Influence of lead and cadmium on amino acids and protein content of pigeonpea seedlings

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SUMMARY

Seeds of pigeonpea were grown in different concentrations of lead and cadmium for 8-days. The studies on the changes in free amino acid composition were confined to 6-day seedlings only. The total quantity of the amino acids increased with increasing lead and cadmium concentrations in both the cultivars of pigeonpea. The studies on protein content revealed the reduction in seedling axes and retention in cotyledons with increasing lead and cadmium concentrations when compared of their respective controls. From this observations it could be emphasized that the cultivar differed in response to two heavy metals.

Key words : Cultivars, Heavy metals, Pigeonpea, Proline, Protein

Lead and cadmium are toxic among the heavy metals. They are supplied to soil, air and water mainly by effluent from industries, mining, burning and leakage of waste and by fertilization with phosphates and sewage sludge. Soils contaminated with these heavy metals affect the growth, development and yield of plants (Tomsett and Thurman, 1988).

Amino acids are the primary products of inorganic nitrogen assimilation. In addition, free amino acids may also be formed by protein hydrolysis. Among the free amino acids, proline plays an important role under stress conditions and also a key role in osmoregulation (Aspinall and Paleg, 1981; Lilibute and Hellebust, 1989) protection of enzyme denaturation acts as a reservoir of carbon and nitrogen source and stabilizes the protein synthesis machinery (Fukutaku and Yamada, 1984; Kadpal and Rao, 1985). During germination and seedling growth, lead and cadmium affect the mobilization and hydrolysis of reserve proteins and their subsequent transport to growing axis (Krupa, 1988). Heavy metal toxicity causes the generation of reactive oxygen species (ROS) and its reaction with lipids, pigments, proteins and amino acids, resulting in membrane damage, inhibition of photosynthesis and enzyme inactivation (Stoeva *et al.*, 2003; Wang *et al*., 2008). The inhibitory action of lead and cadmium on mobilization of seed storage proteins and consequent restricted availability of free amino acids to the growing axis impair the capacity of these tissues to carry out the synthesis of proteins involved in growth.

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Amino acids and proteins are major components of pigeonpea. Pigeonpea is one of the important pulse crops of India. Its seed protein content is about 24% which is at par with any other legume. Seeds of pigeonpea constitute one of the principal sources of vegetable protein to the people of Indian homes hence the objectives of the initial phase of this research was to study the effect of lead and cadmium on free amino acid composition and protein content in pigeonpea cultivars.

MATERIALS AND METHODS

Plant material and its growth conditions :

Seeds of pigeonpea (*Cajanus cajan*(L.)Millspaugh) cv. T21 (medium duration) and cv.LRG30 (long duration) supplied by ICRISAT, Patancheru, India were used in the present study. The seeds of uniform size and free from infection were selected for the experiments. The seeds were surface sterilized by using 0.01M sodium hypochlorite for 2 min, washed thoroughly with distilled water and were placed separately in trays lined with Whatman No.1 filter papers containing 0, 0.5, 1.0 and 1.5mM concentrations of lead (lead acetate: $(CH_3COO)_2Pb.3H_2O$) and cadmium (cadmium chloride: $CdCl_2$ $2.5H_2O$), respectively. Twenty five seeds were taken in each tray. Seeds germinated and seedlings grown in distilled water (zero concentration) served as controls. The seeds were allowed to germinate at 30 ± 2^0C for 8 days under a photoperiod of 12h and at 195m mol m^{-2} s^{-1} PPFD.

Amino acid analysis and total proteins:

Changes in free amino acid composition were studied in the control and treatments of 6-day old pigeonpea seedlings using LKB Automatic Amino Acid Analyser. For amino acid analysis, 200mg of plant material was homogenised in 80% alcohol. The alcohol was evaporated in vacuo and the residue was dissolved in citrate buffer pH 2.2 and was made up to a known volume. The amino acids were loaded and analysed on a cation exchange resin with buffer of carefully defined salt concentrations and pH as described in the 'Hand book and Applications for LKB Biochrom Automatic Amino Acid Analyser' which was used for this analysis. Sodium salt used for buffer preparation was passed through a teflon coil placed in a boiling water bath. Before entering the coil the column effluent was mixed with acetate buffer containing the reduced ninhydrin. This compound reacts with amino acids forming a dye complex. The absorption was determined in a flow photometer and registered on the chart of a recorder. The quantification and identification of the different amino acids were carried out using standard amino acids mixture consisting of aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cysteine, valine, metheonine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, arginine and ammonia. The LKB standard concentration is 2.5μ mole/ml except for cysteine which is 1.25μ mole/ml. Total protein content was estimated by the method of Lowry *et al*., (1951).

RESULTS AND DISCUSSION

Considerable changes in free amino acid composition was observed depending upon the degree of the heavy metal stress in both the cultivars of pigeonpea. Increasing concentrations of heavy metals resulted in increasing concentration of different amino acids in the 6-day old pigeonpea seedlings. The amino acids that showed conspicuous increase in response to lead and cadmium stress in both the pigeonpea cultivars include isoleucine, leucine, metheonine, aspartic acid, proline, glutamic acid, histidine, lysine and cysteine (Table 1). It was reported that heavy metal stress preferentially results in the accumulation of proline, asparagine, isoleucine, leucine, metheonine, lysine, cysteine and valine (Fukutaku and Yamada, 1984; Costa and Morel, 1994). E1- Shintinawy and EI- Ansary (2000) monitored the changes in amino acid metabolism in soybean seedlings exposed to toxic concentrations of cadmium in order to measure the ability of the seedlings to tolerate the heavy metal. The increase of different amino acids in lead and cadmium treated pigeonpea seedlings may be attributed to individual or combined effect of the following possibilities. It might be caused by the malfunctioning of respiratory activity (Lee *et al*., 1976) mostly due to membrane damage (Vazqueze *et al*., 1992), resulting in the accumulation of several krebs cycle compounds such as 2-oxogluterate and pyruvate which may promote the synthesis of specific amino acids. It may also results due to the complexion of lead and cadmium ions with sulfhydryl groups leading to increased metheonine and cysteine. Further, it may be due to reduced protein synthesis or increased protein breakdown (Reese and Winge, 1988; Rauser, 1990). It was interesting to note the conspicuous increase in proline content with increasing heavy metal stress in both the cultivars when compared to their respective controls (Table 1). Most of the proline accumulated in the plant tissues may be the result of net de novo synthesis from glutamic acid (Alia and Saradhi, 1991) or may be due to protein hydrolysis to free amino acids (Mikola and Mikola, 1980) or due to decreased proline oxidation (Huang and Cavalieri, 1979).

The protein content of the seedling axes during seedling growth of pigeonpea cultivars showed a continuous increase associated with a concomitant decrease in their respective colytedons. Though the protein content of the seedling axes of the lead and cadmium treated seedlings exhibited a trend similar to that of controls with age of their values were always lower than their controls. The protein content of the cotyledons of the lead and cadmium treated pigeonpea seedlings showed more retention with increasing lead and cadmium concentrations (Fig. 1). The soluble protein content in plant cells is an important indicator of their physiological state. The retention of proteins in the cotyledons of the treated pigeonpea seedlings is due to the decreased hydrolysis and transport of amino acid products from the cotyledons to the growing seedling axes under heavy metal stress. This has led to the decreased protein content in the seedling axes of the lead and cadmium treated pigeonpea seedlings resulting in decreased growth (Melnichuk *et al*., 1982). The lowered protein content of the pigeonpea seedling axes of the treatments may also be due to breakdown of proteins in response to lead and cadmium stress (Stewart and Larher, 1980). The reduced amount of soluble proteins was most probably a result of the reduced biosynthesis or the accelerated catabolic processes, on the other hand, the accelerated catabolism is probably due to the considerable disturbances in the membrane systems, in response to the metal phytotoxicity (Stoeva *et al.*, 2003).

Between the two heavy metals, cadmium effected the free amino acid and protein content of the seedlings more intensely than lead. In relation to cultivar differences, cv. T21 showed greater values of total free amino acid than cv. LRG30. In cv. T21 the per cent reduction in the protein content of seedling axes and per cent retention in the protein content of cotyledons were relatively more conspicuous when compared to cv. LRG30. The cv.

Lead

T21

A.

Amino acid	Lead Concentrations (mM)			
	0.0	0.5	1.0	1.5
Aspartic acid	0.002	0.097	2.987	3.491
Threonine	1.012	0.526	0.227	0.945
Serine	0.235	1.979	2.650	4.562
Glutamic acid	2.974	1.927	0.358	0.632
Glycine	0.998	1.156	1.162	2.001
Alanine	-	0.984	0.317	0.304
Valine	-	0.079	1.194	0.196
Metheonine	0.001	0.597	1.256	1.494
Isoleucine	0.151	1.821	1.824	1.276
Leucine	0.276	0.694	0.704	1.094
Tyrosine	0.002	0.021	0.398	0.199
Phenylalanine	0.216	0.184	0.987	0.270
Histidine	0.434	0.493	1.762	0.515
Lysine	0.156	1.976	0.954	1.617
Ammonia	1.001	0.394	1.612	0.621
Arginine	0.404	-	0.697	0.856
Proline	-	0.240	0.435	2.382
Cysteine	-	2.567	-	0.459
Total	**7.862**	**15.735**	**19.524**	**23.214**

E.

Amino acid	Lead Concentrations (mM)			
	0.0	0.5	1.0	1.5
Aspartic acid	0.075	2.085	3.126	4.162
Threonine	0.135	0.235	0.135	0.324
Serine	0.216	0.416	0.782	1.692
Glutamic acid	0.306	0.406	0.426	0.349
Glycine	0.901	1.202	1.344	0.492
Alanine	0.899	0.202	0.114	2.216
Valine	0.200	0.207	0.407	0.596
Metheonine	-	1.513	2.009	3.012
Isoleucine	-	1.487	2.127	2.560
Leucine	0.252	2.425	1.321	2.719
Tyrosine	0.492	0.546	0.576	0.672
Phenylalanine	0.124	0.295	0.423	0.694
Histidine	0.687	0.786	0.987	0.262
Lysine	0.721	0.535	0.216	0.495
Ammonia	0.116	0.398	0.297	0.649
Arginine	0.123	0.225	0.145	0.235
Proline	-	1.014	3.113	4.256
Cysteine	0.013	0.402	0.427	0.475
Total	5.260	14.379	15.714	25.863

LRG 30

B.

Amino acid	Lead Concentrations (mM)			
	0.0	0.5	1.0	1.5
Aspartic acid	-	0.032	0.619	1.268
Threonine	1.016	0.483	0.193	0.726
Serine	0.245	1.924	2.350	3.242
Glutamic acid	3.744	1.858	1.168	1.433
Glycine	0.857	1.132	2.066	2.501
Alanine	-	0.762	0.210	0.108
Valine	-	0.034	1.131	0.154
Metheonine	-	0.397	0.993	0.139
Isoleucine	0.162	1.621	1.627	1.216
Leucine	0.281	0.669	0.803	1.053
Tyrosine	-	-	0.192	0.180
Phenylalanine	0.231	0.181	0.956	1.107
Histidine	0.416	0.694	1.458	1.215
Lysine	0.176	1.831	0.755	1.416
Ammonia	0.996	0.279	1.410	1.221
Arginine	0.400	-	0.600	0.758
Proline	-	-	0.281	0.187
Cysteine	-	2.454	-	1.454
Total	8.524	14.351	16.811	19.378

F.

Amino acid	Lead Concentrations (mM)			
	0.0	0.5	1.0	1.5
Aspartic acid	0.057	0.032	1.565	2.483
Threonine	0.145	1.128	0.708	1.226
Serine	0.517	1.857	2.634	3.558
Glutamic acid	0.106	0.477	-	0.955
Glycine	0.200	0.501	0.988	1.001
Alanine	0.901	-	0.072	-
Valine	0.899	-	-	-
Metheonine	-	-	1.001	1.002
Isoleucine	-	0.567	0.155	1.945
Leucine	-	0.214	0.930	1.044
Tyrosine	0.350	0.180	0.036	0.336
Phenylalanine	0.153	0.100	0.453	0.282
Histidine	0.587	0.805	0.620	1.200
Lysine	0.689	0.307	0.072	0.559
Ammonia	0.141	1.531	0.714	1.338
Arginine	-	0.375	0.199	0.508
Proline	-	2.075	3.141	0.154
Cysteine	-	0.510	0.712	0.010
Total	4.745	10.719	14.000	17.601

Cadmium

T21

C.

Amino acid	CadmiumConcentrations (mM)			
	0.0	0.5	1.0	1.5
Aspartic acid	0.002	0.997	4.723	5.343
Threonine	1.012	1.890	2.127	0.798
Serine	0.235	1.776	3.126	4.854
Glutamic acid	2.974	3.806	0.021	1.762
Glycine	0.998	1.497	4.976	5.684
Alanine	-	0.385	0.708	0.792
Valine	-	0.954	0.254	0.121
Metheonine	0.001	0.389	1.573	0.239
Isoleucine	0.151	0.121	0.127	1.708
Leucine	0.276	1.827	3.765	13.001
Tyrosine	0.002	0.425	0.394	1.206
Phenylalanine	0.216	2.467	0.545	0.470
Histidine	0.434	1.975	0.250	0.331
Lysine	0.156	0.872	1.217	0.876
Ammonia	1.001	0.896	0.892	0.121
Arginine	0.404	0.774	0.612	2.890
Proline	-	0.124	0.856	2.900
Cysteine	-	-	0.757	1.430
Total	7.862	21.175	25.917	47.527

G

Amino acid	Cadmium Concentrations (mM)			
	0.0	0.5	1.0	1.5
Aspartic acid	0.075	2.152	3.194	6.232
Threonine	0.135	0.294	0.346	0.472
Serine	0.216	0.786	1.642	2.794
Glutamic acid	0.306	0.569	0.397	0.564
Glycine	0.901	1.396	2.586	4.723
Alanine	0.899	0.498	0.692	0.816
Valine	0.200	0.798	0.894	0.983
Metheonine	-	3.043	4.093	5.193
Isoleucine	-	1.560	1.516	4.126
Leucine	0.252	3.569	4.297	3.586
Tyrosine	0.492	0.297	0.486	0.649
Phenylalanine	0.124	0.897	0.998	0.234
Histidine	0.687	6.129	6.239	6.497
Lysine	0.721	0.689	0.889	0.002
Ammonia	0.116	0.729	0.442	0.242
Arginine	0.123	0.125	0.492	0.649
Proline	-	4.296	4.567	4.672
Cysteine	0.013	0.225	0.349	0.432
Total	5.260	28.052	34.209	45.866

LRG 30

D.

Amino acid	Cadmium Concentrations (mM)			
	0.0	0.5	1.0	1.5
Aspartic acid	-	0.856	1.513	4.318
Threonine	1.016	1.290	3.919	0.548
Serine	0.245	1.240	3.490	3.650
Glutamic acid	3.744	0.106	0.015	1.526
Glycine	0.851	3.247	4.763	5.420
Alanine	-	0.180	0.504	0.774
Valine	-	0.780	0.154	0.060
Metheonine	-	0.357	1.430	2.135
Isoleucine	0.162	0.081	0.181	1.406
Leucine	0.281	1.607	3.214	5.500
Tyrosine	-	0.324	0.192	1.006
Phenylalanine	0.231	2.113	0.503	0.243
Histidine	0.416	1.354	0.153	0.330
Lysine	0.176	0.671	0.945	0.631
Ammonia	0.996	0.750	0.791	0.041
Arginine	0.400	0.574	0.660	4.620
Proline	-	0.120	0.750	3.789
Cysteine	-	-	0.547	2.500
Total	8.524	15.652	23.708	38.497

H.

Amino acid	Cadmium Concentrations (mM)			
	0.0	0.5	1.0	1.5
Aspartic acid	0.057	2.714	3.217	4.043
Threonine	0.145	1.839	1.700	0.946
Serine	0.517	1.061	1.305	1.371
Glutamic acid	0.106	0.358	0.998	1.483
Glycine	0.200	0.569	1.229	1.240
Alanine	0.901	0.215	0.137	0.428
Valine	0.899	0.017	-	-
Metheonine	-	1.001	2.351	1.060
Isoleucine	-	1.743	2.071	1.046
Leucine	-	0.441	0.864	1.054
Tyrosine	0.350	0.036	0.211	0.540
Phenylalanine	0.153	0.090	1.354	0.138
Histidine	0.581	1.888	0.873	0.168
Lysine	0.689	1.957	0.700	1.149
Ammonia	0.141	1.842	1.206	0.450
Arginine	-	1.274	1.084	-
Proline	-	0.156	0.545	0.545
Cysteine	-	2.175	-	-
Total	4.745	19.376	22.980	30.744

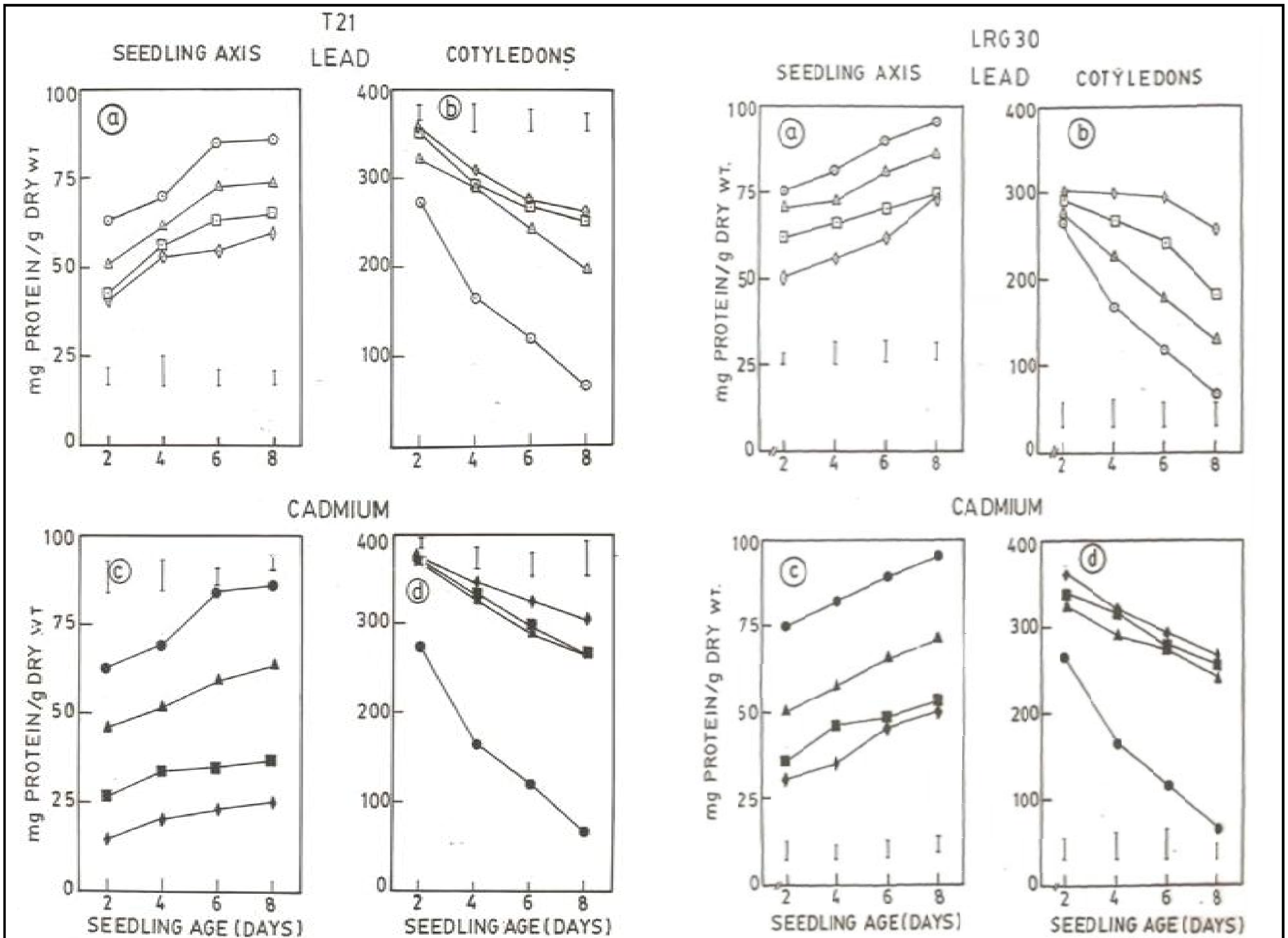
cv. T21 Lead | Seedling axis : A, Cotyledons : E

cv. T21 Cadmium | Seedling axis : C, Cotyledons : G

cv. LRG30 Lead | Seedling axis : B, Cotyledons : F

cv. LRG30 Cadmium | Seedling axis : D, Cotyledons : H

Table 1 : Free amino acid composition («m/f/f wt) of seedling axes of 6-day old pigeonpea cv. T 21 and cv.LRG 30 in response to lead and cadmium stresses (*The data on ammonia, which is not an amino acid, are included for information-not detectable)



Seedling axis : a and c
Cotyledons : b and d
Lead : a and b
Control : ○ - ○; 0.5 : mM Δ- Δ ; 1.0: mM ¯-¯ ; 1.5: mM ◊-◊
Cadmium : c and d
Control : ●-● ; 0.5: mM ▲-▲ ; 1.0: mM ■-■; 1.5: mM ♦-♦

Fig. 1 : Protein content of seedlings of pigeonpea, cv.T21 and cv.LRG30 in response to lead and cadmium stresses (Vertical lines represent S.E.)

LRG30 possessed relatively better tolerance mechanism to elevated concentrations of lead and cadmium.

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