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Effect of aluminium toxicity on biochemical parameters of rice

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ABSTRACT

Experiment were conducted at Bidhan Chandra Krishi Viswavidyalaya to ascertain the intensity of damage in rice due to aluminium toxicity among the moderately resistant and susceptible cultivar Khitish and Shatabdi, respectively. Changes in respect of protein, free amino acid and changes in root and shoot of the crop had been studied. It was observed that the above parameter decreased substantially with the increase of aluminium i.e. from 0.1 mM to 0.6 mM as also with duration of exposure i.e. from 24 hour to 72 hour. However, the intensity of damage was less in Khitish compared to Shatabdi.

Key words: *rice, aluminium, toxicity, amino acid, protein*

Metal ions are essential in maintenance and evolution of all life systems, and mediate all stages of dissemination of genetic information carried out in the genetic code. At the same time metals can, when present in excess, or under wrong conditions, causes severe cellular injuries. The primary source of heavy metals in the environment is from naturally occurring geochemical materials. Uptake of toxic metals by plant roots depends on their availability, which in turn, to a great extent affected by soil pH. Increase in acidity vis-a-vis lowering in pH of soil solution increased availability of metals like Al, Cd, Cr and Pb (Nriagu and Pacyna, 1988). Around 12% of land involved in crop production in acidic and acid soil infertility is a major limitation to crop production on tropical and temperate regions of the world (Von Uexkiill and Mutert, 1995). In India acid soils are found extensively in Himalayan region, Eastern, Northeastern and peninsular India. Aluminium toxicity is the single most important factor, being a major constraint to crop production (Eswaran *et al.*, 1997). Aluminium has been shown to disturb several physiological and biochemical processes and consequently many mechanisms of aluminium toxicity have been proposed from time to time by different authors (Kochian, 1995).

MATERIALS AND METHODS

Seeds of rice *cv.* Khitish and Shatabdi were collected from Rice Research Station, Chinsurah, Hoogly, West Bengal and used for study. The experiments were carried out under laboratory condition in hydroponics system with balance nutrient solution. Seeds of rice were surface sterilized with 0.1% $HgCl_2$ and soaked in distilled water overnight and were sown in the cotton bed in a plastic tray containing 100mM $CaCl_2$ solution and grown upto 7 days. After that it was transplanted to nutrient solution having varying concentrations of aluminium. The nutrient solution used in the experiments was Standard rice culture solution (Yoshida *et al.*, 1971).

Seedling were transplanted/transferred on a special floating nets in glass beaker containing nutrient solution and varying concentration of aluminium in separate containers having 4 replications to study the toxicity effect of aluminium. pH of the control nutrient solution as well as different treatment solutions were kept at 4.0. The pH was adjusted to pH 4.0 by using 0.1N NaOH and 0.1N HCl on each day as required. Seedlings were harvested at an interval of 24 hours, 48 hours, and 72 hours after treatments and root-shoot growth were recorded to ascertain the toxicity of aluminium. Free amino acid content was determined according to the Moore and Stein (1948). Protein estimation was carried out according to Lowry *et al.* (1951).

RESULTS AND DISCUSSION

Treatment with aluminium in solutions increased proline content significantly over the control

in both cultivars. Proline content increased more over control in response to different aluminium treatments (Table 1). Proline content of root in Khitish cultivars in control at 24 hr duration was 0.363 ìmole/g fresh wt, and with application of 0.6mM aluminium concentration in solution it was induced to 2.118 ìmole/g fresh wt. Where as in shoot, proline content of control IET-4094 seedlings was 0.510 ìmole/g fresh wt. at 24 hr exposure, substantial increase in proline was observed under 0.6mM aluminium concentration (Table 2). Proline content in root and shoot of two rice cultivars increased in response to higher concentration of aluminium treatments and longer duration of exposure to aluminium. The results suggest that, as IET-4094 accumulated more proline in response to aluminium concentration than that of IET-4786, the former one confers more resistance to aluminium. The accumulation rate of proline in shoot in response to aluminium treatments also differed in the two cultivars.

Proline accumulation due to aluminium toxicity was possibly due to metal imposed increase in water deficiency rather than direct toxicity effect of metal (Schat *et al*., 1997). Aluminium mediated extensive root damages limited water uptake in plants. The reduced volume of the root system (Clarkson, 1969), decrease of both over all L_{Pr} i.e. root hydraulic conductivity (Barcelo *et al.*, 1996) and L_{Pc} i.e. cortex cell hydraulic conductivity (Zhao *et al*., 1987) also the cause behind water deficit/stress experienced by plants due to aluminium stress.It may be suggested from the above result that functional significance of aluminium induced proline accumulation would lie in its contribution to water balance maintenance and that proline-mediated alleviation of water deficit could substantially contribute to aluminium tolerance of the plants (Schat *et al.*, 1997).

Amino acids, both free á-amino acids and proline content, both are good indicators of toxicity

Table 1. Time duration study of root proline content (µmole g^{-1} fresh wt) of two rice seedlings in response to aluminium toxicity

Treatment (Al^{+3} concI)	Kshitish			Shatabdi		
	24 Hour	48 Hour	72 Hour	24 Hour	48 Hour	72 Hour
Control	0.363 f	0.559 e	0.617 f	0.315 e	0.406 d	0.737 f
0.1 mM	0.426 e	0.774 e	0.931 e	0.386 e	0.481 d	0.922 e
0.2 mM	0.814 d	1.363 d	1.497 d	0.618 d	0.739 c	1.218 d
0.3 mM	1.227 c	1.602 c	1.815 c	0.940 c	1.100 b	1.402 c
0.4 mM	1.814 b	2.327 b	2.543 b	1.344 b	1.494 a	1.937 b
0.6 mM	2.118 a	2.698 a	2.918 a	1.602 a	1.679 a	2.16 a

Values in table represent pooled data of three replicates. Different letters beside the value indicate statistically significant difference at 0.05 level according to DMRT

Table 2. Time duration study of shoot proline content (µmole g^{-1} fresh wt) of two rice seedlings in response to aluminium toxicity

Treatment (Al^{+3} concI)	Kshitish			Shatabdi		
	24 Hour	48 Hour	72 Hour	24 Hour	48 Hour	72 Hour
Control	0.510 f	0.578 f	0.649 f	0.638 f	0.669 f	0.839 f
0.1 mM	0.623 e	0.692 e	0.788 e	0.708 e	0.795 e	0.909 e
0.2 mM	0.738 d	0.803 d	0.909 d	0.801 d	0.885 d	0.972 d
0.3 mM	0.827 c	0.897 c	1.021 c	0.847 c	0.967 c	1.064 c
0.4 mM	0.909 b	1.008 b	1.135 b	0.943 b	1.003 b	1.154 b
0.6 mM	1.020 a	1.183 a	1.341 a	1.129 a	1.169 a	1.296 a

Values in table represent pooled data of three replicates. Different letters beside the value indicate statistically significant difference at 0.05 level according to DMRT

responses (Hare and Cress, 1997). But unlike other toxic metals aluminium treatments decreased free á-amino acid content in both varieties over durations of exposure except 0.1mM Al in solutions at 24 hr exposure (Table 3). Prolong exposure at 72 hr to 0.1mM aluminium treatment though reduced free amino acid content in both cultivars decreased with increasing concentration of aluminium. Aluminium treatments with 0.6mM exert most detrimental effect on free amino acid content. At 24 hr duration free á-amino acid content of root was 0.782 mg g^{-1} in control plants of IET-4094 and that had been reduced to 0.428 mg g^{-1} in response to 0.6 mM aluminium treatments. Over the time period the free á-amino acid content was further reduced to 0.28 mg/g fresh wt. in root. The same was observed in variety IET-4786 where free á-amino acid content of root reduced more in response to treatments over control treatments. In IET-4094 after 72 hr of exposure to aluminium concentration, percent reduction in free á-amino acid content was 53.63 whereas in IET-4786 it was only 60.58%. In case of shoot free á-amino acid content difference had been observed more (Table 4). Whereas 0.6mM aluminium treatments for 72 hr in IET-4786 cultivar there was a reduction in shoot á-amino acid content to the extent of 42%, but it was only 22.26% in IET-4094 (both were measured as changes over control). Such observation was reported on jute (*Corchorus olitorius*) by Mazen (2004), who suggested that prolonged exposure to aluminium perhaps suppresses the metabolic processes including amino acid formation. Another possible reason for this pattern could be that amino acids were chelated with aluminium and free amino acid content might be reduced as because amino acid were reported to play a significant role in metal chelation (Hall, 2002).

In general results observed that root protein content was much lower than shoot protein content in

Table 3. Time duration study of root free amino acid content (mg g^{-1} fresh wt) of two rice seedlings in response to aluminium toxicity

Treatment (Al^{+3} concI)	Kshitish)			Shatabdi		
	24 Hour	48 Hour	72 Hour	24 Hour	48 Hour	72 Hour
0 mM	0.782 a	0.705 a	0.606 a	0.834 a	0.804 a	0.723 a
0.1 mM	0.673 b	0.637 b	0.539 b	0.693 b	0.658 b	0.654 b
0.2 mM	0.681 b	0.605 c	0.481 c	0.656 c	0.623 c	0.583 c
0.3 mM	0.608 c	0.582 d	0.429 d	0.609 d	0.563 d	0.465 d
0.4 mM	0.536 d	0.439 e	0.389 e	0.540 e	0.420 e	0.332 e
0.6 mM	0.428 e	0.387 f	0.281 f	0.410 f	0.384 f	0.285 f

Values in table represent pooled data of three replicates. Different letters beside the value indicate statistically significant difference at 0.05 level according to DMRT

Table 4. Time duration study of shoot free amino acid content (mg g^{-1} fresh wt) of two rice seedlings in response to aluminium toxicity

Treatment (Al^{+3} concI)	Kshitish			Shatabdi		
	24 Hour	48 Hour	72 Hour	24 Hour	48 Hour	72 Hour
0 mM	2.261 a	2.131 a	2.039 a	1.643 a	1.474 a	1.445 a
0.1 mM	2.197 b	2.073 b	1.998 b	1.576 b	1.322 b	1.209 b
0.2 mM	2.104 c	2.004 c	1.843 c	1.471 c	1.236 c	1.118 c
0.3 mM	2.005 d	1.925 d	1.801 d	1.409 d	1.171 d	1.044 d
0.4 mM	1.829 e	1.756 e	1.706 e	1.329 e	1.035 e	0.947 e
0.6 mM	1.773 f	1.684 f	1.585 f	1.246 f	0.986 f	0.838 f

Values in table represent pooled data of three replicates. Different letters beside the value indicate statistically significant difference at 0.05 level according to DMRT

both cultivars exposed to different aluminium concentrations and same has been observed in control plants also. It was further observed between root and shoot protein content, where in root, protein content decreased continuously with longer duration with or without aluminium, while in shoot the reverse pattern has been observed. In response to 0.6mM aluminium concentration the root protein content of Khitish seedlings was reduced to 2.920mg/g fresh wt, which was 51.06% reduction over control (Table 5). In Shatabdi the reduction in root protein content in response to 0.6 mM aluminium treatments was 73.04% over control. Treatments effects within each time period on each cultivar were significantly reflecting the damaging effect of aluminium in root metabolic processes.

aluminium, whereas with 0.6mM aluminium in nutrient solution this change was from 19.45mg g^{-1} fresh weight (24hr) to 21.043mg g^{-1} fresh weight (72hr). So it can be stated that though protein content has been increased over time with or without aluminium the increment in protein content was gradually decreased with increasing concentration of aluminium, which was similar in both the varieties. In response to 0.6mM aluminium treatment for 72hr duration results indicate that shoot protein content of Shatabdi was 46.35% over control, while it was only 28.72 % in case of Khitish. It was also observed that over the each time duration reduction in shoot protein content in response to aluminium treatments higher in Khitish and varietal difference was significant in response to aluminium. Present experimental result

Table 5. Time duration study of root protein content (mg g^{-1} fresh wt) of two rice seedlings in response to aluminium toxicity

Treatment (Al^{+3} concI)	IET 4094 (Kshitish)			IET 4786 (Shatabdi)		
	24 Hour	48 Hour	72 Hour	24 Hour	48 Hour	72 Hour
0 mM	7.027 b	6.880 a	5.967 a	4.707 b	4.540 a	3.980 a
0.1 mM	7.323 a	6.733 b	5.820 b	4.870 a	4.393 b	3.650 b
0.2 mM	6.673 c	6.067 c	5.120 c	4.007 c	3.443 c	2.837 c
0.3 mM	6.017 d	5.337 d	4.493 d	3.833 d	3.137 d	2.293 d
0.4 mM	5.303 e	4.493 e	3.563 e	3.273 e	2.500 e	1.720 e
0.6 mM	4.300 f	3.423 f	2.920 f	2.753 f	2.043 f	1.037 f

Values in table represent pooled data of three replicates. Different letters beside the value indicate statistically significant difference at 0.05 level according to DMRT

In shoot, protein content has been increased over time with or without aluminium, though for a particular duration protein content of shoot decreased with increasing concentration of aluminium treatments (Table 6). Over the durations in Khitish seedlings, shoot protein content increased from 23.677mg/g fresh weight (24hr) to 29.523mg g^{-1} fresh weight (72 hr) without

highly in compliance with Ownby and Hruschka (1991) and Basu *et al*., (1994) who showed aluminium caused greater changes in protein content reported in wheat crop. Rincon and Gonzales (1991) opined that most of the changes in protein content caused by aluminium, mainly due to the inhibition of protein synthesis machinery and that too in the low molecular protein,

Table 6. Time duration study of shoot protein content (mg g^{-1} fresh wt) of two rice seedlings in response to aluminium toxicity

Treatment (Al^{+3} concI)	Kshitish			Shatabdi		
	24 Hour	48 Hour	72 Hour	24 Hour	48 Hour	72 Hour
0 mM	23.677 a	26.933 a	29.523 a	18.867 a	20.500 a	23.853 a
0.1 mM	23.013 b	24.410 b	26.377 b	16.133 b	17.230 b	20.037 b
0.2 mM	22.287 c	23.450 c	25.223 c	14.300 c	14.997 c	16.793 c
0.3 mM	21.477 d	22.400 d	23.670 d	13.583 d	14.197 d	15.390 d
0.4 mM	20.487 e	21.250 e	22.203 e	12.683 e	13.030 e	14.057 e
0.6 mM	19.450 f	20.093 f	21.043 f	11.620 f	12.047 f	12.797 f

Values in table represent pooled data of three replicates. Different letters beside the value indicate statistically significant difference at 0.05 level according to DMRT

which might be the reason for reduction in protein content in response to aluminium stress (Boscolo *et al*., 2003).

Aluminium had damaging effect on protein synthesizing machinery as also on proline accumulation mechanism in both the cultivars of rice but effect was less with IET-4786 due to its inherent capacity to combat aluminium toxicity.

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