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RESEARCH ARTICLE

CADMIUM EFFECTS ON PHOTOSYNTHETIC PIGMENTS OF GERMINATING SEEDLINGS OF PIGEONPEA (*CAJANUS CAJAN* L.)

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Abstract

Cadmium (Cd) is a widespread heavy metal pollutant in the environment with a long biological half-life. It is easily taken up by plants. Treatment of three pigeonpea (*Cajanus cajan* L.) cultivars LRG30, LRG41 and ICPL85063 with different concentrations of cadmium (cadmium chloride: $CdCl_2.H_2O$) representing 0.02, 0.04 and 0.06 mM was used in the present study. Photosynthetic parameters like chlorophyll a, chlorophyll b, total chlorophyll, pheophytin a, pheophytin b, total pheophytin and total carotenoids content were analysed on 6-8 day old seedlings only. Cadmium treatment at all levels tested decreased chlorophyll and total carotenoid content, increased pheophytin content were observed in the order of increasing concentrations of externally supplied Cd in the three pigeonpea cultivars. The pigment composition was affected in the pigeonpea cultivars, LRG41 and ICPL85063 than LRG30 in response to Cd treatment.

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Introduction

The rapid industrialization that took place at the turn of the 20^{th} century, the pollution of air, water, and soil has become a major global problem. As a consequence of the industrial revolution there is an enormous and increasing demand for heavy metals that leads to high anthropogenic emission of heavy metals in the biosphere (Vangronsveld and Cunningham, 1998). Nonradioactive As, Cd, Cu, Hg, Pb and Zn and radioactive Sr, Cs, and U are the most important metallic pollutants (Raskin *et al*., 1997). Among these Cd presence in the soil can cause serious problems to all organisms. Although not essential for plant growth, when bioavailable, cadmium is readily taken up by roots and translocated into aerial organs where it can accumulate to high levels. The most apparent visible symptoms of cadmium toxicity in plants are retardation of plant growth, chlorosis and stunting (Sanità di Toppi and Gabbrielli, 1999; Kabata-Pendias and Pendias, 2001), photosynthesis and alteration in chlorophyll contents (Lagriffoul *et al*., 1998; Sandalio *et al*., 2001). The damage to photosynthesis occurs mainly from an impaired efficiency of the RUBISCO activity and a decrease in chlorophyll content (Vassilev *et al*., 2003). Cadmium ions directly affect the biosynthesis of photosynthetic pigments and the associated photosynthetic processes, leading to impaired carbon-utilization and respiratory processes (Sersen and Kralova, 2001; Sanita di Toppi *et al*., 2003; Prasad, 2004a) in plants.

The photosynthetic process has been reported to be very sensitive to Cd. As the reserve substances continue depleting in the storage tissue, the growing leaves starts supporting the developing seedling through photosynthesis. Cd toxicity may decrease stomatal density and conductance to CO_2, thus reducing leaf photosynthesis (Poschenrieder and Barceló, 1999; Baryla *et al*., 2001). Direct effects of heavy metals on light and dark reactions and indirect effects caused by them decreasing the photosynthetic pigment content are involved, as well as changes in stomata function. It seems that nearly all of the components of the photosynthetic apparatus are influenced by almost all heavy metals, including chlorophyll and carotenoid content, chloroplast membrane structure, light-harvesting and oxygen-evolving complexes, photosystems and constituents of the photosynthetic electron transport chain (Barcelo and Poschenrieder, 2004; Mysliwa-Kurdziel *et al*., 2004). The effects of Cd ions on the photosynthetic parameters are not adequately established (Sheoran *et al*., 1990a; Krupa *et al*., 1993). Measuring the photosynthetic activity is a good screening method for detecting possible stress situations, perhaps including those involving heavy metals. Cadmium damages the photosynthetic apparatus and causes a decrease in chlorophyll and carotenoid content (Clemens, 2006). Together, these Cd effects are responsible for lowering the photosynthetic quantum yield.

Heavy metals decrease the formation of chlorophyll by interacting with sulphydryl requiring enzyme like aminolevulinic acid synthase, aminolevulinic acid dehydratase and porphobilinogen deaminase leading to accumulation of intermediates of chlorophyll synthesis. Chlorophyll level might also be decreased due to increased activity of chlorophyllase in the presence of Zn, Cu and Hg (Padmaja *et al*., 1990; Keshan and Mukherji, 1992). Apart from inhibition of synthetic enzymes of chlorophyll formation the increased activity of lipoxygenase may also contribute to the decreased level of chlorophyll under Cd, Zn or Pb treatments (Somashekaraiah *et al*., 1992; Deepledge *et al*., 1994). Therefore it is focused to elucidate the effect of cadmium on various aspects of photosynthetic pigments of pigeonpea (*Cajanus cajan* (L.) Millspaugh) is one of the most important and major pulse crop, which is considered to be a profitable and popular crop of India.

Materials and methods

Seed materials

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.

Seed Germination

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 what man 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 studies on the changes in activity of various photosynthetic parameters were confined to 6-8 day old seedlings only. Five replicates were used for each treatment.

Statistical analysis: The data collected were computed and analysed by using statistical analysis IBM-SPSS (Version 21.0). Means and standard errors (SE) were calculated along with Analysis of variance (ANOVA) test for comparing the significance of the differences between means ($P < 0.05$). The data was used to determine whether treatment and cultivar differences were statistically significant by comparing the growth performance of three pigeonpea cultivars between increasing concentrations of Cd supplied.

Pigment composition

a) Chlorophylls

Chlorophyll contents (chlorophyll a, chlorophyll b and total chlorophyll) were estimated using the method of Arnon (1949).

b) Pheophytins

Pheophytin contents (pheophytin a, pheophytin b and total pheophytin) were determined as per the method of Lichtenthaler (1987).

c) Total carotenoids (xanthophylls + β-carotene; x+c)

Total carotenoids content was also determined in the same chloroplast pigment 80% acetone extract by measuring the absorbance at 470 nm and the amount of total carotenoids was calculated according to the equation given by Lichtenthaler (1987).

Results and Discussion

Chlorophyll content

The chlorophyll a, chlorophyll b, and total chlorophyll contents of the shoots of the Cd-treated germinating seeds of pigeonpea increased from 6 to 8 days of seedling growth. However, the chlorophyll contents of the Cd treatments always registered lower values when compared to their controls and decrease in chlorophyll a, chlorophyll b, and total chlorophyll contents became more evident with increasing concentrations of Cd supplied (Fig.1).

Photosynthetic pigments are important to plants mainly for harvesting light and production of reducing powers. The chlorophyll a, b and carotenoids are helping the plants to withstand adversaries. Along with the growth of plants under increasing concentration of heavy metals, the entire metabolic activity of the plants under metal stress was affected resulting in reduced metabolic activities. Under the metal stress, the levels of photosynthetic pigments, namely chlorophyll 'a', chlorophyll 'b' and carotenoids decreases as the concentrations of Cd in soil increases. Photosynthesis in higher plants is highly sensitive to heavy metals inhibiting chlorophyll biosynthesis and accessory pigments (Vassilev *et al*., 1993; Costa and Morel, 1994; Zhang *et al*., 2003).

Observed decrease in chlorophyll content in response to Cd supply may be due to disorders in chlorophyll biosynthesis as well as early ageing process of the photosynthetic apparatus (Astolfi *et al*., 2004). Cadmium-induced reduction of chlorophyll amount has been reported in Indian mustard, mung bean, gram and sorghum (Šimonováe *et al*., 2007; Tantrey and Agnihotri, 2010; Da-lin *et al*., 2011) and *Phaseolus vulgaris* (Zengin and Munzuroglu, 2005).

Among the three cultivars of pigeonpea, greatest reduction in chlorophyll content was noted in the seedlings of LRG41 and ICPL85063 treated with different concentrations of Cd. Heavy metal treatment inhibited the levels of photosynthetic pigments, chlorophylls and carotenoids in *Phaseolus vulgaris*, *Vigna sinensis*, *Vigna radiata* and *Abelmoscus esculentus* leaves (Keshan and Mukherji, 1992; Somashekaraiah *et al*., 1992). The photosynthetic process seems to be the primary target of lead and cadmium toxicity (Greger and Ogren, 1991; Greger and Bertell, 1992). Various photosynthetic parameters affected by the heavy metals include the stomatal conductance, chlorophyll content, several enzymes and electron flow. The reduction in chlorophyll content under cadmium treatments may be due to their impact on synthesis by affecting δ-aminolevulinic acid dehydratase and porphobilinogen deaminase leading to accumulation of intermediates of chlorophyll synthesis such as ALA and porphyris (Allan *et al*., 1985; Stobart *et al*., 1985). The decrease in chlorophyll content under cadmium treatments were more in cv.LRG41 and ICPL85063 than in LRG30, indicating that the latter was more tolerant than the former.

Among the three cultivars of pigeonpea studied greatest reduction in chlorophyll contents were noted in the seedlings of LRG41 and ICPL85063 than in LRG30 treated with different concentrations of Cd.

Chlorophyll a content showed a 0.05 level of significance in the three pigeonpea cultivars and chlorophyll b showed 0.05 level of significance in cv.LRG30 and 0.01 level of significance in LRG41 and ICPL85063 and total chlorophyll contents showed 0.05 level of significance in cv.LRG30 and 0.01 level of significance in LRG41 and ICPL85063 with the external concentrations of Cd ions supplied (Table-1).

Pheophytin content

Pheophytin a, pheophytin b and total pheophytin contents of the three pigeonpea cultivars grown in different concentrations of Cd exhibited an increasing trend was observed from 6 to 8 days of seedling growth. In addition, the pheophytin contents of the three pigeonpea cultivars increased in the order of increasing concentrations of externally supplied Cd. Low concentration of Cd (0.02 mM) did not have any appreciable effects on pheophytin a, but the content of this pigment was considerably increased at 0.04 and 0.06 mM Cd concentrations when compared to the controls (Fig.2). However, the content of pheophytin b increased sharply with increasing concentrations of externally supplied Cd.

Under stress conditions, part of the chlorophylls might have converted to their magnesium free derivatives, the pheophytins. It is considered that chlorophyll a is more sensitive to pheophytinization than chlorophyll b (Lichtenthaler, 1987), eventhough the increase in pheophytin contents might not show any proportionate balance with the decrease in the chlorophyll content in response to Cd treatment. It was evident by the observation of lower levels of chlorophyll content in the pigeonpea cultivars, LRG41 and ICPL85063 where as higher levels of pheophytin content in LRG30 in response to Cd treatment. This might be presumed that, due to toxic effects of cadmium ions, the biosynthesis of chlorophyll itself get affected as indicated by the lower activity of δ-ALAD enzyme and was more pronounced in cv.LRG41 and ICPL85063 than in LRG30. Therefore, the pigeonpea cultivars, LRG41 and ICPL85063 registered lower levels of both chlorophyll and pheophytin contents in addition to the reduced activity of δ-ALAD enzyme when compared to cv.LRG30.

Of the three cultivars of pigeonpea studied, LRG41 and ICPL85063 grown in different concentrations of Cd showed lower levels and LRG30 showed greater levels of pheophytin content.

Pheophytin a content was not significant, whereas pheophytin b showed 0.05 level of significance in the three pigeonpea cultivars and total pheophytin contents of the cv.LRG30 and LRG41 showed 0.05 level of significance and not significant in cv. ICPL85063 with the external concentrations of Cd ions supplied (Table-1).

Total carotenoids

The total carotenoid content of the pigeonpea cultivars increased from 6 to 8 days of seedling growth in all the Cd treatments. However, the total carotenoid content of the pigeonpea seedlings grown in different concentrations of Cd showed a drastic reduction in comparison to their controls and their levels tend to decrease with increasing concentrations of Cd supplied (Fig.3).

Total carotenoid content of the three pigeonpea cultivars increased from 6 to 8 days of seedling growth. However, the total carotenoid content of the Cd treated pigeonpea seedlings showed a drastic reduction when compared to their controls. The decrease becomes conspicuous with increasing concentrations of Cd supplied. The decrease in the levels of chlorophylls and carotenoids by Pb, Cd, Hg, Cu and Ni treatment suggest that these metal ions interfere with the pigment metabolism (Prasad and Prasad, 1987; Muthuchelian and Rani, 1988).

In relation to the cultivar differences, LRG41 and ICPL85063 registered lower levels of carotenoid content than LRG30.

The carotenoid contents of the pigeonpea cv.LRG30 was not significant and cv. LRG41 and ICPL85063 showed 0.05 level of significance with the external concentrations of Cd ions supplied (Table-1).

Table-1

The significant values between treatments and control of chloroplast pigments and with increasing concentrations of externally supplied Cd, extracted from the shoots of 6-day old seedlings of three pigeonpea cultivars, LRG30, LRG41 and ICPL85063 were statistically evaluated by one-way ANOVA.

SHOOTS

	LRG30	**LRG41**	**ICPL85063**
Chlorophyll a	.018*	.022*	.022*
Chlorophyll b	.019*	.002**	.002**
Total chlorophylls	.017*	.002**	.005**
Pheophytin a	.072$^{@}$	.067$^{@}$	.070$^{@}$
Pheophytin b	.020*	.017*	.027*
Total pheophytins	.029*	.045*	.052$^{@}$
Carotenoids	.062$^{@}$	.048*	.047*

**1% Level of Significant (P <0.01)
*5% Level of Significant (P <0.05)
$^{@}$ Not Significant

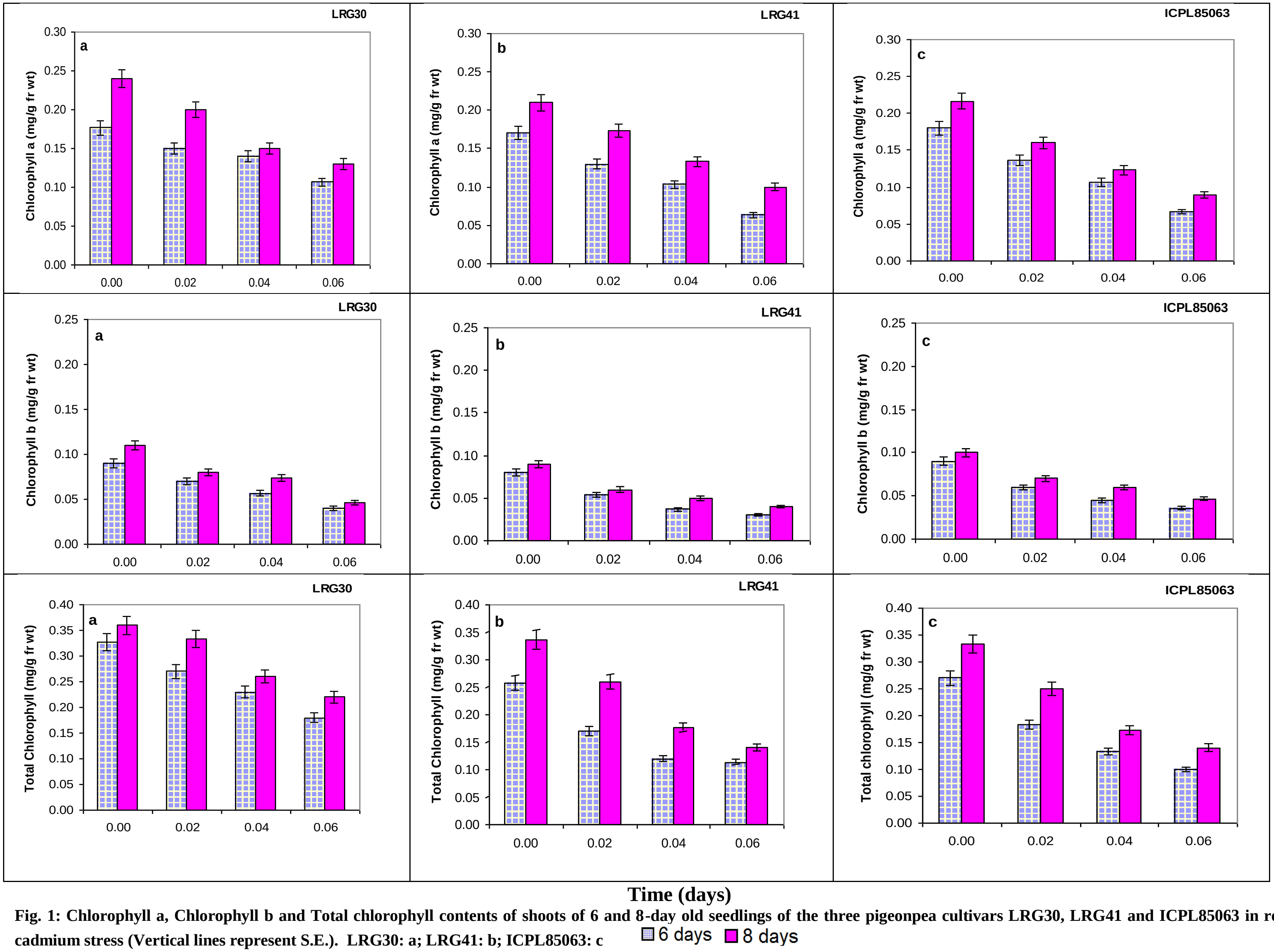

Fig. 1: Chlorophyll a, Chlorophyll b and Total chlorophyll contents of shoots of 6 and 8-day old seedlings of the three pigeonpea cultivars LRG30, LRG41 and ICPL85063 in response to cadmium stress (Vertical lines represent S.E.). LRG30: a; LRG41: b; ICPL85063: c

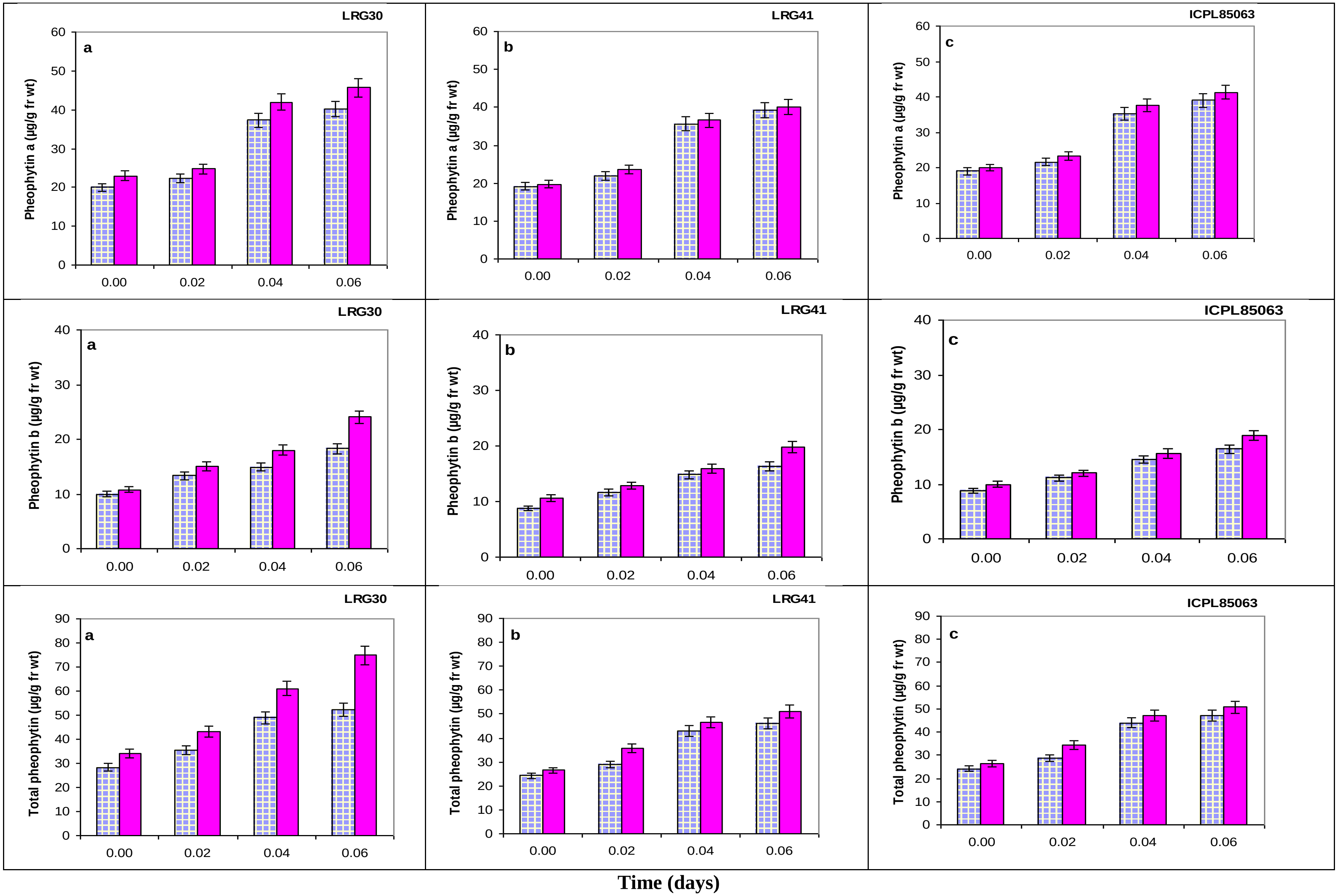

Fig. 2: Pheophytin a, Pheophytin b and Total pheophytin contents of shoots of 6 and 8-day old seedlings of the three pigeonpea cultivars LRG30, LRG41 and ICPL85063 in response to cadmium stress (Vertical lines represent S.E.). LRG30: a; LRG41: b; ICPL85063: c 6 days 8 days

SHOOTS

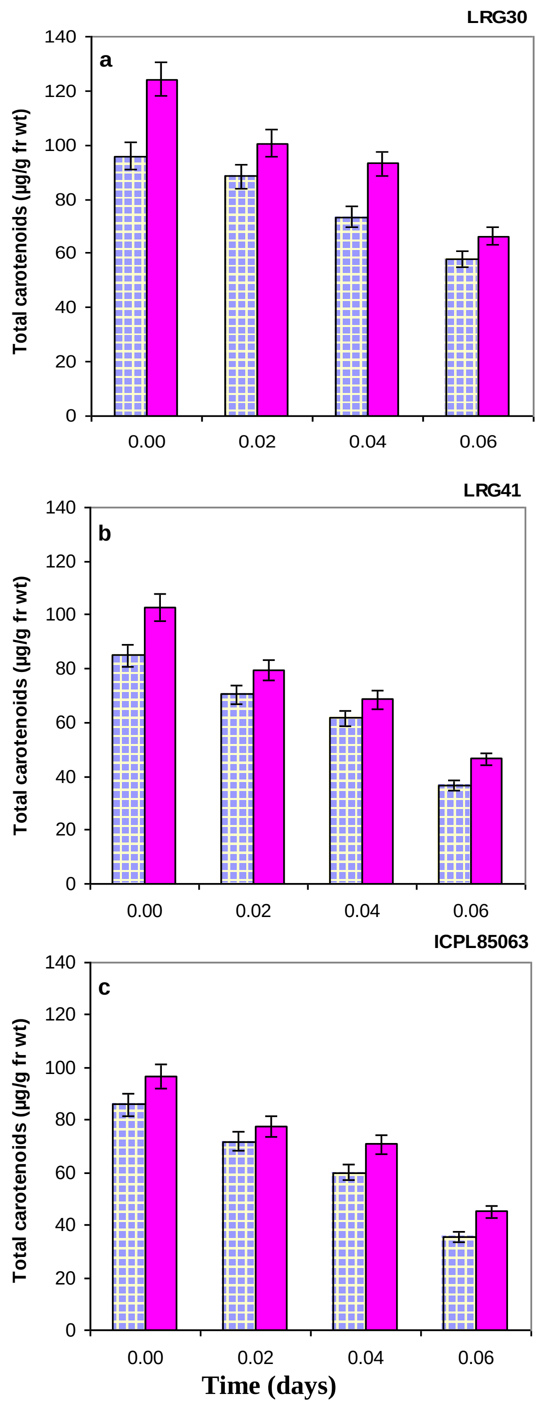

Fig. 3 : Total carotenoid content of shoots of 6 and 8-day old seedlings of the three pigeonpea cultivars LRG30, LRG41 and ICPL85063 in response to cadmium stress (Vertical lines represent S.E.).

LRG30:	**a**	6 days	8 days
LRG41:	**b**	6 days	8 days
ICPL85063:	**c**	6 days	8 days

Conclusions

From the present investigation it can be concluded that the cadmium treatment decreased the chlorophyll and carotenoid contents, and increased the pheophytin content of the three pigeonpea cultivars. The cv.LRG41 and ICPL85063 registered lower levels of all the pigment fractions than LRG30 in response to Cd treatment. The pigment composition was affected in the pigeonpea cultivars, LRG41 and ICPL85063 in response to Cd treatment.

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