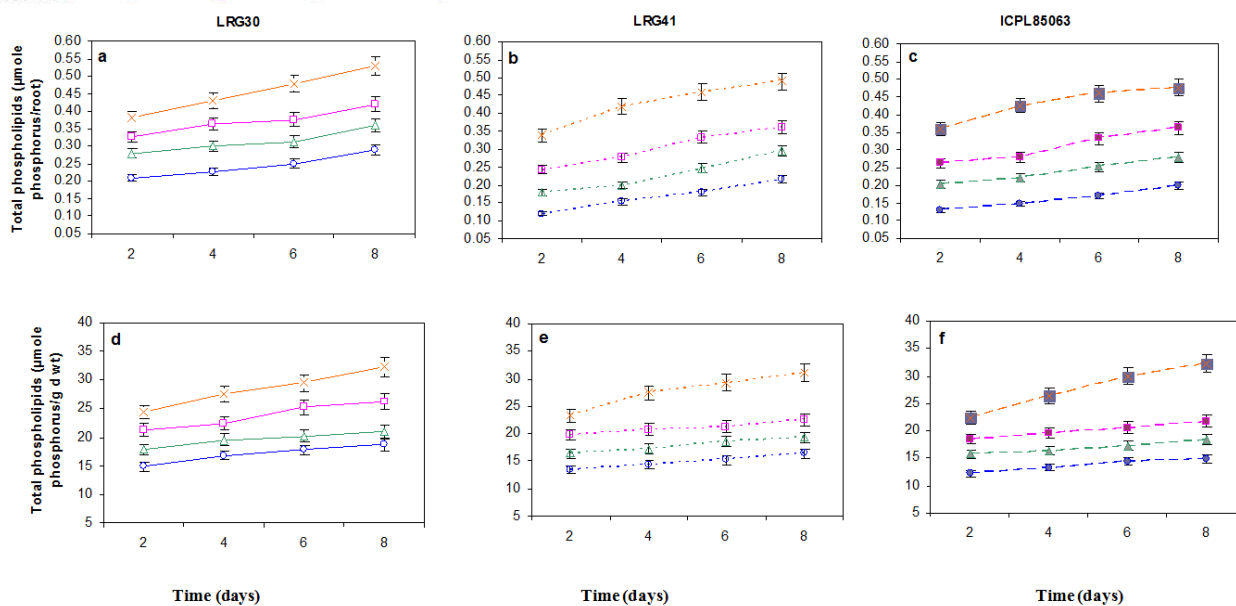

Fig 7 Total phospholipid content of roots of seedlings of the three pigeonpea cultivars LRG30, LRG41 and ICPL85063 in response to cadmium stress (Vertical lines represent S.E.).

The total lipid content of the cotyledons of the corresponding Cd treated germinating seeds showed a reduction of 21.93, 36.90 and 49.20% in LRG30; 24.58, 42.38 and 66.53% in LRG41 and 31.98, 42.52 and 63.57% in ICPL85063 when compared to their controls. Among the three cultivars of pigeonpea, LRG30 registered higher values of total lipid content than the LRG41 and ICPL85063 in response to Cd treatment. On dry weight basis, the changes in lipid content of the roots, shoots and cotyledons of the three pigeonpea cultivars exhibited a trend similar to per organ basis both with increasing seedling growth and with increasing concentrations

of externally supplied metal ions (Fig.4d, e, f; 5d, e, f and 6d, e, f).

Total phospholipids

The total phospholipid content of the roots of the pigeonpea seedlings grown in different concentrations of Cd showed a slight increase with an advance in seedling growth. However, the phospholipid content of the roots decreased with increasing concentrations of externally supplied Cd and the values always exhibited lower when compared to their controls (Fig.7a, b, c). The phospholipid content of the shoots and cotyledons of the pigeonpea seedlings exhibited a trend similar to roots with

increasing concentrations of externally supplied Cd (Fig. 8a, b, c and 9a, b, c). On the other hand, the phospholipid content of the shoots increased and the cotyledons decreased with increasing seedling age.

controls. The phospholipid content of the cotyledons of the respective Cd treated germinating seeds of pigeonpea showed

Fig 8 Total phospholipid content of shoots of seedlings of the three pigeonpea cultivars LRG30, LRG41 and ICPL85063 in response to cadmium stress (Vertical lines represent S.E.).
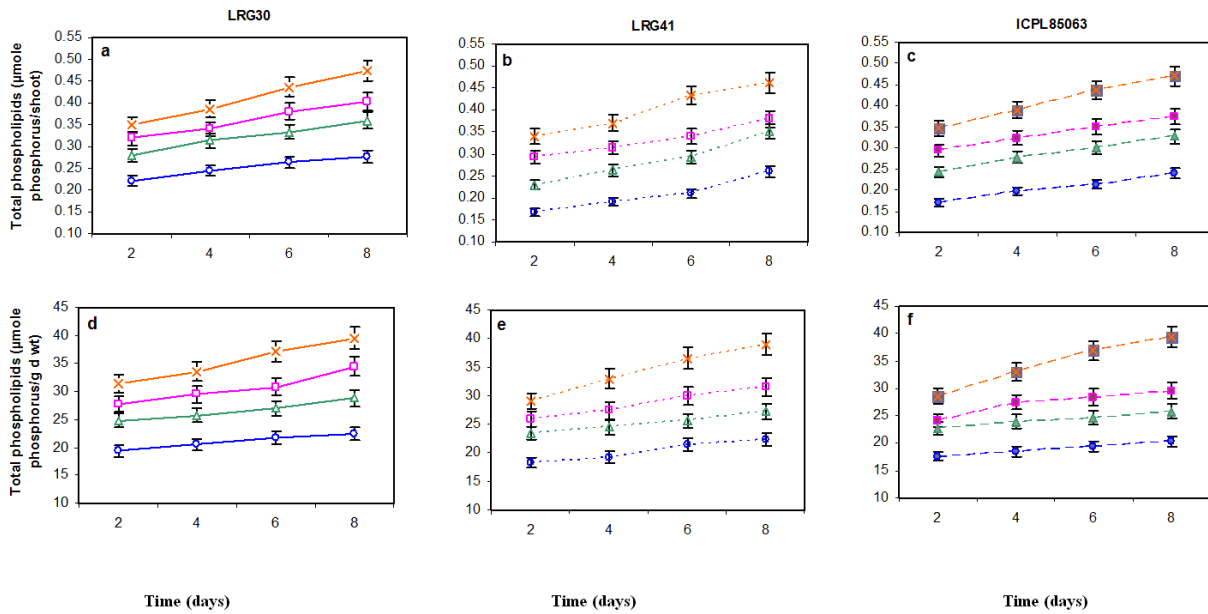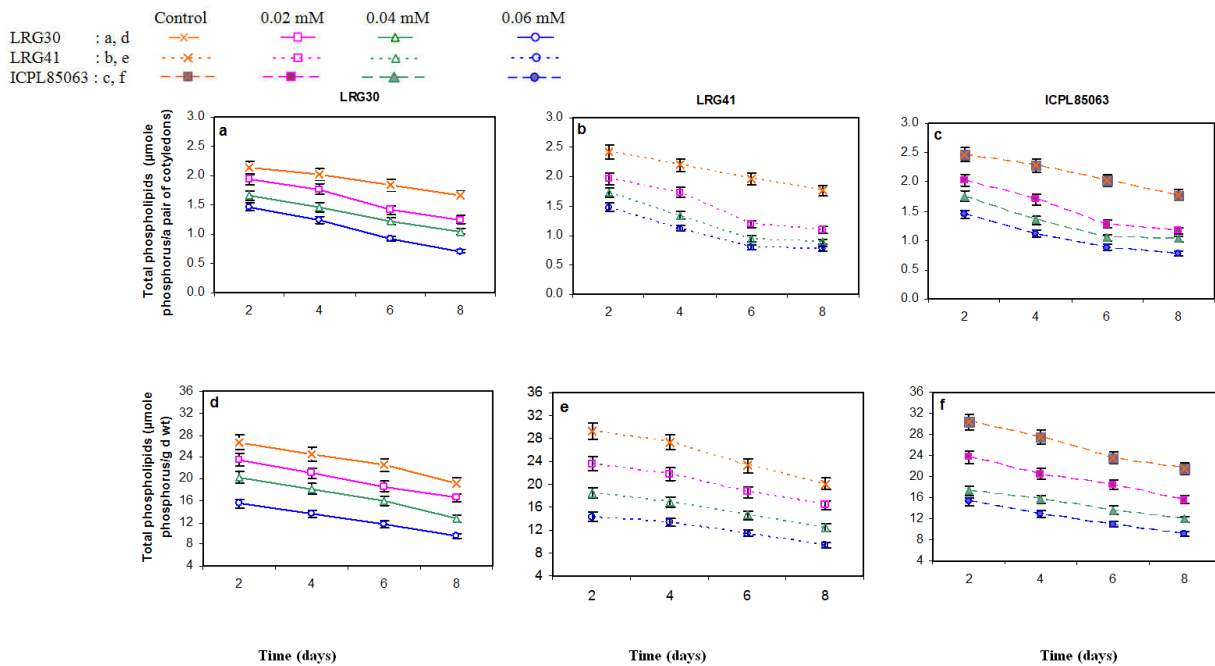

Fig 9 Total phospholipid content of cotyledons of seedlings of the three pigeonpea cultivars LRG30, LRG41 and ICPL85063 in response to cadmium stress (Vertical lines represent S.E.).

The per cent decrease in the phospholipid content of the roots of 6-day old pigeonpea seedlings germinated and grown in 0.02, 0.04 and 0.06 mM Cd concentrations were 20.84, 35.42 and 47.92% in LRG30; 28.27, 45.66 and 60.87% in LRG41 and 30.44, 45.65 and 63.05% in ICPL85063 when compared to their controls. The phospholipid content of the shoots of the 6-day old pigeonpea seedlings grown in the respective Cd concentrations showed a decrease of 13.64, 25 and 40.91% in LRG30; 20.93, 32.56 and 51.17% in LRG41 and 20.46, 31.82 and 52.28% in ICPL85063 with compared to their appropriate

a reduction of 22.83, 33.70 and 50% in LRG30; 39.80, 52.04 and 59.69% in LRG41 and 36.95, 48.28 and 56.66% in ICPL85063 when compared to their appropriate controls. Among the three cultivars of pigeonpea, LRG30 exhibited higher values of phospholipids than the LRG41 and ICPL85063 at all stages of seedling growth in response to Cd treatment. On dry weight basis, the changes in the phospholipid content of the roots, shoots and cotyledons of the three pigeonpea cultivars exhibited a trend similar to per organ basis with increasing seedling growth as well as with

increasing concentrations of externally supplied metal ions (Fig.7d, e, f; 8d, e, f and 9d, e, f).

DISCUSSION

The changes in the permeability of membranes to nonelectrolytes were studied in three pigeonpea cultivars in response to Cd treatments. The increase in the leakage of nonelectrolytes (sugars, organic acids and amino acids) from the three pigeonpea cultivars was observed with increasing concentrations of externally supplied metal ions (Fig.1-3). The main harmful effect of metals at the cellular level might be the alteration in the plasma membrane permeability (De Vos *et al.*, 1991). The evidence for metal ion-induced changes in membrane structure was obtained from the studies quantifying ion leakage from roots or cells exposed to a variety of metal ions. Metal exposure alters membrane structure, leading to metal ion permeation via nonbilayer structures. Metals may additionally transverse the plasma membrane via cationic and anionic carriers, subsequently depolarizes the cell electrical potential and may acidify the cytoplasm. This change in transplasma membrane electrical potential which acts as a general stimulus leading to net K^+ efflux (Silver and Misra, 1988; Cumming and Taylor, 1990). The control of membrane integrity is critical under heavy metal exposure due to oxidation and crosslinking of protein thiols and inhibition of plasmalemma ATPase which in turn inhibits proton efflux (Meharg, 1993; Vangronsveld and Clijsters, 1994). The interaction between Cd and the membrane constituents may result in the alteration of membrane fluidity, which in turn effects the activities of membrane bound enzymes (Ros *et al.*, 1992). Kennedy and Gonsalves (1989) showed that the supply of cations like Zn^{2+}, Cd^{2+}, Hg^{2+}, Cu^{2+} and Pb^{2+} to maize roots causes an inhibition of Mg^{2+}-ATPase activity and also affects ATPase related processes such as H^+ ion efflux and transmembrane and transroot potentials. Pedersen and Carafoli (1987) suggested that the leakage of potassium and other ions through plasma membranes may be attributed to the decreased activity of plasma membrane ATPase. Dhindsa *et al.* (1981) reported that the regulation of plasma membrane permeability depends on the degree of lipid peroxidation. The loss of membrane controlled permeability is initiated by free radicals such as OH generated in the Haber-Weiss reaction and also due to peroxy and alkoxy radicals, which arise from the decomposition of organic hydroperoxides. Heavy metals initiate the formation of these harmful free radicals and might therefore cause oxidative stress in cells (Halliwell and Gutteridge, 1984; Aust *et al.*, 1985; Girotti, 1985). The pigeonpea cultivar, LRG30 exhibited comparatively low levels of nonelectrolyte leakage than LRG41 and ICPL85063 suggesting that LRG30 was less effected by Cd treatment.

Lipids form an important component of cell membranes. Changes in membrane lipid composition under heavy metal stress conditions can alter the fluidity of membranes integrity. Changes in lipid composition by heavy metal treatment were studied by several workers (Maksymiec and Baszynski, 1988). Under a variety of stress conditions, the lipid composition of root cells is known to alter markedly, which includes changes in the level of unsaturation of the fatty acids and changes in the total and relative abundance of the various phospholipid classes of cells and plasma membranes (Svenningsson and Lilijenberg, 1986; Norberg and Lilijenberg, 1991). Such changes inevitably affect the phase transition temperature of the membranes which in turn, could influence the activity of a

number of membrane bound enzymes (Cook *et al.*, 1989). Cadmium may induce premature senescence of leaves and it results in the progressive degradation of thylakoid membrane lipids (Fong and Heath, 1977). In addition to the lipid component, phospholipids also play an important role in the structure and functions of plant cells. It is presumed that the reduction of lipids and phospholipids in response to Cd exposure may be a consequence of their reduced synthesis or increased lipase activity or lipid peroxidation, either individually or in different combinations (Summerfield and Tappel, 1984). The loss in membranes integrity is often caused by an increase in saturation of membrane phospholipids and a decrease in lipid and phospholipids contents. The studies on the cultivars of pigeonpea clearly suggest that membrane deterioration as indicated by a decline in total lipids and phospholipids and consequent loss of membrane integrity represents an important physiological event associated with Cd. The total lipid content of the roots and shoots of the three pigeonpea cultivars decreased with increasing concentrations of externally supplied Cd ions. With increasing age of the seedlings the total lipid content of the roots and shoots however, increased slightly (Fig.4 and 5). On the other hand, the total lipid content of the cotyledons decreased with increasing age of the seedlings as well as with increasing concentrations of externally supplied metal ions (Fig.6). The primary target for metal action at the cellular level might be the plasma membrane since this barrier was the first functional structure encountered by a metal ion penetration into the cell and thus causing membrane destabilisation by interfering with the membrane proteins and lipids (De Vos *et al.*, 1989; 1991). This effect is generally attributed to lipid peroxidation (Mayak *et al.*, 1983). In addition, certain changes in the membrane lipids were reported to be an adaptive response to different environmental stresses, in order to restore optimum physical membrane properties (Ben Ammar *et al.* 2007; Nouairi *et al.* 2006; Morsy *et al.* 2012).

The total phospholipid content of the three pigeonpea cultivars was also decreased considerably in response to Cd treatments and the values were always lower than their respective controls (Fig.7-9). Previous results indicated that cadmium stress decreased the total lipids and phospholipids in pepper (Jemal *et al.* 2000) and cucumber (Janicka *et al.* 2008). Changes in plasma membrane structure and selectivity could also result from the interaction of metal ions with membrane phospholipids. The presence of calcium as a structural component of membrane phospholipids reduces nonselective permeability and its displacement by metal ions may affect membrane function (Korner *et al.*, 1985; Landau and Loshem, 1988). Among the three cultivars of pigeonpea, LRG30 registered higher values of both total lipid and phospholipid contents than the LRG41 and ICPL85063 in response to Cd treatment.

CONCLUSIONS

Leakage of nonelecrolytes increased in the three pigeonpea cultivars with increasing age of the seedlings as well as increasing concentrations of externally supplied Cd ions. The total lipid and phospholipid contents of the roots and shoots of the three pigeonpea cultivars increased slightly with increasing age of the seedlings and decreased sharply with increasing concentrations of externally supplied Cd. The total lipid and phospholipid contents of the cotyledons decreased with increasing age of the seedlings and with increasing

concentrations of externally supplied Cd ions. The decrease of lipid and phospholipid contents was more conspicuous in cv.LRG41 and ICPL85063 than LRG30.

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