International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2013): 4.438

Toxicity of Cadmium on Mineral Nutrition of Seedlings of Pigeonpea (*Cajanus Cajan* (L.) Millspaugh) Cultivars

B. Priyadarshini[1], B. Sujatha[2]

Department of Botany, Andhra University, Visakhapatnam-530003, A.P., India

Abstract: ***Heavy metals are naturally occurring in the earth's crust but anthropogenic and industrial activities have led to drastic environmental pollutions in distinct areas. Cadmium (Cd) toxicity was investigated with different concentrations representing 0, 0.02, 0.04 and 0.06 mM was used in three pigeonpea cultivars (LRG30, LRG41 and ICPL85063) on the changes in activity of mineral composition of roots, shoots and cotyledons. Except for the Cd accumulation in the respective metal-treated seedlings sodium, magnesium, potassium, calcium, iron, copper, zinc, manganese and molybdenum contents of the roots and shoots decreased with increasing concentrations of externally supplied metal ions and registered lower values when compared to their controls. However, the cotyledons of the three pigeonpea cultivars registered higher values when compared to their controls and the retention of various elements in the cotyledons increased with increasing concentration of externally supplied heavy metal ions. The decrease in the contents of different mineral elements was more in cv.LRG41 and ICPL85063 than in LRG30 in response to Cd treatment.***

Keywords: Cadmium, environmental pollution, mineral composition, pigeonpea cultivars, toxicity.

1. Introduction

Cadmium belongs to the most intensively studied metal in terms of their impact on plants. Although the metal is toxic to plants, it is known for its easy uptake (Kabata-Pendias, 2010; Priyadarshini and Sujatha, 2011). Cadmium is widely studied in the context of environmental pollution and its' impact on human health (Sanita and Gabbrielli, 1999). Cadmium is one the most frequent and dangerous inorganic pollutants. A balance of inorganic nutrients is required by plants for maximum growth and development under optimal and stressful environments. Mineral deficiencies or imbalances and depression of plant growth can result from excessive Cd toxicity that affects the rate of uptake and distribution of certain nutrients in plants. Accumulation of Pb^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}, and Ni^{2+} caused significant effects on whole plant mineral composition (Walker *et al.*, 1977; Chamel and Heuman, 1987). Heavy metals affect mineral uptake and their long distance transport (Piccini and Malavolta, 1992). Iwai *et al.* (1975) reported that the decrease in nitrogen, iron, manganese and zinc contents in corn shoots was due to increased accumulation of Cd. Most of the studies indicated that the excess availability of Cd, Zn, and Ni resulted in severe disturbances in the nutrition status of plants (Woolhouse, 1983; Ruano *et al.*, 1987; Clarkson and Luttge, 1989; Loneragan and Webb, 1993). In general, Cd has been shown to interfere with the uptake, transport, and use of several elements (Ca, Mg, P, and K) and water by plants (Das *et al*., 1997).

Several plant nutrients have many direct as well as indirect effects on Cd availability and toxicity. Direct effects include decreased Cd solubility in soil by favouring precipitation and adsorption (Matusik *et al.*, 2008), competition between Cd and plant nutrients for the same membrane transporters (Zhao *et al.*, 2005), and Cd sequestration in the vegetative parts to avoid its accumulation in the grain/edible parts (Hall, 2002). Indirect effects include dilution of Cd concentration by increasing plant biomass and alleviation of physiological stress. However, the amount of Cd deposited into the root, shoot and inter-veins of leaves considerably differed among different species (Abu-Muriefah, 2008; Wahid *et al*., 2008). Therefore to understand the multifarious effects of Cd toxicity on germinating seeds of pigeonpea, the most important and major pulse crop in India, this study is carried on individual organs of different plant cultivars to provide a comprehensive picture on the mineral nutrition of pigeonpea.

2. Materials and Methods

2.1 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.

2.2 Seed Germination and Metal Exposure

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 mineral composition were confined to 6-day old seedlings only. Five replicates were used for each treatment.

2.3 Statistical Analysis

The data collected were computed and analyzed 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 three pigeonpea cultivars between increasing concentrations of Cd supplied.

3. Mineral Composition

3.1 Quantification of Sodium, Magnesium, Potassium and Calcium

One gram of dried and powdered material of different parts of control and treated germinating seeds were ashed between 500 to 550°C in a muffle furnace for 3 h. The ash was dissolved in a few ml of 25% HCl and then filtered through Whatmann No.44 filter paper and the filtrate was made up to 25 ml in a volumetric flask with deionised double distilled water. Sodium, Magnesium, Potassium and Calcium in the extract were estimated by using Corning EEL Model 100 Flame photometer and the results were expressed as mg nutrient per gram dry weight. The standard solutions of Sodium, Magnesium, Potassium and Calcium were prepared using Sodium chloride (NaCl), Magnesium chloride ($MgCl_2$), Potassium chloride (KCl) and Calcium carbonate ($CaCO_3$) respectively.

3.2 Trace elements and heavy metal analysis

One gram of dried and powdered samples belonging to different parts of control and treated 6-day old germinating seeds were ashed between 450-550°C in a muffle furnace for 3 h. The ash was dissolved in a few ml of 25% HCl and then filtered through whatman No.44 filter paper and made up to 25 ml in a volumetric flask using double distilled water. Iron, copper, zinc, manganese, molybdenum and cadmium were analyzed using inductively coupled plasma-mass spectrometer (ICP-MS).

4. Results and Discussion

The studies on the mineral composition of the three pigeonpea cultivars were confined to 6-day old seedlings only. The contents of the various elements such as potassium, calcium, magnesium, sodium, iron, copper, zinc, manganese, molybdenum and cadmium were estimated in the roots, shoots and cotyledons of the three pigeonpea cultivars treated with different concentrations of Cd. The increasing concentrations of Cd significantly influenced the elemental composition of the three cultivars (Figure 1-9). Except for the Cd content, all the other elements analysed in the roots and shoots decreased with increasing concentrations of externally supplied metal ions and registered lower values when compared to their controls. However, the cotyledons of the three cultivars retained higher quantities when compared to their controls and the retention of various elements increased with increasing concentrations of the externally supplied heavy metal ion (Figure 10). Among the three cultivars, LRG41 and ICPL85063 recorded greater levels of cadmium accumulation with increasing concentrations of cadmium than LRG30.

The significant values between the contents of various mineral elements were expressed in three pigeonpea cultivars. The Cd content of both the roots and shoots of the Cd-treatment showed 0.05 level of significance with the external concentrations of Cd supplied. However, the Cd content of the cotyledons of three pigeonpea cultivars was significant at P=0.05 level in the three pigeonpea cultivars with the external concentrations in response to Cd treatment (Table 1).

The inhibition of Cd stress on plant growth is related to its effect on nutrient uptake and distribution. The uptake and translocation of mineral nutrients such as Fe, Zn, Cu and Mn under Cd stress have been reported in crops, such as soybean (Cataldo *et al.*, 1983; Dražić *et al.*, 2004) and rice (Rubio *et al.*, 1994; Liu *et al.*, 2003). The different heavy metals interact significantly with the activities such as translocation, compartmentation and circulation of nutrients within the plants. Some changes in elemental composition upon heavy metal stress were so severe that secondary adverse effects on the plants can be noted on the basis of the changed elemental composition of the shoots or roots (Brune and Dietz, 1995). In sugar beet, deficiency of Fe in roots induced by Cd was observed (Chang *et al.*, 2003). A decrease in uptake of Ca and K by Cd has been found in a Cd-hyperaccumulator, *Atriplex halimus* subsp. *schweinfurthii* (Nedjimi and Daoud, 2009).

Gussarssonn *et al.*, 1994 suggested that trace elements including Cd may interfere with nutrient uptake, including K, by altering the plasma membrane permeability and by affecting element transport processes across the membrane. According to Jalil *et al.* (1994), Cd addition decreased the concentration of K, Zn and Mn in wheat root and shoot, while Fe and Cu concentrations in shoot and root were not affected. In this experiment, we found that the effect of Cd on mineral nutrient concentration varied with plant parts and genotypes. It has been shown that the influence of cadmium on the content of mineral nutrients in plants can depend on the concentration of cadmium, growth conditions, species, or ecotype being investigated or even on plant organ under study (Cai *et al.*, 2010). Interactions between cadmium and essential mineral elements have been studied in potatoes and different ecotypes of wheat (Zhang *et al.*, 2002; Gonçalves *et al.*, 2009). Metwally *et al.* (2005) indicated that toxic Cd levels inhibited uptake of nutrient elements such as P, K, S, Ca, Zn, Mn, and B by plants in an organ-and genotype-specific manner in pea. Cd toxicity also affected concentration of some nutrients in barley (Wu and Zhang, 2002; Wu *et al.*, 2003; Huang *et al.*, 2007). In the current study, we found that Cd stress decrease in the contents of various elements was more in cv.LRG41 and ICPL85063 than in LRG30 in response to Cd treatment.

5. Conclusions

The mineral elements of the roots and shoots of the three pigeonpea cultivars studied decreased with increasing concentrations of externally supplied Cd ions. On the other hand, the retention of the mineral elements with increasing concentrations was observed in the cotyledons of the three cultivars. The decrease in the contents of different mineral elements were more in cv.LRG41 and ICPL85063 than in LRG30 in response to Cd treatment suggesting that heavy metals are affecting the transport of mineral elements from cotyledons to growing axis. To reverse the negative effect of Cd stress, plants need to either inhibit its accumulation or enhance its tolerance capacity to Cd for survival. The severity of Cd toxicity, however, can be reduced through the optimization of these nutrients. Sufficient availability of nutrients may reduce the accumulation of a metal in plants and decrease its toxicity by inducing several physiological processes.

References

[1] **Abu-Muriefah SS., 2008.** Growth parameters and elemental status of cucumber (*Cucumus sativus*) seedlings in response to cadmium accumulation. *Int. J. Agric. Biol.*, **10**:261-266.

[2] **Brune A and Dietz KJ., 1995.** A comparative analysis of element composition of barley roots and leaves under cadmium, molybdenum, nickel and zinc-stress. *J. Plant Nutr*., **18**:853-868.

[3] **Cai Y, Lin L, Cheng W, Zhang G., and Wu, F., 2010.** Genotypic dependent effect of exogenous glutathione on Cd-induced changes in cadmium and mineral uptake and accumulation in rice seedlings (*Oryza sativa*). *Plant, Soil and Environ.*, **56**:516-525.

[4] **Cataldo DA, Garland TR and Wildung RE., 1983.** Cadmium uptake kinetics in intact soybean plants. *Plant Physiol.,* **73**:844-848.

[5] **Chamel A and Heuman P., 1987.** Foliar absorption of nickel: Determination of its cuticular behavior using isolated cuticles. *J. Plant Nutr*., **10**:99-111.

[6] **Chang Y, Zouari M, Gogorcena Y, Lucena JJ and Abadía J., 2003.** Effects of cadmium and lead on ferric chelate reductase activities in sugar beet roots. *Plant Physiol. Biochem.*, **41**:999-1005.

[7] **Clarkson DT and Luttge U., 1989.** Mineral nutrition. Divalent cations, transport and compartmentalization. *Prog. Bot*., **51**:93-112.

[8] **Das P, Samantaray S and Rout GR., 1997.** Studies on cadmium toxicity in plants: A review. *Environ. Pollu.,* **98**:29-36.

[9] **Dražić G, Mihailović N, Stojanović Z. 2004.** Cadmium toxicity: the effect on macro and micro-nutrient contents in soybean seedlings. *Biol Plant*. **48**(4):605-607.

[10] **Gonçalves JF, Antes FG, Maldaner J, Pereira LB, Tabaldi LA, Rauber R., et al. 2009.** Cadmium and mineral nutrient accumulation in potato plantlets grown under cadmium stress in two different experimental culture conditions. *Plant Physiol. Biochem*., **47**:814-821.

[11] **Gussarsson M. 1994.** Cadmium-induced alterations in nutrient composition and growth of *Betula pendula* seedlings: the significance of fine roots as a primary target for cadmium toxicity. *J. Plant Nutr*. **17**:2151-2163.

[12] **Hall JL., 2002.** Cellular mechanisms for heavy metal detoxification and tolerance. *J. Exp. Bot.*, **53**:1-11.

[13] **Huang You-Zong, Wei Kang, Yang Juan, Dai Fei, Zhang Guo-ping, 2007.** Interaction of salinity and cadmium stresses on mineral nutrients, sodium, and cadmium accumulation in four barley genotypes. *J. Zhejiang Univ Sci B,* **8**(7):476-485.

[14] **Iwai I, Hara T and Sonoda Y., 1975.** Factors affecting cadmium uptake by the corn plant. *Soil Sci. Plant Nutr.*, **21**:37-46.

[15] **Jalil A, Selles F, and Clarke JM., 1994.** Effect of cadmium on growth and the uptake of cadmium and other elements by durum wheat. *J. Plant Nutr*. **17**:1839-1858.

[16] **Kabata-Pendias A. 2010.** Trace elements in soils and plants. Boca Raton: CRC Press.

[17] **Liu JG, Liang JS, Li KQ, Zhang ZJ, Yu BY, Lu XL, Yang JC and Zhu QS., 2003.** Correlations between cadmium and mineral nutrients in absorption and accumulation in various genotypes of rice under cadmium stress. *Chemosphere*, **52**:1467-1473.

[18] **Loneragan JF and Webb MJ., 1993.** Interactions between zinc and other nutrients affecting the growth of plants. In: Robson AD, editor. Zinc in Soils and Plants. Dordrecht. The Netherlands: Kluwer Academic Publishers. Australia, pp. 119-134.

[19] **Matusik J, Bajda T and Manecki M., 2008.** Immobilization of aqueous cadmium by addition of phosphates. *J. Hazardous Materials*, **152**:1332-1339.

[20] **Metwally A, Safronova VI, Belimov AA and Dietz KJ., 2005.** Genotypic variation of the response to cadmium toxicity in *Pisum sativum* L. *J. Exp. Bot*., **56**:167-178.

[21] **Nedjimi B and Daoud Y., 2009.** Cadmium accumulation in *Atriplex halimus subsp. schweinfurthii* and its influence on growth, proline, root hydraulic conductivity and nutrient uptake. Flora-Morphology, Distribution. *Func. Eco. Plants*, **204**(4):316-324.

[22] **Piccini DF and Malavolta E., 1992.** Effect of nickel on two common bean cultivars. *J. Plant. Nutr*., **15**:2343-2350.

[23] **Priyadarshini B and Sujatha B., 2011.** Cadmium-induced plant stress investigated by scanning electron microscopy in *Cajanus cajan. J. Pharm. Res.* **4**(3):938-941.

[24] **Ruano A, Barcelo J and Poschenrieder C., 1987.** Zinc toxicity-induced variation of mineral element composition in hydroponically grown bush bean plants. *J. Plant Nutr*., **10**(4):373-384.

[25] **Rubio MI, Escrig I, Martinez-Cortina C, López-Benet FJ and Sanz A., 1994.** Cadmium and nickel accumulation in rice plants. Effects on mineral nutrition and possible interactions of abscisic and gibberellic acids. *Plant Growth Regul*., **14**:151-157.

[26] **Sanita di Toppi L and Gabbrielli R., 1999.** Response to cadmium in higher plants. *Env. Exp. Bot*., **41**:105-130.

[27] **Wahid A, Ghani A and Javed F., 2008.** Effect of cadmium on photosynthesis, nutrition and growth of mung bean. *Agron. Sustain. Dev.,* **28**:273-280.

[28] **Walker WM, Miller JE and Hassett JJ, 1977.** Effect of lead and cadmium upon the calcium, magnesium, potassium, and phosphorus concentration in young corn plants. *Soil Sci.*, **124**:145-151.

[29] **Woolhouse HW., 1983.** Toxicity and tolerance in the response of plants to metals, in: O.L. Lange, P.S. Nobel, C.B. Osmond, M. Ziegfer (Eds.), Encyclopedia of Plant Physiology, Responses to the Chemical and Biological Environment, Springer, Berlin. **12**(c):245-300.

[30] **Wu FB and Zhang GP., 2002.** Differences between barley cultivars in seedling growth and in uptake of cadmium and nutrients under various Cd levels. *Chin. J. Appl. Ecol*., **13**(12):1595-1599.

[31] **Wu FB, Zhang GP and Yu JS, 2003.** Interaction of cadmium and four microelements for uptake and translocation in

different barley genotypes. *Commun. Soil Sci. Plant Anal.*, **34**(13-14):2003-2020.

[32] **Zhang GP, Fukami M and Sekimoto H., 2002.** Influence of cadmium on mineral concentrations and yield components in wheat genotypes differing in Cd tolerance at seedling stage. *Field Crops Research*, **77**:93-99.

[33] **Zhao Z, Zhu YG and Cai YL, 2005.** Effects of zinc on cadmium uptake by spring wheat (*Triticum aestivum*, L.): long-time hydroponic study and short-time ^{109}Cd tracing study. *Journal of Zhejiang University-Science*, **6A**: 643-648.

Table 1: The significant values between the contents of various mineral elements and with increasing concentrations of externally supplied Cd or dry weight of 6-day old seedlings of the different parts of three pigeonpea cultivars, LRG30, LRG41 and ICPL85063 were statistically evaluated by one-way ANOVA.

	LRG30	LRG41	ICPL85063
		ROOTS	
Sodium	.032*	.015*	.014*
Iron	.017*	.008**	.009**
Copper	.020*	.010*	.011*
Calcium	.039*	.019*	.017*
Manganese	.018*	.006**	.005**
Magnesium	.004**	.006**	.005**
Potassium	.026*	.004**	.004**
Zinc	.008**	.008**	.004**
Molybdenum	.000**	.001**	.000**
Cadmium	.020*	.027*	.024*
		SHOOTS	
Sodium	.030*	.007**	.068@
Iron	.019*	.006**	.006**
Copper	.031*	.015*	.017*
Calcium	.019*	.009**	.012*
Manganese	.016*	.010*	.008**
Magnesium	.005**	.007**	.005**
Potassium	.046*	.011*	.014*
Zinc	.014*	.020*	.025*
Molybdenum	.046*	.001**	.001**
Cadmium	.039*	.047*	.047*
		COTYLEDONS	
Sodium	.023*	.015*	.013*
Iron	.047*	.032*	.030*
Copper	.070@	.023*	.024*
Calcium	.035*	.021*	.020*
Manganese	.012*	.006**	.005**
Magnesium	.014*	.010*	.016*
Potassium	.057@	.062@	.060@
Zinc	.038*	.040*	.040*
Molybdenum	.005**	.012*	.009**
Cadmium	.026*	.026*	.026*

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

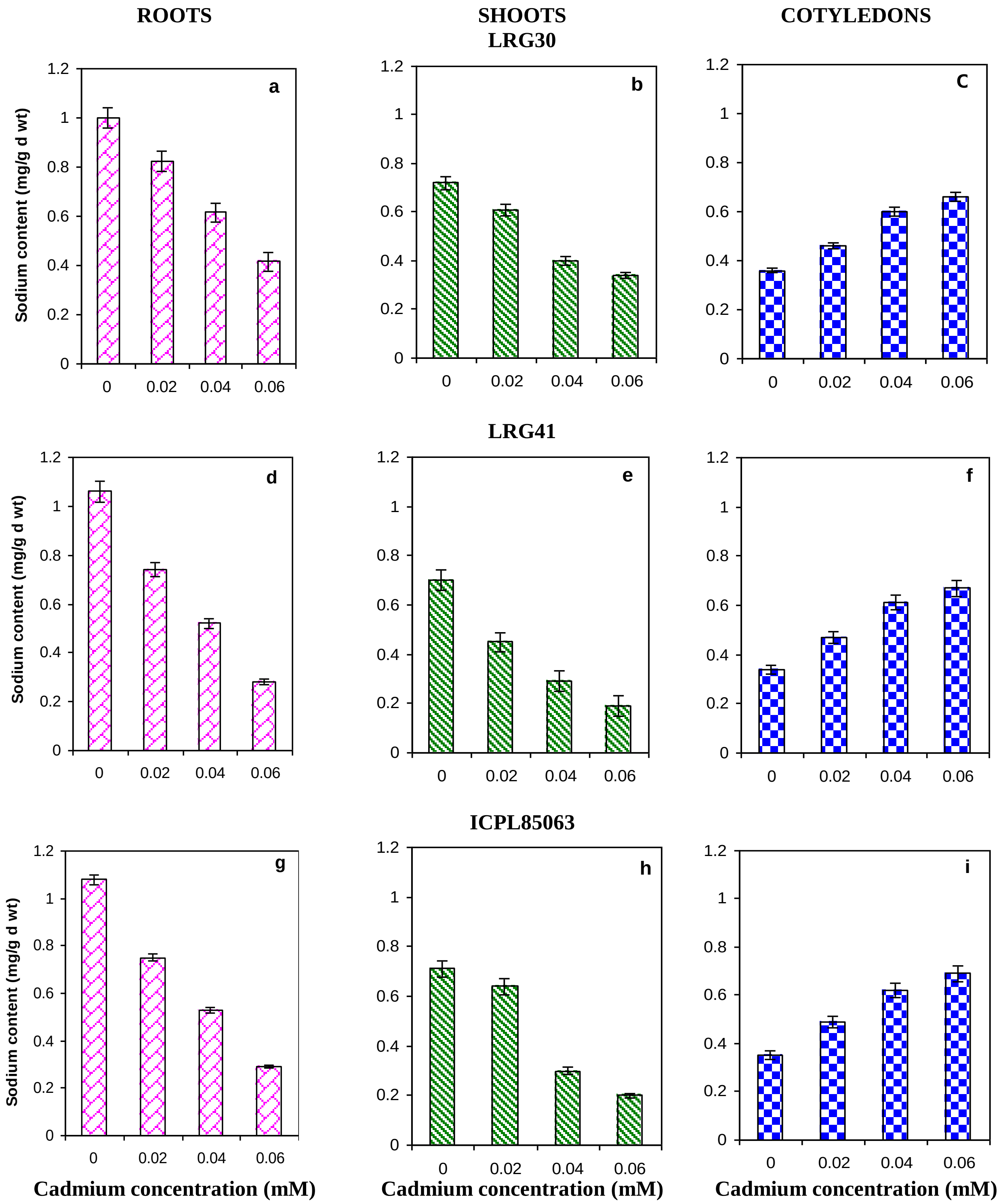


Figure 1: Sodium content of roots, shoots and cotyledons of 6-day old seedlings of the three pigeonpea cultivars LRG30, LRG41 and ICPL85063 in response to cadmium stress (Vertical lines represent S.E.).

		LRG30	LRG41	ICPL85063	
Roots	:	a	d	g	-
Shoots	:	b	e	h	-
Cotyledons	:	c	f	i	-

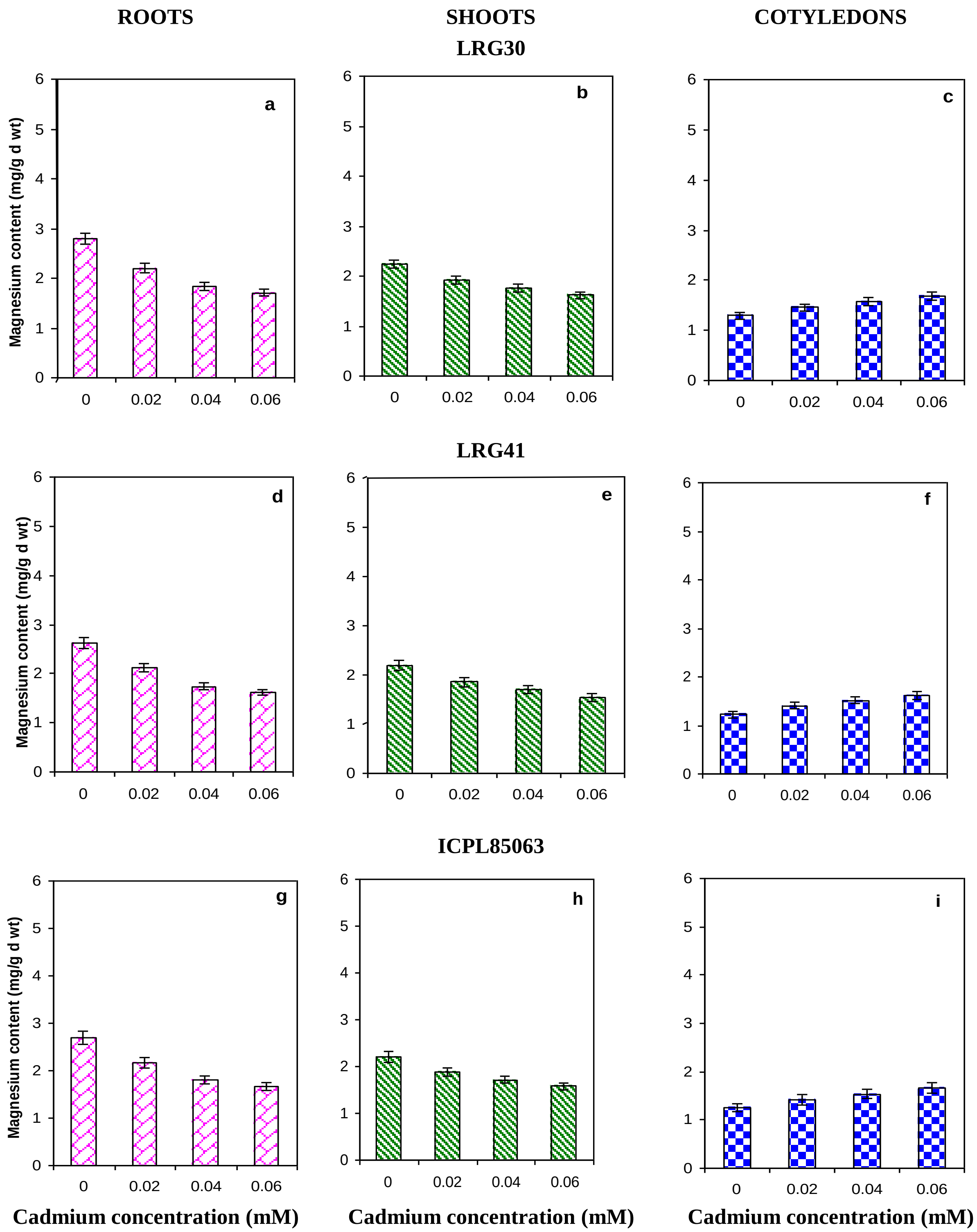


Figure 2: Magnesium content of roots, shoots and cotyledons of 6-day old seedlings of the three pigeonpea cultivars LRG30, LRG41 and ICPL85063 in response to cadmium stress (Vertical lines represent S.E.).

		LRG30	LRG41	ICPL85063		
Roots	:	a	d	g	-	
Shoots	:	b	e	h	-	
Cotyledons	:	c	f	i	-	

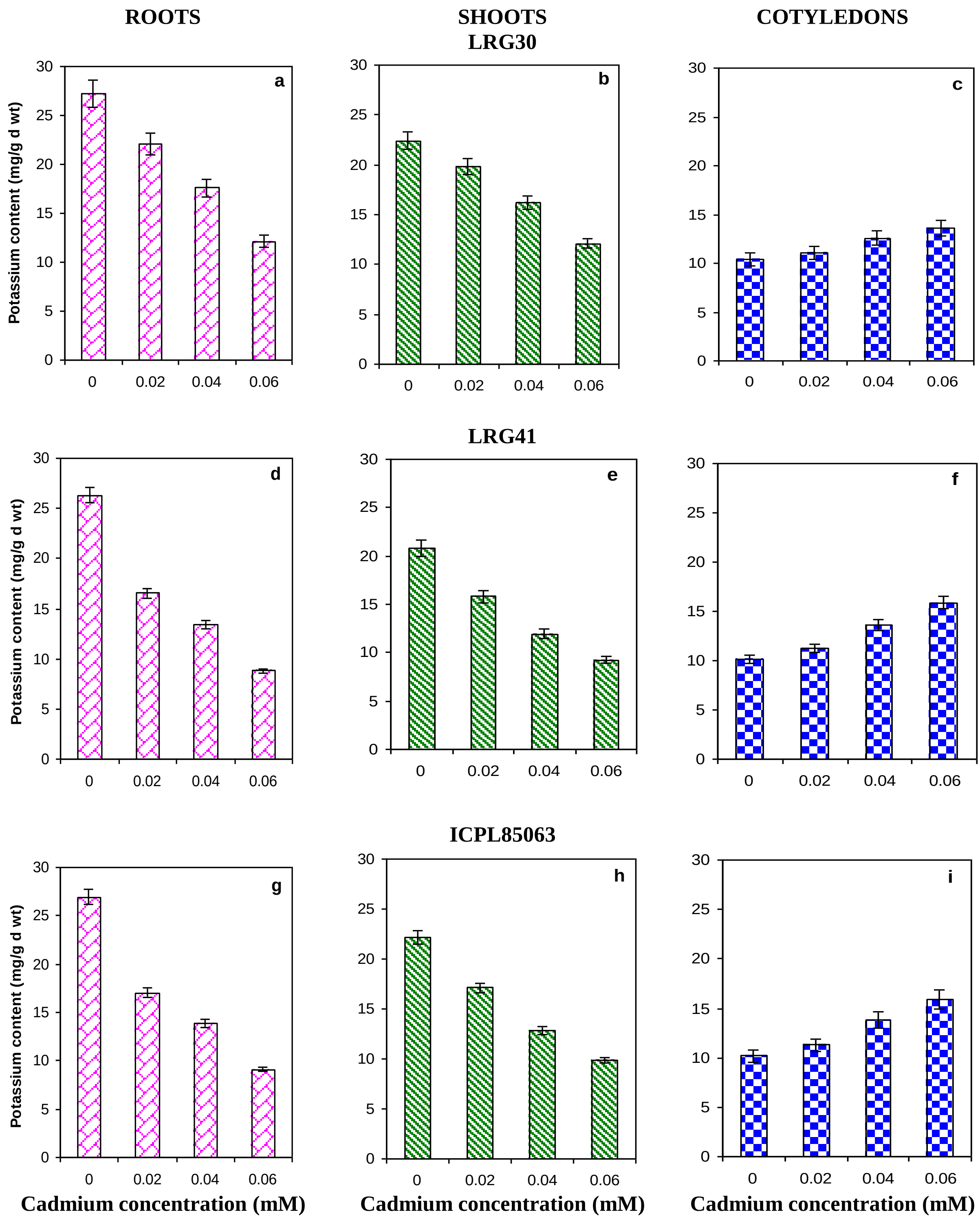
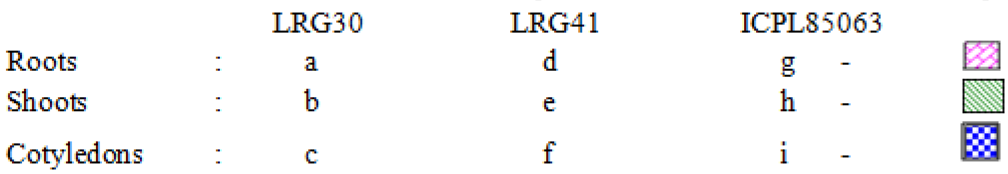


Figure 3: Potassium content of roots, shoots and cotyledons of 6-day old seedlings of the three pigeonpea cultivars LRG30, LRG41 and ICPL85063 in response to cadmium stress (Vertical lines represent S.E.).



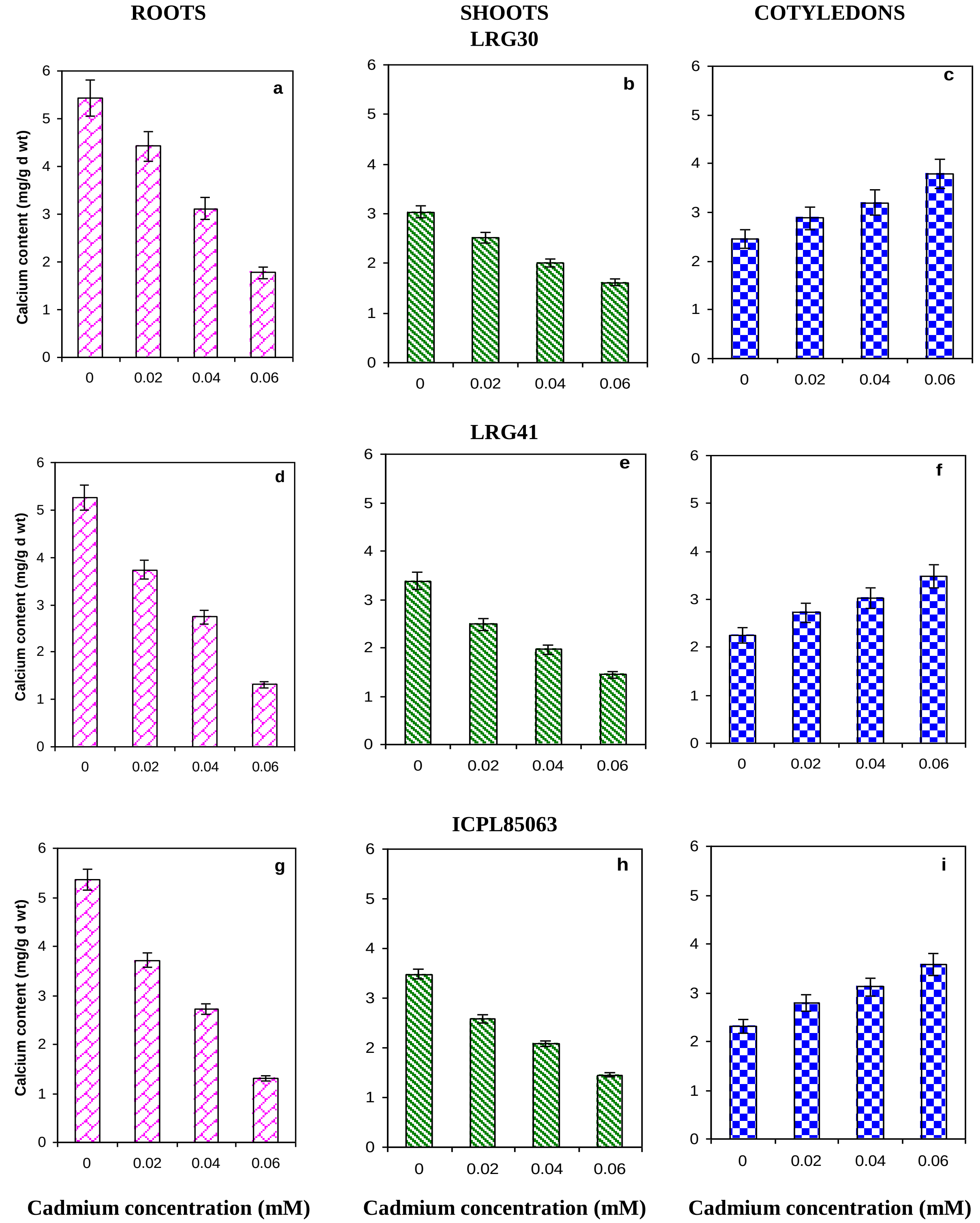


Figure 4: Calcium content of roots, shoots and cotyledons of 6-day old seedlings of the three pigeonpea cultivars LRG30, LRG41 and ICPL85063 in response to cadmium stress (Vertical lines represent S.E.).

		LRG30	LRG41	ICPL85063	
Roots	:	a	d	g	-
Shoots	:	b	e	h	-
Cotyledons	:	c	f	i	-

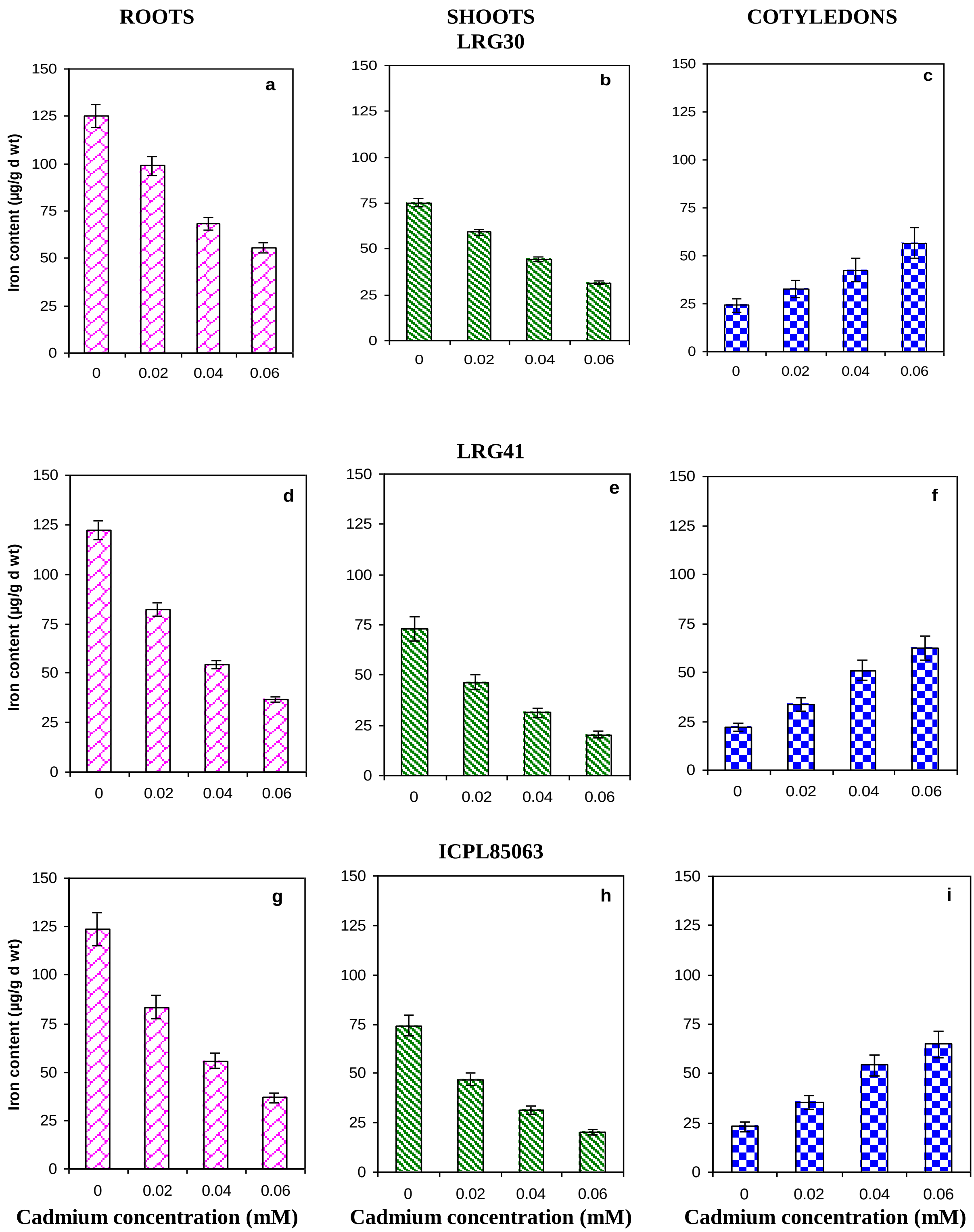


Figure 5: Iron content of roots, shoots and cotyledons of 6-day old seedlings of the three pigeonpea cultivars LRG30, LRG41 and ICPL85063 in response to cadmium stress (Vertical lines represent S.E.).

		LRG30	LRG41	ICPL85063	
Roots	:	a	d	g	-
Shoots	:	b	e	h	-
Cotyledons	:	c	f	i	-

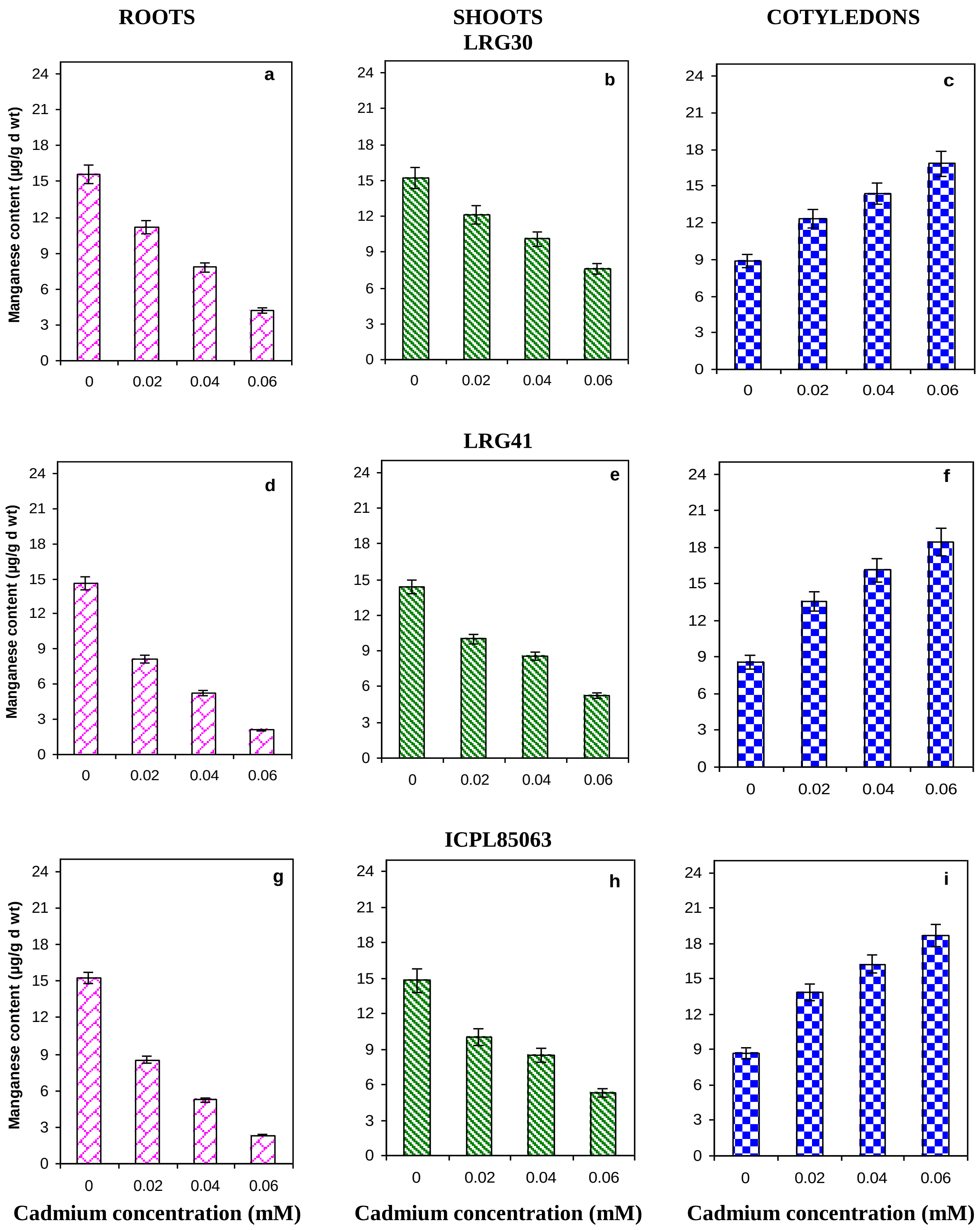


Figure 6: Manganese content of roots, shoots and cotyledons of 6-day old seedlings of the three pigeonpea cultivars LRG30, LRG41 and ICPL85063 in response to cadmium stress (Vertical lines represent S.E.).

		LRG30	LRG41	ICPL85063	
Roots	:	a	d	g	-
Shoots	:	b	e	h	-
Cotyledons	:	c	f	i	-

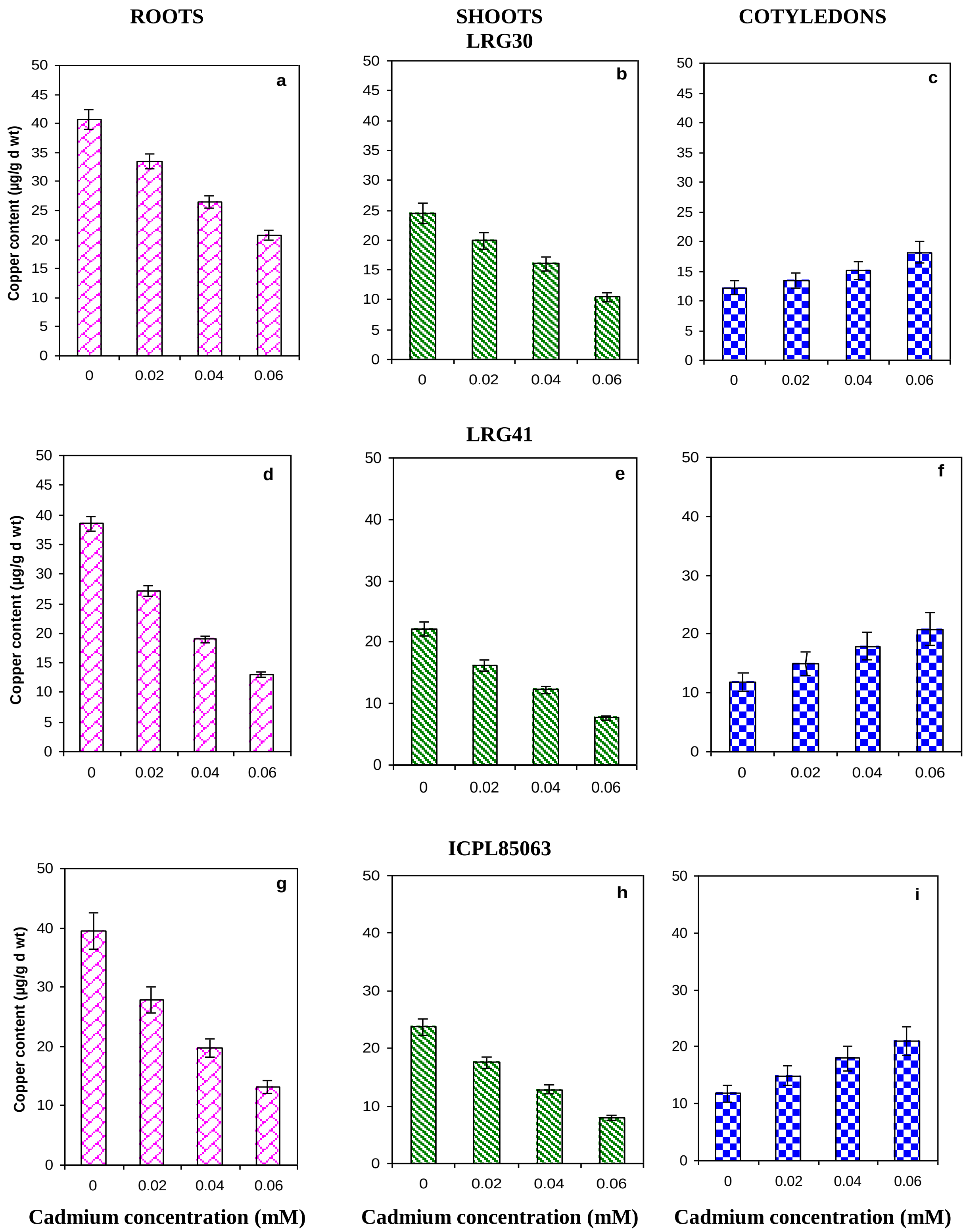


Figure 7: Copper content of roots, shoots and cotyledons of 6-day old seedlings of the three pigeonpea cultivars LRG30, LRG41 and ICPL85063 in response to cadmium stress (Vertical lines represent S.E.).

		LRG30	LRG41	ICPL85063	
Roots	:	a	d	g	-
Shoots	:	b	e	h	-
Cotyledons	:	c	f	i	-

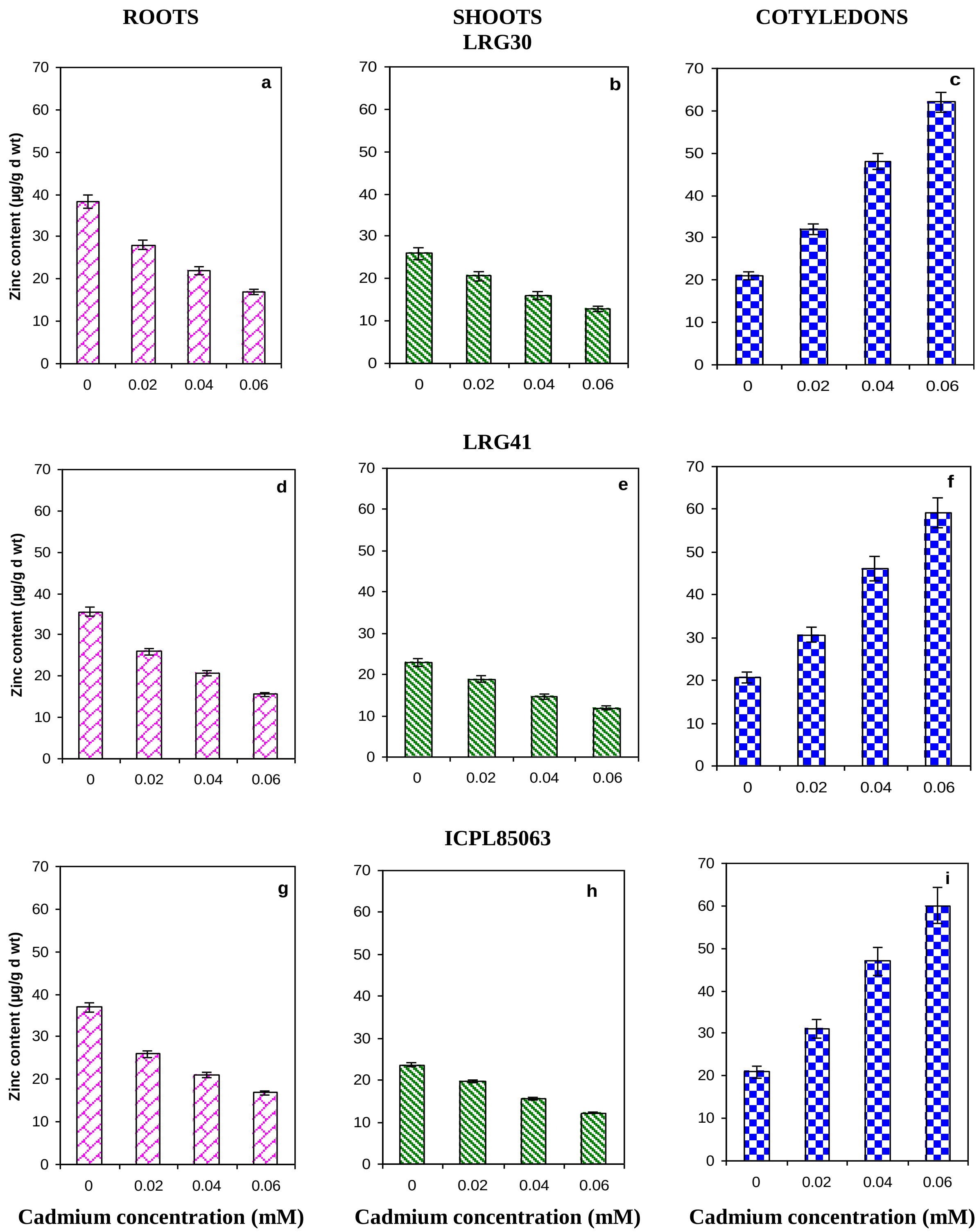


Figure 8: Zinc content of roots, shoots and cotyledons of 6-day old seedlings of the three pigeonpea cultivars LRG30, LRG41 and ICPL85063 in response to cadmium stress (Vertical lines represent S.E.).

		LRG30	LRG41	ICPL85063	
Roots	:	a	d	g	-
Shoots	:	b	e	h	-
Cotyledons	:	c	f	i	-

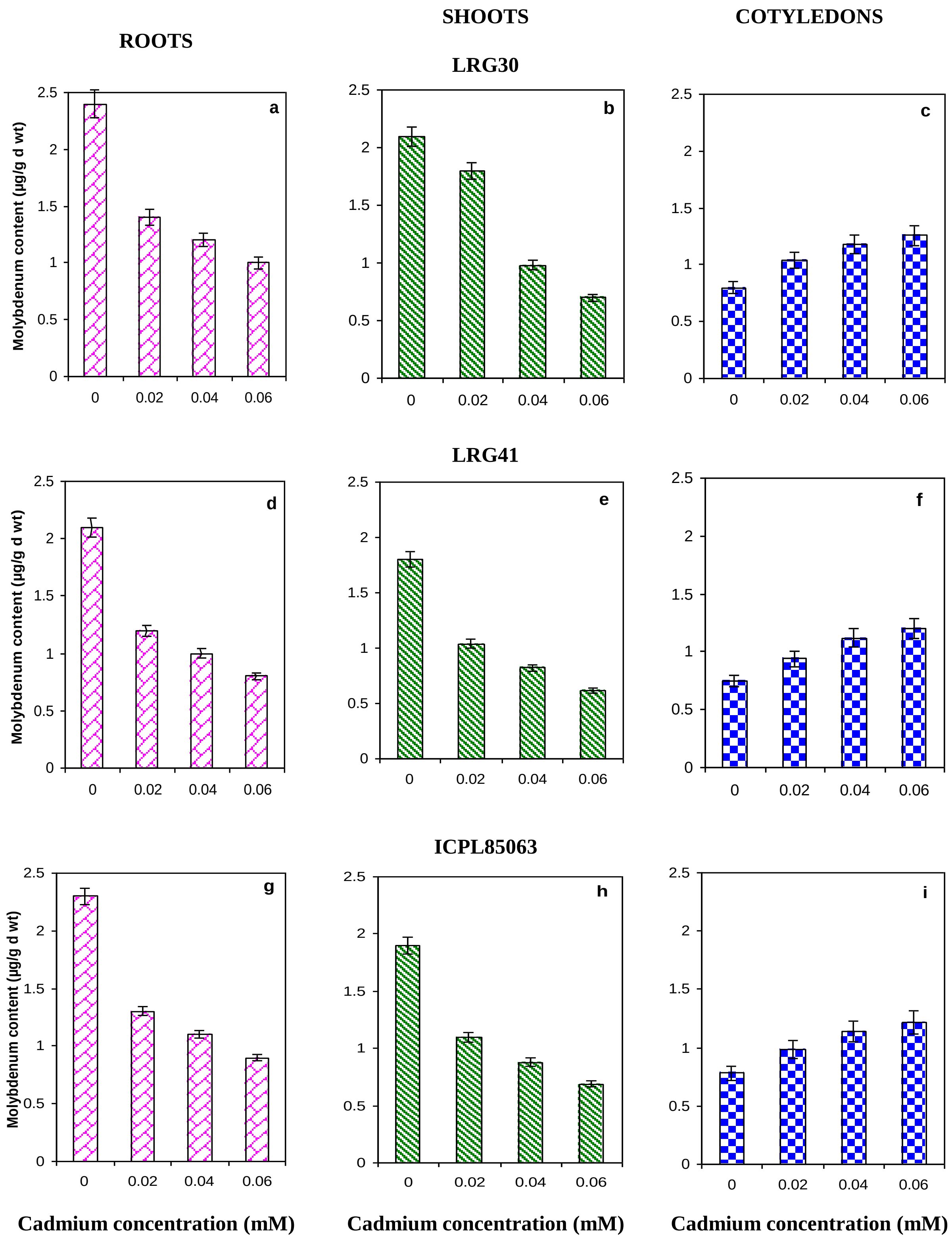


Figure 9: Molybdenum content of roots, shoots and cotyledons of 6-day old seedlings of the three pigeonpea cultivars LRG30, LRG41 and ICPL85063 in response to cadmium stress (Vertical lines represent S.E.).

		LRG30	LRG41	ICPL85063	
Roots	:	a	d	g	-
Shoots	:	b	e	h	-
Cotyledons	:	c	f	i	-

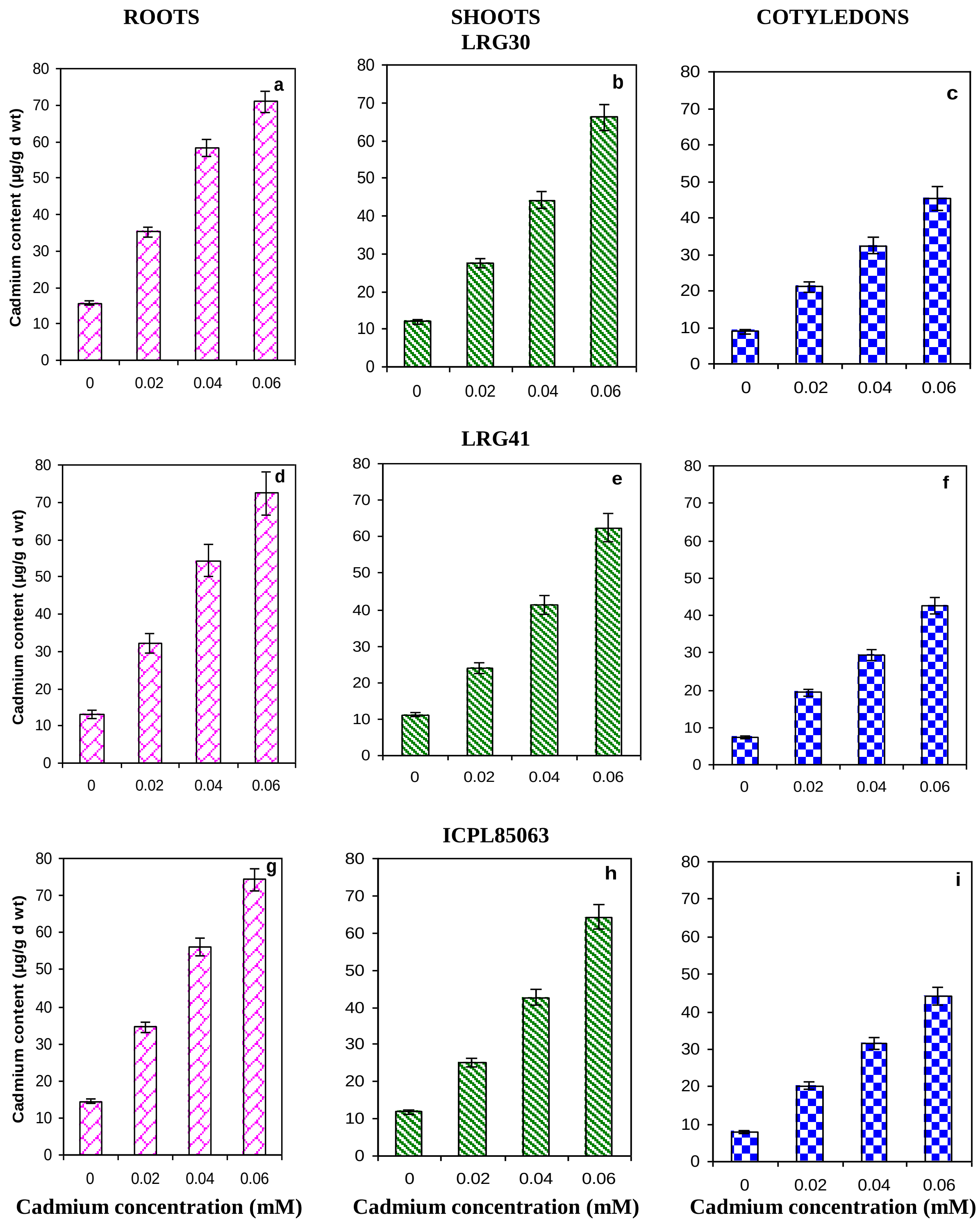


Figure 10: Cadmium content of roots, shoots and cotyledons of 6-day old seedlings of the three pigeonpea cultivars LRG30, LRG41 and ICPL85063 in response to cadmium stress (Vertical lines represent S.E.).

		LRG30	LRG41	ICPL85063	
Roots	:	a	d	g	-
Shoots	:	b	e	h	-
Cotyledons	:	c	f	i	-