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CHANGES IN NITROGEN, PROTEIN, AMINO ACID AND PROLINE CONTENT IN MUNGBEAN (*VIGNA RADIATA* L. WILCZEK) SEEDLINGS AS AFFECTED BY TOXIC LEVELS OF ALUMINIUM

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SUMMARY

The toxic effect of aluminium was studied in germinating seedlings of mungbean (*Vigna radiata*) by taking different concentrations viz. 8×10^{-4}, 10^{-3} and 5×10^{-3}M of aluminium sulphate. Nitrogen content declined with an increase in aluminium concentration. The buffer soluble fraction of total protein, however, showed an increasing trend due to Al-toxicity. The increased intensity of three major polypeptides (R_{mf} 0.14, R_{mf} 0.51 and R_{mf} 0.68) in SDS PAGE profile, however, was in accordance with increased status of protein level in Al-toxicity induced germinated seedlings. Free amino acids and free proline increased with increase in concentration of $Al_2(SO_4)_3$, a phenomenon common with any type of stress influence.

INTRODUCTION

Aluminium toxicity is probably one of the most important factors responsible to limit plant growth in acid soil. Aluminium at concentration 148 and 297 µM is reported to enhance nitrogen concentration in both leaves and roots of *Trifolium repens* (Lee and Pritchard 1984) while reports are available of lowering nitrogen concentration in aerial parts of *Vigna unguiculata* (Mayz and Cartwright 1984) and groundnut (Shamsuddin *et al.* 1992). The soil aluminium, has significant bearing on the protein content. The decrease in protein content by aluminium had been found in rice (Sarkunan *et al.* 1984). Aluminium appeared to cause greater change in protein content associated with cytoplasm, while little change in protein content is observed in other cellular compartments. Of the approximate 600 proteins examined in hard red winter wheat, 14 cytoplasmic and 8 microsomal proteins had been induced or enhanced by aluminium treatment, whereas 9 cytoplasmic and 12 microsomal proteins had been either diminished or repressed (Ownby and Hruschka 1991). Taylor (1997) has reported that treatment with Al led to accumulation of a 23 kD polypeptide in root exudates of an aluminium-resistant cultivar of *Triticum aestivum*. Free amino acid has direct correlation with Al uptake. Higher amount of amino acid accumulation in plants due to toxic levels of aluminium has been reported by Cambraia *et al.* (1983). The increase in free amino acid pool could be the result of enhanced protein degradation (Possingham 1956). Of the total amino acid pool the percentage of asparagine declined but glutamine is found to increase with Al level in the nutrient solution, indicating its interference in synthesis and /or interconversion of these amides (Bauer *et al.* 1977). Proline is used as a stress marker and as an index of stress resistance. Plants exposed to higher Al concentrations, in majority of the experiments, show an increased proline content justifying the stress condition. Leaves of chickpea seedling have been reported to accumulate maximum amount of proline at 1mM $Al_2(SO_4)_3$ concentration (Satakopan *et al.* 1992a). On the contrary, Galvez *et al.* (1991) have reported that free proline level in leaves and roots of *Sorghum* is not much affected as aluminium in solution is increased. Ecological studies also support the idea that proline accumulation is

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important in plant adaptation to adverse environments and is related to survival.

In order to increase our understanding about aluminium toxicity, the present study was undertaken to observe the changes in nitrogen, protein, amino acid and proline content in relation to the varying concentrations of aluminium.

MATERIALS AND METHODS

Seeds of mungbean (*Vigna radiata*) were germinated in petri plates in presence of different concentrations of aluminium sulphate, while seeds germinating in water served as control. The leaves of 7 days old seedlings were harvested and subject to different biochemical analyses. For the estimation of total nitrogen, Micro Kjeldahl method was followed. The total nitrogen content was expressed in terms of mg nitrogen per g dry weight basis. Protein was estimated according to method of Lowry *et al.* (1951), and result was expressed as mg protein per g fresh tissue. The separation of proteins by SDS-PAGE was carried out according to method of Laemmli (1970) with some modifications. The electrophoretic gel after staining and destaining was photographed. All the protein bands of the gel were drawn diagrammatically and mobility of protein bands calculated. Then the relative mobility factor (distance migrated by the protein band/distance migrated by the front line) was calculated for each band. The scanning was done in gel scanner (Bio Rad Gel Doc 1000). The free amino acid content was estimated according to the ninhydrin method of Lee and Takahashi (1966) with some modifications. The colour intensity was measured at 570 nm in Hitachi-U-2000 Spectrophotometer. The results were expressed in terms of mg amino acid for per g fresh tisue. Proline was estimated according to method of Bates *et al.* (1973), and the optical density was recorded in spectrophotometer at 520 nm. The results were expressed as mg proline per g fresh tissue.

RESULTS AND DISCUSSION

Nitrogen content was reduced significantly ($P<0.01$) by 34%, 41% and 56% with 8×10^{-4}M, 10^{-3}M and 5×10^{-3} $Al_2(SO_4)_3$ respectively, and maximum reduction was noticed with the highest $Al_2(SO_4)_3$ concentration i.e. 5×10^{-3}M, (Table 1). The significant negative correlation between nitrogen content and aluminium serves to support the experimental findings (Table 2). Similar observation of lowered nitrogen concentration in the presence of Al in aerial parts of *Vigna unguiculata* (Gomes *et al.* 1985) was reported. Plant nitrogen concentration plays a key role in crop development which is crucial for CO_2 fixation and dry matter production (Sivsankar *et al.* 1993). Thus low nitrogen content in mungbean in the presence of aluminium indicates low CO_2 fixation and reduced crop growth.

Different concentrations of $Al_2(SO_4)_3$ contributed to significant increase ($P<0.01$) in buffer soluble protein, increase being 11%, 29% and 81% with 8×10^{-4}M, 10^{-3}M and 5×10^{-3}M respectively (Table 1). The SDS PAGE profile showed 3 major bands, viz. band (i) R_{mf}0.14), band (ii) R_{mf}(0.51) and band (iii) R_{mf} 0.68). No change in number of major bands was recorded due to $Al_2(SO_4)_3$ treatment. The intensity of three bands increased with increasing concentrations of $Al_2(SO_4)_3$, increase being

Table 1. Effect of aluminium on nitrogen, buffer soluble protein, free amino acid and proline content of leaves of 7 days old seedings of *Vigna radiata* germinated in petri plates.

Concentrations $Al_2(SO_4)_3$ (M)	Nitrogen content mg N released/g d.w.	Buffer soluble Protein mg/g f.w.	Free amino acid content mg/g f.w.	Free proline content
Water Control	7.94	1.86	3.57	0.059
8×10^{-4}	5.26	2.06	5.95	0.107
10^{-3}	4.65	2.40	6.94	0.151
5×10^{-3}	3.46	3.36	8.76	0.176
S.E. (Mean)	0.140	0.138	0.152	0.002
C.D. at 5%	0.456	0.449	0.495	0.006

Table 2. Multiple correlation between concentrations of $Al_2(SO_4)_3$, nitrogen, buffer soluble protein, free amino acid and proline content.

	Aluminium	Nitrogen	Amino acid	Protein
Nitrogen	-0.738*			
Amino acid	0.863*	-0.917*		
Protein	0.93*	-0.752*	0.859*	
Proline	-0.412	0.810*	-0.766*	-0.424

*Significant at 1% level

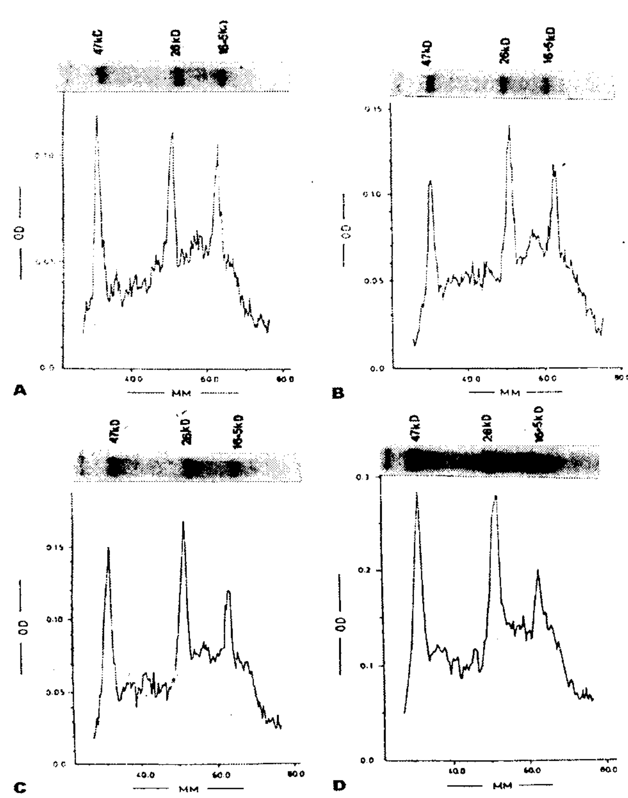

Fig. 1. SDS-PAGE profile of soluble protein in the leaves of control and $Al_2(SO_4)_3$ treated 7 days old mungbean seedlings (A) control (B) $8x10^{-4}$ M $Al_2(SO_4)_3$ (C) 10^{-3} M $Al_2(SO_4)_3$ (D) $5x10^{-3}$ M $Al_2(SO_4)_3$

7%, 42% and 95% in case of band (i), 47%, 97% and 168%in case of band (ii) and 60%, 142% and 304 in case of band (iii) due to the effect of 8×10^{-4}M, 10^{-3}M and 5×10^{-3}M $Al_2(SO_4)_3$ respectively. The increase in intensity of the 3 bands was gradual in case of 8×10^{-4}M and 10^{-3}M though a drastic increase in band intensities was noticed in case of 5×10^{-3}M $Al_2(SO_4)_3$ (Fig 1). A general trend of reduction of total protein content has been reported in many species due to effect of abiotic stresses and metal toxicity (Osman and Esmat-Elwy 1989). The buffer soluble fraction of total protein, however, showed an increasing trend due to Al-toxicity in the present study. The result is further justified from statistical point of view as positive correlation is obtained between concentration of Al and buffer soluble protein content. The increased intensity of three major polypeptides (R_{mf} 0.14, R_{mf} 0.51 and R_{mf} 0.68) in SDS PAGE profile, however, was in accordance with increased status of protein level in Al-toxicity induced germinated seedlings. A possible explanation is that there may be stimulation of some type of protein synthesis in response to an inhibitor which is not directly concerned with growth. It can be inferred that there may be chelation of the proteins by metals forming metal-protein complex, thereby leading to dysfunction of enzyme protein. Thus the overall effect was an increase in the level of soluble protein.

Amino acid content increased significantly ($P<0.01$) with increasing concentrations of $Al_2(SO_4)_3$, the increase being to 66%, 94% and 145% as affected by 8×10^{-4}M, 10^{-3}M and 5×10^{-3}M $Al_2(SO_4)_3$ respectively (Table 1). A similar result was observed by Mosquim (1977) where high amount of amino acid accumulated in *Stylosanthes humilis* because of toxic levels of aluminium. More proline accumulated with increasing concentrations of $Al_2(SO_4)_3$ which amounted to 82%, 158% and 200% in presence of 8×10^{-4}M 10^{-3}M and 5×10^{-3}M $Al_2(SO_4)_3$ respectively (Table 1). There was a trend of increase of both total free amino acids and free proline with increase in concentration of $Al_2(SO_4)_3$. This happened as free amino acids and particularly proline, have been reported to accumulate unequivocally in diverse plant species by any type of stress imposition (Satakopan *et al.* 1992b). Accumulation of proline in plants under various environmental stresses including drought and temperature stress is well documented (Gzik 1996). It is now evident from the present study that an increase in proline content may serve as a means of protection of plant tissue against stress. This increase in proline content is thought to act as a hydrophobic protectant for enzymes and subcellular organelles (Lerudulier *et al.* 1994) thus helping the plants to tolerate or adapt to the stress condition. From statistical point of view, increment of $Al_2(SO_4)_3$ concentrations was found to be negatively correlated with proline content while that was positively correlated with content of amino acid justifying the experimental findings (Table 2).

It was observed in our work that all the biochemical constituents estimated underwent considerable change with the increase in aluminium concentration. Increased buffer soluble protein, free amino acid and proline content corresponding with an increase in aluminium concentration, characterised the unfavourable situation and served as biochemical indicator of stress condition.

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