ISSN: 0976-3376

Available Online at http://www.journalajst.com

ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

Asian Journal of Science and Technology
Vol. 08, Issue, 08, pp.5276-5284, August, 2017

RESEARCH ARTICLE

DIFFERENTIAL TOXICITY OF CADMIUM ON NITROGEN METABOLISM AND ENZYMATIC ACTIVITIES IN *CAJANUS CAJAN* L. SEEDLINGS

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ARTICLE INFO

Article History:
Received 20th May, 2017
Received in revised form
18th June, 2017
Accepted 20th July 2017
Published online 31st August, 2017

Key words:

Cadmium, Nitrate reductase,
Nitrogen metabolism,
Pigeonpea cultivars, Stress.

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 total nitrogen content, nitrate reductase, malate dehydrogenase and glycolate oxidase activities were studied. Results obtained show that the pigeonpea cultivar, LRG30 registered higher values of nitrogen content in response to Cd treatment. The nitrate reductase activity and glycolate oxidase were affected more in the pigeonpea cultivars LRG41 and ICPL85063 in response to Cd treatment. The malate dehydrogenase, exhibited lower values in the pigeonpea cultivars, LRG41 and ICPL85063 than in LRG30 in response to Cd treatments. The effects were more conspicuous under Cd treatment.

INTRODUCTION

Industrial activity combined with a low conscience of the consequences of environmental pollution during a long period created a worldwide problem of soil, air and water contamination with various pollutants. Heavy metals are among the most widespread soil contaminants. The effects of certain heavy metals such as Cd, Hg and Pb on cell systems have received attention in recent decades due to the increasing exposure of living organisms to these metals in the environment (Cavallini *et al*., 1999). Among these, cadmium is generally considered as a non-essential heavy metal and its presence in the environment is essentially due to anthropogenic activities (Stohs and Bagchi, 1995). Cadmium has been considered as an extremely significant pollutant due to its high toxicity and great solubility in water. It can reach high levels in agricultural soils and is easily assimilated by plants (Lagriffoul *et al*., 1998). During seed germination seed proteins are extensively hydrolysed and the released amino acids are translocated to embryonic axis for the synthesis of protein and other nitrogenous compounds. Consequently in germinating seeds the total nitrogen in the storage tissues get depleted with an accompanying increase in the embryonic axis (Mayer and Poljakoff-Mayber, 1975).

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Nitrogen plays important roles in plant growth and development, and its metabolism affects all levels of plant function. However, nitrogen metabolism requires a complex series of biochemical reactions. For example, in nitrate assimilation, the nitrate is converted to NO_2^- by nitrate reductase (NR), and NO_2^- is converted to NH_4^- N by nitrite reductase (NiR) (Stitt *et al.*, 2002; Mokhele *et al.*, 2012). In plants, Cd toxicity is associated with growth inhibition and imbalances in many macro- and micronutrient levels (Priyadarshini and Sujatha, 2015). The presence of Cd in plants results in many physiological alterations affecting nitrogen metabolism (Hernandez *et al*., 1997; Chaffei *et al*., 2003). It has been shown that enzymes of nitrogen metabolism are differently affected by Cd stress (Petrovic *et al*., 1990; Chugh *et al*., 1992; Singh *et al*., 1994). Glutamine plays a central role in nitrogen metabolism of germinating seeds. The available evidence suggests that nitrogen from cotyledons is translocated to embryonic axis mostly in the form of glutamine. The nitrate reductase (NR) activity was considered as a rate limiting step in the overall process of nitrate assimilation (Srivastava, 1980; Singh *et al*., 1988). Pronounced inhibition of *in vivo* NR activity in the leaves of *Helianthus pennesetum* (Venketramana *et al*., 1978) and *Zea mays* (Sinha *et al*., 1988) has been observed under toxic levels of Cd. Nitrate reductase activity is significantly decreased, leading to reduced nitrate assimilation by plants. The changes observed in nitrogen metabolism in Cd-stressed plants are similar to

changes observed during natural senescence (Mae *et al*., 1983; Masclaux *et al*., 2000). Binding of heavy metals to nitrate reductase and it is interesting to note the vacuolar storage of nitrate in relation to heavy metal treatment (Gransted and Huffaker, 1982; Rauser and Ackerley, 1987; Jain and Gadre, 1997). Inhibition of absorption and assimilation processes of mineral nitrogen constitute one of the reasons that may be attributed to heavy metal phytotoxicity, but studies undertaken in this field remain rather limited. Some studies have shown the existence of an inhibitive effect of cadmium (Cd) upon the content of nitrogen as well as upon enzyme activity such as NR (Chugh *et al*., 1992; Boussama *et al*., 1996, 1999; Ouariti *et al*., 1997; Hernandez *et al*., 1997; Gouia *et al*., 2000). $NADP^+$ and NAD^+- dependent malate dehydrogenase (MDH) enzyme is likely to play an important role in the synthesis of metabolite, implicated in the amino-acid production under stress condition. Glycolate oxidase is a key enzyme for photorespiration that is metabolically coupled with photosynthetic CO_2 assimilation (i.e. the Calvin cycle). Glycolate oxidase catalyses the oxidation of glycolate with equimolar amounts of glyoxylate and hydrogen peroxide produced. As characterized by CO_2 release, photorespiration is apparently counterproductive to the Calvin cycle and may account for at least 20% loss of net CO_2 assimilation in C_3 plants (Peterson, 1983; Sharkey, 1988). However, the related research on the responses of pigeonpea (*Cajanus cajan* (L.) Millspaugh) to Cd stress is scarce. Thus, it is of importance, to study the effects of Cd exposure related to nitrogen metabolism and enzymatic activities of NR, MDH and glycolate oxidase in different pigeonpea cultivars.

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 µmol $m^{-2}s^{-1}$. 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.

Total nitrogen content

Total nitrogen was determined according to the method of Markham (1942). One gram of dried and powdered material was taken in a 25 ml micro-kjeldhal flask taking care not to allow the material to stick to the sides of the flask. One gram of catalyst (a mixture of 1 g copper sulphate, 9 g potassium sulphate and 1 g selenium dioxide) was added for aiding digestion. Three ml of nitrogen-free analar sulphuric acid and 1ml of hydrogen peroxide were also added to the sample and it was digested on a hot plate until a clear colourless solution was obtained. The volume of the solution was made up to 25 ml in a volumetric flask after digestion. Blank with reagents alone was also carried out simultaneously. Five ml of the aliquot of the digest was transferred to the distillation unit and 10 ml of 40% sodium hydroxide was added. This solution was distilled for 20 min in the micro-kjeldhal distillation apparatus. The ammonia liberated was absorbed into 2 ml of boric acid indicator mixture kept below in a conical flask. The completion of the distillation was recognized by the change in pH of the indicator in the receiver. The indicator solution was pink in the beginning and turned green at the end of complete distillation. The solution containing the indicator was titrated against N/100 HCl until pink colour reappears. The amount of nitrogen present in the sample was calculated thus:

1ml of N/100 HCl = 0.14 mg of nitrogen.

Preparation of boric acid indicator mixture: The boric acid indicator mixture was prepared by mixing 10 g of boric acid, 200 ml of absolute alcohol and 20 ml of indicator solution (indicator solution was prepared by mixing 0.033 g of bromocresol green and 0.666 g of methyl red in 100 ml of absolute alcohol) in a litre flask and the final volume was made to 1 litre with distilled water. The pH of the solution was then adjusted to 5.0 to 5.1.

Nitrate reductase (*in vivo*) activity (E.C.1.6.6.1) Nitrate reductase (*in vivo*) activity of control and treated samples were estimated according to the method of Jaworski (1971) as modified by Dykstra (1974). Five hundred mg of material was chopped into pieces and placed in a 5 ml of incubation medium consisting of 0.1 M K_2HPO_4, 0.2 M KNO_3, 0.5% w/v PVP (polyvinyl pyrrolidone) and 5% (v/v) isopropanol having pH of 7.5. The material along with incubation medium was kept in dark for 2 h at room temperature. Then the reaction was stopped by adding 1 ml of 0.02% (w/v) N, 1-napthylethylenediamine-2HCl and 1 ml of 1.0% (w/v) sulphanilic acid in 1.5 N HCl. After 20 min the absorbance of the solution was read at 540 nm on Milton Roy Spectronic 1201 UV spectrophotometer. The nitrate reductase activity was expressed as µ moles of nitrite formed per g tissue per hour. The standard curve was prepared by using analar $NaNO_2$.

Malate dehydrogenase (E.C.1.1.1.37)

The enzyme extract was prepared according to the method followed by Crookston *et al*., (1974) and the assay was carried out by the method of Heddley and Stoddart (1971). The readings were taken on Milton Roy Spectronic 1201 UV-spectrophotometer. The activity was expressed as µ moles per min per mg protein.

Glycolate oxidase (E.C.1.1.3.1)

The enzyme extract was prepared according to the method followed by Crookston *et al*., (1974) and the assay was carried out by the method of Heddley and Stoddart (1971). The assay mixture consisted of 2.5 ml of 0.1 M KH_2PO_4 buffer (pH 7.4), 0.3 ml of 0.05 M phenylhydrazine HCl and 0.05 ml of 0.1 M glycolate. To this, 0.5 ml of enzyme extract was added and the production of phenylhydrazone was measured at 324 nm on Milton Roy Spectronic 1201 UV-spectrophotometer.

Statistical analysis

The data presented in this work are the average of at least five replicates per treatment; means ± standard error (S.E) are given in the figures. Each experiment was carried out in duplicate. According to the Tukey test, values ± 0.05 were considered significantly different.

RESULTS

Total nitrogen content

The total nitrogen content of the roots of the Cd treated pigeonpea cultivars showed an increase from 2 to 8 days of seedling growth. However, the total nitrogen content of the roots of the pigeonpea seedlings decreased in relation to increasing concentrations of externally supplied Cd and the content always registered lower values when compared to their controls (Fig.1 A,B,C). The nitrogen content of the shoots of the Cd treated germinating seeds of pigeonpea exhibited a trend similar to that exhibited by roots both with increasing seedling age and with increasing concentrations of externally supplied Cd (Fig. 2 A,B,C).

The nitrogen content of the cotyledons of the Cd treated germinating seeds of three pigeonpea cultivars declined gradually, with increasing seedling growth and however, retained in them higher amounts when compared to their controls (Fig. 3 A,B,C). The per cent decrease in the total nitrogen 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 10.72, 28.58 and 39.29 in cv. LRG30, 12.0, 28.0, 36.0 in cv. LRG41 and 40.0, 52.0, and 60.0 in cv. ICPL85063 respectively in relation to their controls.

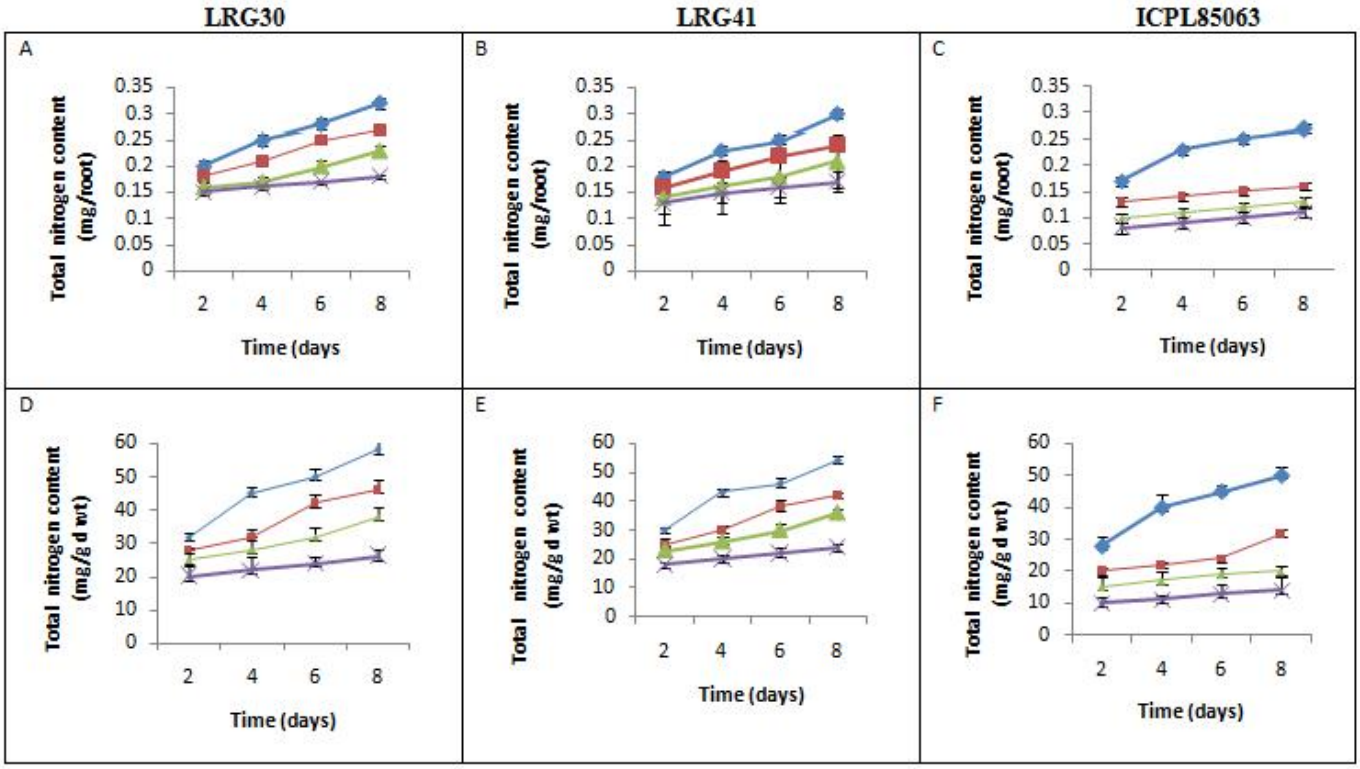

Figure 1. Total nitrogen content of roots of seedlings of the three pigeonpea cultivars LRG30, LRG41 and ICPL85063 in response to cadmium stress (Vertical lines represent S.E.)

The total nitrogen content of the shoots of the respective Cd treated germinating seeds of three pigeonpea cultivars showed a decrease of 20.0, 30.0 and 40.0% in LRG30, 21.43, 32.15, 42.86 in cv. LRG41 and 33.34, 44.45 and 51.86% in ICPL85063 in relation to their controls. The retention in the nitrogen content of the cotyledons of the respective Cd treated germinating seeds of pigeonpea showed an increase of 1.83, 2.67 and 3.33 folds in cv. LRG30, 1.8, 2.8, 3.6 cv. LRG41 and 1.45, 2.0 and 2.67 folds in cv. ICPL85063 when compared to their corresponding controls. The pigeonpea cultivar, LRG30 registered higher values of nitrogen content when compared to cv. LRG41 and ICPL85063 in response to Cd treatments. On per unit dry weight basis, the changes in the total nitrogen content of the roots, shoots and cotyledons of the three pigeonpea cultivars exhibited a trend similar to per organ basis with increasing seedling age as well as with increasing concentrations of externally supplied metal ions (Figs. 1 D,E,F; 2 D,E,F and 3 D,E,F).

Nitrate reductase activity

The *in vivo* nitrate reductase activity of the roots of the control seedlings increased continuously from 2 to 8 days of seedling growth.

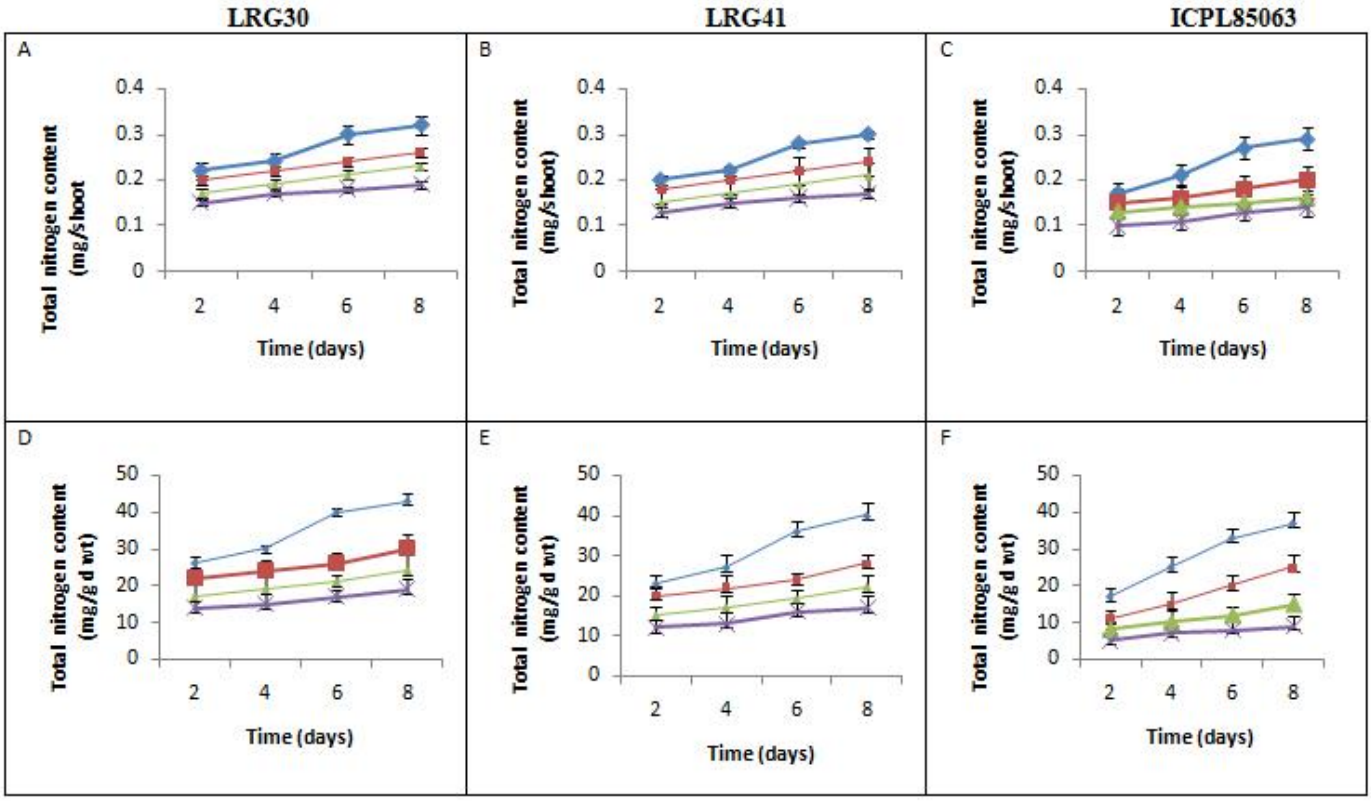

Figure 2. Total nitrogen content of shoots of seedlings of the three pigeonpea cultivars LRG30, LRG41 and ICPL85063 in response to cadmium stress (Vertical lines represent S.E.)

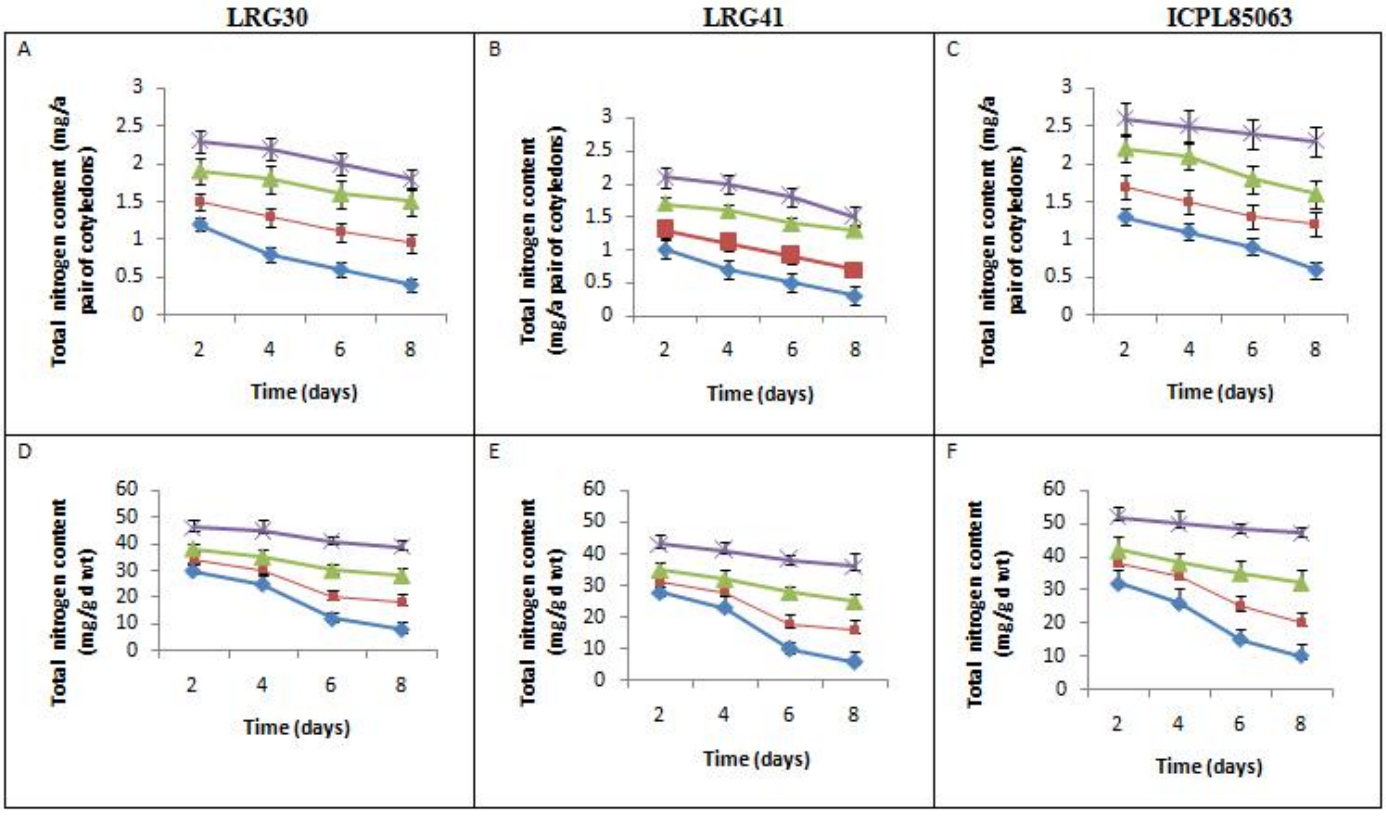

Figure 3. Total nitrogen content of cotyledons of seedlings of the three pigeonpea cultivars LRG30, LRG41 and ICPL85063 in response to cadmium stress (Vertical lines represent S.E.)

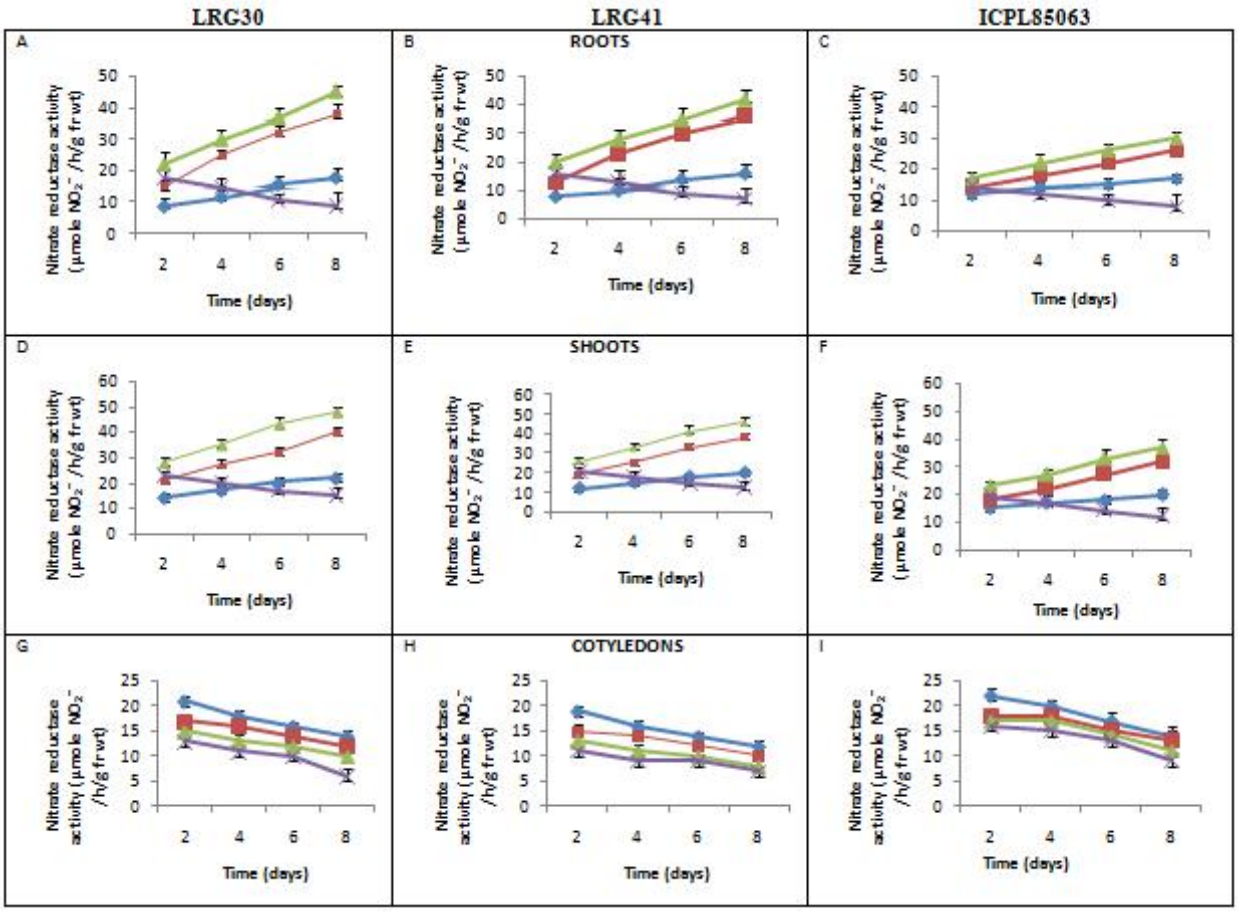

Figure 4. Nitrate reductase activity of roots (A,B,C), shoots (D,E,F) and cotyledons (G,H,I) of seedlings of the three pigeonpea cultivars LRG30, LRG41 and ICPL85063 in response to cadmium stress (Vertical lines represent S.E.)

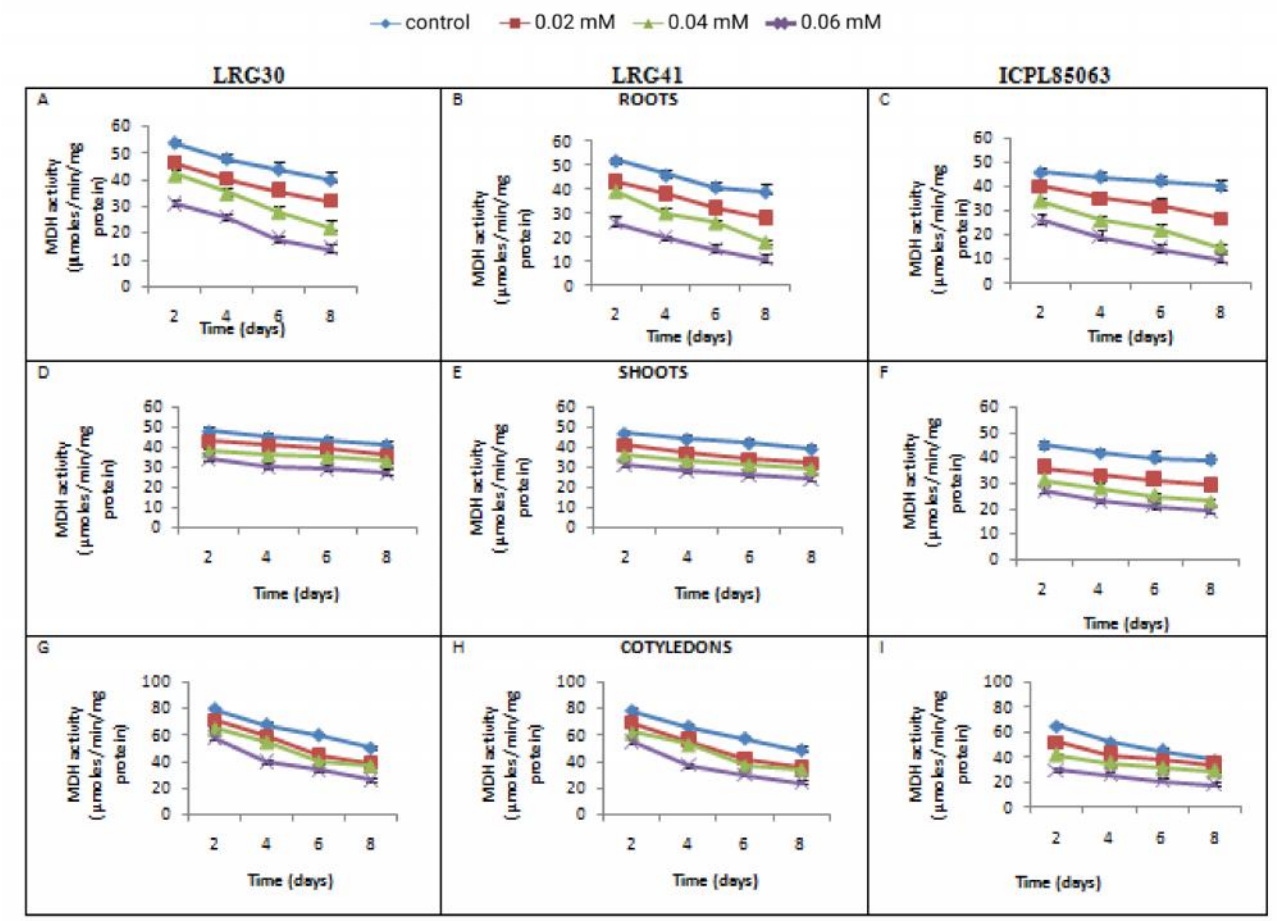

Figure 5. Malate dehydrogenase activity of roots (A,B,C), shoots (D,E,F) and cotyledons (G,H,I) of seedlings of the three pigeonpea cultivars LRG30, LRG41 and ICPL85063 in response to cadmium stress (Vertical lines represent S.E.)

control 0.02 mM 0.04 mM 0.06 mM

Among the Cd treated germinating seeds of pigeonpea the 0.02 and 0.04 mM Cd concentrations of a continuous increase in the *in vivo* nitrate reductase activity of the roots. Cd at 0.06 mM concentration exhibited a decrease in the nitrate reductase activity of roots with increasing seedling growth (Fig. 4 A,B,C). Nitrate reductase activity of the roots of the Cd treated germinating seeds of three pigeonpea cultivars registered higher values at all stages of seedling growth when compared to their corresponding controls. However, the roots of 0.06 mM Cd treated germinating seeds of pigeonpea showed lower levels of nitrate reductase activity in 6- and 8-day old seedlings of cv.LRG30 and 4-, 6- and 8-day old seedlings of cv.LRG41 and ICPL85063 when compared to their respective controls (Fig. 4 A,B,C). The changes in the *in vivo* nitrate reductase activity of the shoots of the three pigeonpea cultivars exhibited a trend similar to that observed for roots both with increasing seedling growth as well as with increasing concentrations of the externally supplied Cd. However, the shoots of the 0.06 mM Cd treated germinating seeds of the three pigeonpea cultivars showed lower levels of enzyme activity in 6- and 8-day old seedlings when compared to their appropriate controls (Fig. 4 D,E,F). On the other hand the nitrate reductase activity of the cotyledons of the three pigeonpea cultivars decreased with increasing seedling age as well as with increasing concentrations of externally supplied Cd and registered lower levels when compared to their controls (Fig. 4 G,H,I).

The per cent increase(+) or decrease(-) in the *in vivo* nitrate reductase activity of the roots of 6 day old pigeonpea seedlings germinated and grown in 0.02, 0.04 and 0.06 mM Cd concentrations were +100.0, +131.25 and -31.25 in cv.LRG30, +114.28, +150.0, -35.72 in cv.LRG41 and +46.67, +73.34 and -33.34 in cv. ICPL85063 respectively with respect to their corresponding controls. The per cent increase (+) or decrease (-) in the nitrate reductase activity of the shoots of the respective Cd treated germinating seeds were +60.0, +115.0 and -15.0 in cv. LRG30, +83.34, +127.78, -16.67 in cv.LRG41 and +50.0, +83.34 and -22.23 in cv. ICPL85063 when compared to their controls. The nitrate reductase activity of the cotyledons of the respective Cd treated germinating seeds of pigeonpea showed a reduction of 12.5, 25.0 and 37.5% in cv. LRG30, 14.3, 28.6, 35.72 in cv.LRG41 and 11.77, 17.65 and 23.53% in cv. ICPL85063 in relation to their controls. The *in vivo* nitrate reductase was more active in cv. LRG30 when compared to cv. LRG41 and ICPL85063 in response to Cd treatment.

Malate dehydrogenase activity

A gradual decline in the malate dehydrogenase activity of the roots of Cd-treated germinating seeds of pigeonpea was observed from 2 to 8 days of seedlings germinated. The malate dehydrogenase activity of the roots decreased with increasing concentrations of externally supplied Cd and registered lower values when compared to their controls (Fig. 5 A,B,C). The malate dehydrogenase activity of the shoots and cotyledons of the three pigeonpea cultivars grown in different concentrations of Cd exhibited a trend similar to that exhibited by roots. (Fig.5 D,E,F,G,H,I). The per cent decrease in the malate dehydrogenase activity of the roots of 6-day old seedlings of the pigeonpea cultivar LRG30 germinated and grown in 0.02, 0.04 and 0.06 mM Cd concentration were 18.2, 36.37 and 59.1, 21.96, 36.6 and 63.42 in cv.LRG41 and the roots of cv. ICPL85063 showed a decrease of 23.8, 47.62 and 66.67, respectively in relation to their controls. The malate dehydrogenase activity of the shoots of the respective Cd treated germinating seeds exhibited a per cent reduction of 9.3, 18.6 and 32.56 in cv. LRG30, 19.05, 26.2 and 38.1 in cv.LRG41 and 22.5, 37.50 and 47.5 in cv. ICPL85063 when compared to their controls. The cotyledons of the respective Cd treated as also showed a decrease in the malate dehydrogenase activity with a reduction of 25.0, 33.34 and 45.0% in cv. LRG30, 26.32, 35.1 and 47.37 in cv.LRG41 and 15.56, 31.12 and 53.34% in cv. ICPL85063 in relation to their controls. Comparatively the different parts of the pigeonpea cultivars LRG41 and ICPL85063 exhibited lower levels of malate dehydrogenase activity in response to Cd treatment.

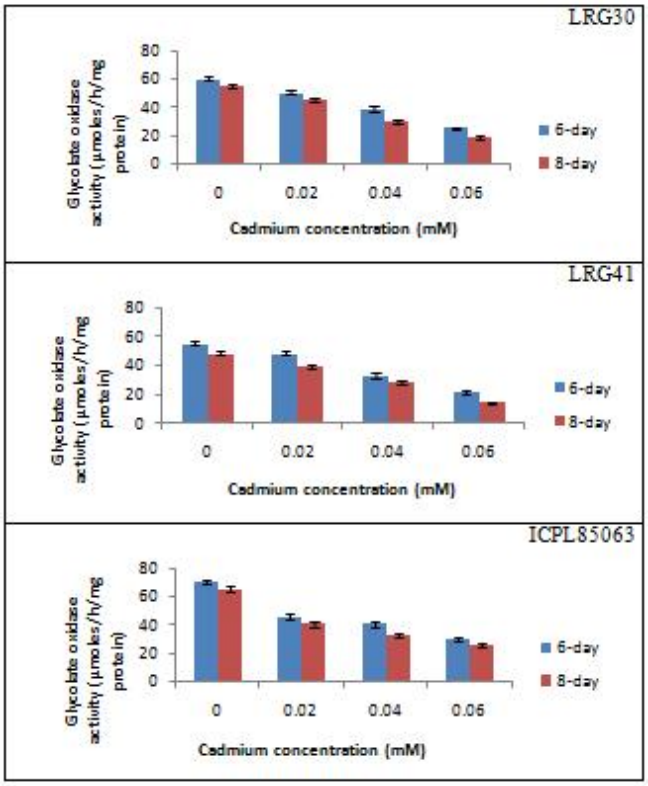

Figure 6. Glycolate oxidase activity of shoots of seedlings of the three pigeonpea cultivars LRG30, LRG41 and ICPL85063 in response to cadmium stress (Vertical lines represent S.E.)

Glycolate oxidase activity

Glycolate oxidase activity was decreased in all the Cd treatments from 6 to 8 days of seedling growth. Cd treatment led to a sharp decrease in the glycolate oxidase activity of the three pigeonpea cultivars when compared to their controls and it became more conspicuous with increasing concentration of externally supplied Cd. The changes in the glycolate oxidase activity of the shoots of pigeonpea exhibited a trend similar to that observed for Cd treatments (Fig. 6). The per cent decrease in the glycolate oxidase activity of the shoots of 6-day old pigeonpea seedlings germinated and grown in 0.02, 0.04 and 0.06 mM Cd concentrations were 16.67, 36.67 and 58.34 in cv. LRG30, 12.73, 41.82 and 61.82 in cv.LRG41 and 35.72, 42.86 and 57.15% in cv. ICPL85063 respectively in relation to their controls. The reduction of glycolate oxidase activity was more evident in cv. LRG41 and ICPL85063 than in cv.LRG30 in response to Cd treatment.

DISCUSSION

Nitrogen metabolism is a prerequisite for normal plant growth and development because almost all 'vital' biomolecules are nitrogenous substances (Mokhele *et al.,* 2012). The forms and concentrations of nitrogen within a plant vary widely according to the organ, developmental stage, and environmental conditions. The root is obviously the predominant organ where many exchanges of a variety of nitrogen forms occur between root cells and the soil solution (Stitt *et al.,* 2002). The total nitrogen content of the roots and shoots of the three pigeonpea cultivars increased slightly in relation to increasing seedling age but decreased in relation to increasing concentrations of externally supplied Cd (Figs. 1 and 2). NO_3^-, NO_2^- and NH_4^+ concentrations in leaves are significantly reduced by heavy metal stresses in tobacco, and spinach plants (Wu *et al.,* 2008; Maaroufi Dguimi *et al.,* 2009). The total nitrogen content in the cotyledons of the Cd treated germinating seeds of three pigeonpea cultivars decreased gradually with an advance in seedling growth and retained in them greater quantity of nitrogen when compared to their controls (Fig. 3). Mittal and Sawhney (1990) reported that the diminished activity of glutamine synthetase due to heavy metal treatment would impair the capacity of the seeds to synthesize glutamine and would consequently interfere with the supply of nitrogen from cotyledons to the growing enzyme activity. In addition, the heavy metal would cause disturbances in nitrogen metabolism due to deleterious effects on the activities of aminotransferases and proteases (Mittal and sawhney, 1990). The pigeonpea cultivars, LRG30 registered higher values of nitrogen content when compared to cv. LRG41 and ICPL85063 in response to Cd treatment.

Nitrate reduction, catalysed by nitrate reductase utilizing NADH as reducing agent is the primary entry point for the incorporation of inorganic nitrogen (Srivastava, 1980; Singh *et al*., 1988). The *in vivo* nitrate reductase activity of the roots and shoots of the three pigeonpea cultivars increased of the lower concentration and decreased at the higher concentration of Cd employed (Figs. 4 A,B,C,D,E,F). However, the nitrate reductase activity of the cotyledons decreased in all the Cd treatments and registered lower values when compared to their controls (Fig. 4 G,H,I). Inhibition of nitrate reductase by heavy metal ions has been a general observation in several plant species (Sinha *et al*., 1988, 1989; Burzynski, 1990; Kumar *et al*., 1993; Bharti *et al*., 1996). The inhibition of nitrate reductase may be due to reduced supply of NADH (Gengen Bach *et al*., 1973), disorganization of chloroplasts (Rebechini and Hanzely, 1974), water stress created by the metal, decreased NO_3^{2-} supply to the site of the enzyme synthesis (Burzynski and Grabowski, 1984) and a direct effect of the metal on the enzyme related protein synthesis as it is a strong affinity for any functional SH-group of the enzyme (Prasad and Prasad, 1987). Reduction of *in vivo* production of NADH due to reduced rates of photosynthesis (Bazzaz and Govindjee, 1974) and respiration (Reese and Roberts, 1985) in the presence of Cd has been reported. Moreover, Cd may stimulate NADH oxidation (Bittell *et al*., 1974) and subsequent reduction in NADH pool availability to the nitrate reductase activity. Further, the phytotoxic response to Cd was more pronounced in the roots than in the shoots, which may be attributed to a restricted translocation of the metal to the site of enzyme activity in the shoots (Fujita and Kawanishi, 1987). It is interesting to note vacuolar storage of nitrate in relation to heavy metal accumulation. Vacuoles are the major storage pools for nitrate (Gransted and Huffaker, 1982) and vacuolar accumulation of heavy metals after exposure of plants to high dosed has also been reported (Rauser and Ackerley, 1987; Sresty and Madhava Rao, 1999). Hence the heavy metal accumulation may lead to increased vacuolar storage of nitrate there by reducing its mobilization form storage to metabolic pool, indicating limitation in NO^{3-} availability to the enzyme (Jain and Gadre, 1997). The nitrate reductase activity was more active in cv. LRG30 than in cv. LRG41 and ICPL85063in response to Cd treatment.

Malate dehydrogenase, one of the important respiratory enzymes exhibited a decline in its activity under conditions of Cd stress. The decline was closely associated with increasing concentration and duration of treatments. In all the treatments the malate dehydrogenase activity decreased from 2 to 8 days of seedling growth as well as with increasing concentrations of externally supplied Cd and always registered lower values when compared to their controls (Fig. 5). Guo *et al*. (2016) found that significant decreases ($p<0.05$) NADP-MDH activity in all *Miscanthus* spp. exposed to Cd stress. Comparatively the different parts of pigeonpea cultivars, LRG41 and ICPL85063 exhibited lower levels of malate dehydrogenase activity in response to Cd treatment than LRG30. In between Cd treatments, the decrease in the malate dehydrogenase activity was more under 0.06 mM Cd exposure. Glycolate oxidase is the first enzyme of the photorespiratory pathway reactions that take place in peroxisomes, and converts glycolate into glyoxylate with concomitant production of H_2O_2. Yamaguchi and Nishimura (2000) had also observed that the glycolate oxidase downstream enzymes were little affected in the glycolate oxidase-suppressed tobacco plants, and thus they considered photorespiration could be regulated by more than one mechanism. Cadmium treatments led to a sharp decrease in the glycolate oxidase activity of the three pigeonpea cultivars when compared to their controls and it became more conspicuous with increasing concentrations of externally supplied metal ions. Among the three pigeonpea cultivars the reduction in glycolate oxidase activity was more evident in cv. LRG41 and ICPL85063 than in LRG30 in response to Cd treatment (Figs. 6).

CONCLUSIONS

The total nitrogen content of the roots and shoots of the Cd treated germinating seeds of the three pigeonpea cultivars increased with increasing seedling growth and recorded lower values when compared to their corresponding controls. The nitrogen content of the cotyledons declined gradually with increasing seedling growth and retained higher amounts over their controls. The pigeonpea cultivar, LRG30 registered higher values of nitrogen content in response to Cd treatment. The *in vivo* nirate reductase activity of the roots and shoots increased at lower concentrations and decreased at higher concentrations of Cd supplied with increasing age of the seedlings. On the other hand, the nitrate reductase activity of the cotyledons decreased with increasing seedling growth as well as with increasing concentrations of externally supplied metal ions. The nitrate reductase was more active in cv. LRG30 in response to Cd treatment. Nitrate reductase activity was effected more in the pigeonpea cultivar ICPL85063 in response to Cd treatment. The malate dehydrogenase activity

of the different parts of the three pigeonpea cultivars decreased with increasing seedling age as well as with increasing concentrations of externally supplied Cd ions. Comparatively cv. ICPL85063 exhibited lower levels of malate dehydrogenase activity in response to Cd treatments. The decrease in the enzyme activities were more drastic in response to Cd treatment. The glycolate oxidase, activity of the three pigeonpea cultivars decreased with increasing concentrations of externally supplied Cd ions. These activities were conspicuously affected in cv. ICPL85063 and appeared more sensitive to Cd treatment.

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