Oryza Vol. 48. No. 4, 2011 (360-365)

Effect of aluminium toxicity on protein content of rice plant

P.K. Bandopadhyay*, S.N. Gupta, N. Karmakar and A. Chakravorty

Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia (West Bengal) 741 252

ABSTRACT

Experiments were conducted in the Department of Plant Physiology, B.C.K.V. to ascertain the intensity of damage in rice due to aluminium toxicity among the moderately resistant and susceptible cultivar IET-4094 and IET-4786, respectively. Changes in respect of protein, free amino acid and proline 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 IET-4786 compared to IET-4094. From the above result it might conclude that 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.

Key words : *rice, aluminium, proline, 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, in wrong places, causes severe cellular injuries and in extreme cases, death of organism. 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). An estimated 30-40% of the world's arable soils and up to 70% of world's potentially arable land has a pH below 5.5 (Haug, 1983). Currently, 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 under diverse conditions of landscape, geology, climate and vegetation.

Aluminium toxicity is the single most important factor, being a major constraint to crop production (Eswaran *et al*., 1997) and in general, mineral soils contain large amounts of aluminium, which depend upon soil acidification, a fraction of this aluminium becomes soluble and potentially toxic to plants.

From the physiological point at the cellular level, aluminum toxicity results in excessive ROS production, including superoxide radical (O_2^{*-}), hydroxyl radical (*OH) and hydrogen peroxide (H_2O_2), which can cause oxidative damage to biomolecules such as lipid, protein and nucleic acids, and disrupt cellular metabolism (Cakmak and Horst, 1991). 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). A study was undertaken to ascertain the edxtent damage in rice plant due to aluminium toxicity.

MATERIALS AND METHODS

Seeds of rice cv. IET-4094 (Khitish) and IET-4786 (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. Then the seeds were sown in the cotton bed in a plastic tray containing 100 M $CaCl_2$ solution and grown upto 7 days. After that it was transplanted to nutrient solution having varying concentrations of aluminium. The nutrient solution was 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 solution 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.

The estimation of proline content was carried out according to the Bates *et al*. (1973). Plant material was crushed in 3% aqueous sulphosalicylic acid. The homogenate was centrifuged at 5,000 rpm for 10 minutes, extraction was repeated and the supernatant was pooled. This supernatant used for the estimation of proline content. Reaction mixture of 6 ml, containing 2 ml of supernatant, glacial acetic acid and ninhydrin reagent was kept over boiling water bath for 1 hour and then terminate the reaction by placing on ice bath. 4 ml of toluene was added, mixed vigorously for 20-30 seconds, chromosphore layer was aspirated and adjusted to room temperature. Absorbance measurement was done at 520nm against a reagent blank. Amount of proline was calculated in the sample using a standard curve prepared from pure proline and express on fresh weight basis of sample.

Free amino acid content was determined by according to the Moore and Stein (1948), the plant material was crushed in 80% ethanol. The homogenate was centrifuged in 5,000 rpm for 10 minutes; repeatation of extraction was done twice and pooled all the supernatant. Volume was reduced by evaporation and extract was used for estimation of free amino acid. 0.1ml of extract and 1ml of ninhydrin reagent was added and mixed and volume was made upto 2 ml with distilled water, it was wormed in boiling water bath for 20 minute, after that 5 ml of propanol-water mixture was added as diluents, while still on the water bath and mix. After boiling for 15 minutes the tube was cooled in running water and after cooling reading was taken against blank at 570 nm, blank was prepared by taking 0.1 ml of 80% ethanol instead of extract. Calculations were done of total free amino acids using standard curves prepared from leucine. Results were expressed as mg/gm.

Protein estimation was carried out according to Lowry *et al*. (1951). The plant material was grinned in a mortar and pestle with 0.1 M sodium phosphate buffer (pH 7.0). The homogenate was centrifuged at 5,000 rpm for 10 minute and supernatant was used for the estimation of protein. 0.1 ml supernatant was taken and volume was made up to 1 ml with distilled water. 5 ml of reagent C (2% sodium carbonate in 0.1N sodium hydroxide with 0.5% cupper sulphate in 1% potassium sodium tartrate) was added and mixed thoroughly and incubated at room temperature for 10 minutes. 0.5 ml of reagent D (Folin-ciocalteaue reagent) was added and mixed well immediately and incubated at room temperature in dark for 30 minute. Reading was taken at 660 nm in spectophotometer against blank. Blank prepared similarly in which 0.1 ml phosphate buffer was used instead of extract. Protein was calculated in the samples using a standard curve prepared from bovine serum albumin. The amount of protein was expressed as mg/gm.

RESULTS AND DISCUSSION

Treatment with aluminium in solutions increased proline content significantly over the control in both cultivars. Though root proline content increased more over control in response to different aluminium treatments (Table 1). Proline content of root in IET-4094 cultivars in control at 24 hr duration was 0.363 µmole/g fresh wt, and with application of 0.6mM aluminium concentration in solution it was induced to 2.118 µmole/g fresh wt. Where as in shoot, proline content of control IET-4094 seedlings was 0.510 µmole/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

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

Treatment	IET 4094 (Kshitish)			IET 4786 (Shatabdi)		
(Al^{+3} concI)	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

confers more resistance to aluminium. The accumulation rate of proline in shoot in response to aluminium treatments also differs in two cultivars.

The proline accumulation due to aluminium toxicity possibly due to metal imposed increased in water deficiency rather than direct toxicity effect of metal (Schat *et al*., 1997).

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 α-amino acids and proline content, both are good indicators of toxicity responses (Hare and Cress, 1997). But unlike other toxic metals aluminium treatments decreased free α-

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

Treatment	IET 4094 (Kshitish)			IET 4786 (Shatabdi)		
(Al^{+3} concI)	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

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

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- α-amino acid content in both cultivars decreased with increasing concentration of aluminium. Aluminium treatments with 0.6mM exert most detrimental effect on free α-amino acid content. At 24 hr duration free α-amino acid content of root was 0.782 mg/g in control plants of IET-4094 and that had been reduced to 0.428 mg/g in response to 0.6 mM aluminium treatments. Over the

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

Treatment	IET 4094 (Kshitish)			IET 4786 (Shatabdi)		
(Al^{+3} concI)	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

time period the free α-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 α-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 α-amino acid content was 53.63 whereas in IET-4786 it was only 60.58%. In case of shoot free α-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 α-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).

Protein content of roots and shoots were estimated between the varieties growing in nutrient solutions with and without aluminium, which resulted in a highly significant variety X aluminium interaction. Comparisons were done between aluminium concentrations within each varieties and a time course changes of protein content of different variety at each aluminium concentrations. These comparisons revealed wide range of aluminium sensitivity in the two cultivars studied. In general results observed that root protein content was much lower than shoot protein content in both cultivars exposed to different aluminium concentrations and same has been observed in control plants also. Very interesting difference was observed between root and shoot protein content, where in root,

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

Treatment	IET 4094 (Kshitish)			IET 4786 (Shatabdi)		
(Al^{+3} concI)	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

protein content has been 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 IET-4094 seedlings was reduced to 2.920mg/g fresh wt, which was 51.06% reduction over control (Table 5). In IET-4786 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.

solution this change was from 19.45mg/g fresh weight (24hr) to 21.043mg/g 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 IET-4786 was 46.35% over control, while it was only 28.72 % in case of IET-4094. It was also observed that over the each time duration reduction in shoot protein content in response to aluminium

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

Treatment	IET 4094 (Kshitish)			IET 4786 (Shatabdi)		
(Al^{+3} concI)	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 IET-4094 seedlings, shoot protein content increased from 23.677mg/g fresh weight (24hr) to 29.523mg/g fresh weight (72 hr) without aluminium, whereas with 0.6mM aluminium in nutrient

treatments higher in IET-4094 and varietal difference was significant in response to aluminium. Present experimental result is highly in compliance with Ownby and Hruschka (1991) and Basu *et al.*, (1994) who showed aluminium caused greater changes in protein content in wheat crop. Rincon and Gonzales (1991) opined that most of the changes in protein content caused by aluminium was mainly due to the inhibition

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

Treatment	IET 4094 (Kshitish)			IET 4786 (Shatabdi)		
(Al^{+3} concI)	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

of protein synthesis machinery and that too in the low molecular protein, which might be the reason for reduction in protein content in response to aluminium stress. Aluminium-induced ROS mediated protein oxidation might be another cause of protein depletion (Boscolo *et al*., 2003).

REFERENCES

Barcelo J, Poschenrieder CH, Vazquez MD and Gunse B 1996. Aluminum phytotoxicity: A challenge for plant scientists. Fertilizer Research, 43: 217-223

Basu U, Basu A and Taylor GJ 1994. Differential exudation of polypeptides by roots of aluminum-resistant and aluminum-sensitive cultivars of Triticum aestivum L in response to aluminum stress. Plant Physiology, 106: 151-158

Bates LS, Waldren RP and Thomas CE 1973. Rapid determination of free proline for water stress studies. Plant and Soil, 39: 205-207

Boscolo PRS, Menossi M and Jorge RA 2003. Aluminum induced oxidative stress in maize. Phytochemistry, 62: 181-189

Cakmak I and Horst WJ 1991. Effect of Al on lipid peroxidation, superoxide dismutase, catalase and peroxidase activities in root tips of soybean (Glycine max). Physiologia Plantarum, 83: 463-468

Clarkson DT 1969. Metabolic aspects of aluminium toxicity and some possible mechanism for resistance: In IH Rorison, ed, Ecological Aspects of the Mineral Nutrition of Plants Oxford, UK, 381-397

Eswaran H, Reich P and Beinroth F 1997. Global distribution of soils with acidity: In Plant-Soil Interactions at Low pH eds., Moniz, AC *et al* Brazilian Soil Science Society, 159-164

Hall JL 2002. Cellular mechanisms for heavy metal detoxification and tolerance. Journal of Experimental Botany, 53: 1-11

Hare PD and Cress WA 1997. Metabolic implications of stress-induced proline accumulation in plants. Plant Growth Regulation, 21: 79-102,

Haug A 1983. Molecular aspects of aluminum toxicity. CRC Critical Review on Plant Science, 1: 345-373

Kochian LV 1995. Cellular mechanism of aluminium toxicity and resistance in plant. Annual Rev. Pl. Physiol. Pl. Mol. Biol., 46: 237 - 260.

Lowry OH, Rosebrough NJ, Farr AL and Randall RJ 1951. Protein measurement with the folin phenol reagent. Journal of Biological Chemistry, 193: 265-272

Mazen AMA 2004. Accumulation of four metals in tissues of Corchorus olitorius and possible mechanism of their tolerance. Biologia Plantarum, 48: 267-272.

Moore S and Stein WH 1948. In: Methods in enzymol (eds. Colowick, S.P. and Kaplan, N.D.), Academic Press, New York, 3: 468.

Nriagu JO and Pacyna JM 1998. Quantitative assessment of worldwide contamination of air, water and soils by trace metals. Nature, 333: 134-139

Ownby JD and Hruschka WR 1991. Quantitative changes in cytoplasmic and microsomal proteins associated with aluminium toxicity in two cultivars of winter wheat. Plant Cell & Environment, 14: 303-309

Rincon M and Gonzales A 1991. Aluminum partitioning intact roots of aluminum-tolerant and aluminum-sensitive wheat (*Triticum aestivum* L) cultivars. Plant Physiology, 99: 1021-1028

Schat H, Sharma SS and Vooijs R 1997. Heavy metal induced accumulation of free proline in metal tolerant and a non tolerant ecotype of Silene vulgaris. Plant Physiology, 101: 477-82

Von Uexküll HR and Mutert E 1995. Global extent, development and economic impact of acid soils. Plant and Soil, 171: 1-15

Yoshida S, Forno DA and Cock JH 1976. Routine procedure for growing rice plants in culture solution. In: Laboratory Manual for Physiological Studies of Rice. IRRI, LosBanos, Philippines, 61-66

Zhao XJ, Sucoff E and Stadelmann EJ 1987. Al^{+3} and Ca^{+2} alteration of membrane permeability of Querus rubra root cortex cells. Plant Physiology, 83: 159-162