

## EFFECTS OF ALUMINIUM TOXICITY ON SOME BIOCHEMICAL COMPONENTS OF RICE (*ORYZA SATIVA* L.)

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### Abstract

An experiment was conducted to investigate the effect of aluminium (Al) toxicity on reducing and total sugar, proline, total amino acid and protein in rice seedlings grown in solution culture and phenolic compounds, chlorophyll and carotenoids in rice plants grown in sand culture. Exposure of rice seedlings to different concentrations of aluminium (10 - 150  $\mu$ M) led to a stimulation of reducing and total sugar in the root and the shoot. Similarly, Al stress increased proline and total amino acid contents in different parts of rice seedlings. Aluminium toxicity caused a significant increase in phenolic compounds in rice plants. On the other hand, aluminium stress resulted in a reduction of chlorophyll-a, chlorophyll-b and carotenoid contents in the leaves of rice plants.

### Introduction

Aluminium (Al) is the most abundant metal in the earth's crust, comprising about 7% of its mass. Aluminium is non-essential element for plant growth and can be dangerous for plants at low pH<sup>(1)</sup>. It is dissolved in the soil in various ionic forms, among these Al<sup>3+</sup> is the most toxic form<sup>(2)</sup>. About 50% of the world's potentially arable lands are acidic<sup>(3)</sup>. The production of staple food crops, in particular grain crops, is negatively influenced by acid soils<sup>(4)</sup>.

The concentration of glucose was found to increase in Al-treated root of *Quercus serrata*<sup>(5)</sup>. Al-treated cells decreased soluble sugar by one third in tobacco<sup>(6)</sup>. The content of proline was found to be induced in germinating seeds of pigeonpea<sup>(7)</sup>. There was a decrease in the content of total soluble proteins in plant subjected to Al-stress<sup>(8)</sup>.

Roots of maize exposed to Al exuded 20-folds more phenolics than organic anions<sup>(9)</sup>. Al-toxicity reduced total chlorophyll content of spinach<sup>(10)</sup> and decreased carotenoid content in *Vigna radiata*<sup>(11)</sup>.

Reports on the effect of Al-stress in rice on biochemical parameters are limited. Hence, in here, the effect of aluminium toxicity on reducing and total sugar, proline, total amino acid, protein, phenolic compounds, chlorophyll and carotenoids in rice seedlings are reported.

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## Materials and Methods

Seeds of rice (*O. sativa* var. BRRI Dhan-53) were collected from Bangladesh Rice Research Institute (BRRI) used in this experiment.

The seeds were surface sterilized with 5.25% sodium hypochlorite solution. For solution culture, the sterilized seeds were spread over a cotton gauge placed in a lid having holes (1 cm in diameter) and the lid with seeds was placed on a beaker containing 500 ml of distilled water. The beakers were covered by black plastic sheet to avoid the exposure of light to the roots.

After germination, the seedlings were subjected to modified half-strength Hoagland solution<sup>(12)</sup> and the beakers with the seedlings were placed in a light bank. Rice seedlings were grown at a day/night temperature of 30°C ± 1°C /25°C ± 1°C and a photoperiod of 14/10 hrs day/night. Light intensity was 160 μ-einstein m<sup>-2</sup>s<sup>-1</sup>. The solution was continuously aerated through bubbler with the help of air compressor (Rockyvac 320). The solution was replenished every 48 hours. Seven days old seedlings were exposed to 10, 50, 100 and 150 μM Al at pH 4.2, supplied as AlCl<sub>3</sub>. Half-strength Hoagland solution (pH 4.2) was used as control. Samples were collected after 3, 6, 24, 48, 72 and 96 h of treatment.

To determine phenolic compounds, chlorophyll and carotenoid contents, plants were grown in sand culture<sup>(13)</sup>. Sterilized seeds were sown in pots filled with purified quartz sand. The sand was soaked with half-strength Hoagland solution (adjusted to pH 4.2). After germination of seeds, different aluminium treatments (50, 100 and 150 μM) were applied. The sand was always moistened with solution in every 24 hours. Samples were collected at 7, 14, 21 and 28 days of treatment.

Reducing and total sugar were determined by Somogyi-Nelson method<sup>(14-15)</sup> and Dubois *et al.* method<sup>(16)</sup>, respectively. Determination of proline was done according to the method of Bates *et al.*<sup>(17)</sup>. For the assay of total amino acid, ninhydrin reagent was used following the method of Lee and Takahasi<sup>(18)</sup>. Protein was measured by the method of Lowry *et al.*<sup>(19)</sup>.

Phenolic compounds were extracted in 80% ethanol according to the method of Malik and Singh<sup>(20)</sup>. Chlorophyll content of leaves was measured using specific absorption co-efficient method of Mckinney<sup>(21)</sup> and the formula of Maclachlan and Zalik<sup>(22)</sup>. The amount of carotenoid was determined by the equation of Von Wettstein<sup>(23)</sup>.

## Results and Discussion

Aluminium (10 to 150 μM) increased reducing sugar content in the root of rice from 48 to 96 hrs of treatment except an initial inhibition. The degree of stimulation increased with the increase in Al concentration from 10 to 150 μM. The maximum stimulation of reducing sugar accumulation in the root of rice ranged from 71.0% to 2.6-folds following 150 μM Al application over a period of 48 to 96 hrs of exposure (Fig. 1a).

In the shoot of rice, Al (10 - 150  $\mu\text{M}$ ) increased the reducing sugar content except an initial inhibition (Fig. 1b). 150  $\mu\text{M}$  Al increased reducing sugar content in the shoot by 69.0 and 67.9% at 72 and 96 hrs of treatment, respectively, except a decrease in that by 29.0 to 46.8% from 6 to 48 hrs of treatment (Fig. 1b). Similarly, lower concentration of Al was found to increase reducing sugar content in barley<sup>(24)</sup>.

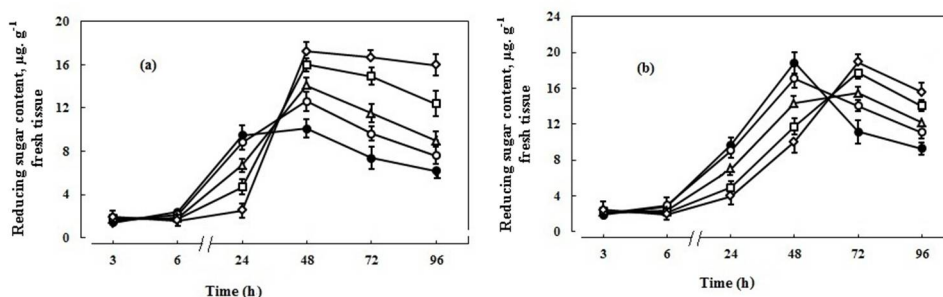


Fig. 1. The effect of different concentrations of aluminium on the accumulation of reducing sugar in the (a) root and (b) shoot of rice seedlings grown in solution culture. ● represents control; ○ 10  $\mu\text{M}$  Al; △ 50  $\mu\text{M}$  Al; □ 100  $\mu\text{M}$  Al; ◇ 150  $\mu\text{M}$  Al. Each value is the mean of three replicates  $\pm$  standard error.

Aluminium (10 to 150  $\mu\text{M}$ ) caused an increase in total sugar content in the root of rice from 24 to 96 hrs of treatment (Fig. 2a). At a concentration of 10  $\mu\text{M}$ , aluminium increased total sugar in the root from 3.7 to 30.0% from 24 to 96 hrs of treatment. Stimulatory effect was the highest (28.7 to 86.0%) in root of rice seedlings exposed to 150  $\mu\text{M}$  Al (Fig. 2a).

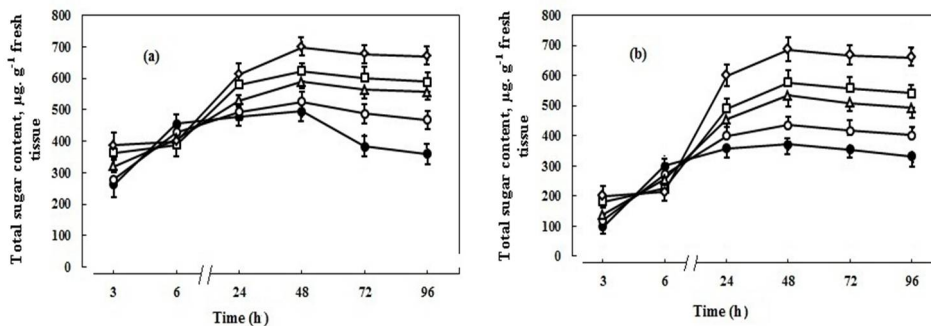


Fig. 2. The effect of different concentrations of aluminium on the accumulation of total sugar in the (a) root and (b) shoot of rice seedlings grown in solution culture. Otherwise as Fig. 1.

Aluminium concentration as low as 10  $\mu\text{M}$ , increased total sugar content in the shoot of rice by 11.8 to 21.0% from 24 to 96 hrs of treatment. The stimulation of total sugar content in the shoot gradually increased with the increase in Al concentration from 50 to 150  $\mu\text{M}$  which ranged from 67.8 to 98.9% from 24 to 96 hrs of application (Fig. 2b). This result is in agreement with the work of Cambraia *et al.*<sup>(25)</sup> who found that Al increased the total sugar content in sorghum.

Proline content in the root of rice seedlings was increased in all the concentrations of Al (10 - 150  $\mu\text{M}$ ) used. At a concentration of 150  $\mu\text{M}$ , Al caused a maximum of 13.0 to 60.7% increase in proline content in the root from 3 to 96 hrs of treatment (Fig. 3a).

Aluminium (10 - 150  $\mu\text{M}$ ) also increased proline content in the shoot of rice but the rate of stimulation was lesser in the shoot than that of the root. 150  $\mu\text{M}$  Al caused a 4.0 to 11.0% increase in proline content in the shoot from 3 to 96 hrs of treatment (Fig. 3b). Similarly, Al was found to increase proline content in *Stylosanthes guianensis* and *S. macrocephala*<sup>(26)</sup>.

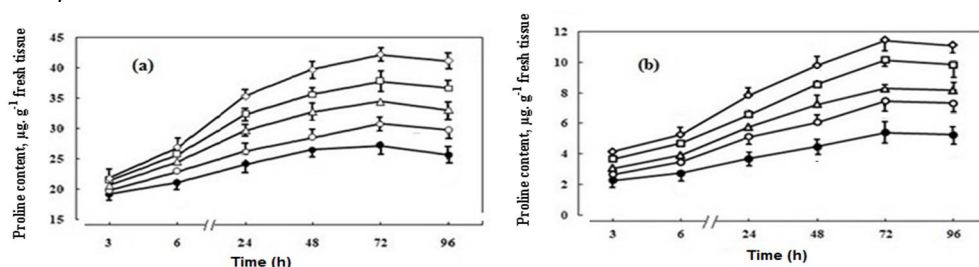


Fig. 3. The effect of different concentrations of aluminium on the accumulation of proline in the (a) root and (b) shoot of rice seedlings grown in solution culture. Otherwise as Fig. 1.

Aluminium (50  $\mu\text{M}$ ) caused a 2-folds increase in total amino acid in the root at 3 hrs of treatment and the stimulatory effect declined from 6 to 96 hrs of treatment. Exposure of rice seedlings to 150  $\mu\text{M}$  Al resulted in a 3.5- to 2.3-folds increase in total amino acid in the root from 3 to 72 hrs of treatment (Fig. 4a).

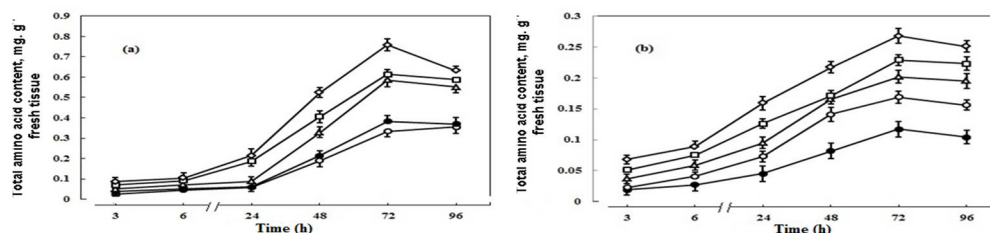


Fig. 4. The effect of different concentrations of aluminium on total amino acid content in the (a) root and (b) shoot of rice seedlings grown in solution culture. Otherwise as Fig. 1.

In the shoot of rice seedlings, 10  $\mu\text{M}$  Al increased total amino acid content by 21.0 to 50% from 3 to 72 hrs of treatment. 50 and 100  $\mu\text{M}$  Al caused a 94.0% to 2-folds and 2.7- to 2.1-folds increase in total amino acid, respectively, in the shoot from 3 to 48 hrs of treatment. A maximum of 3.6-fold increase in total amino acid in the shoot was observed at 3 hrs following 150  $\mu\text{M}$  Al treatment and this high stimulation was sustained up to 96 hrs of exposure (Fig. 4b). On the contrary, Al caused reduction in free amino acids in roots of *Lotus corniculatus*<sup>(27)</sup>.

Aluminium concentrations of 10 to 150  $\mu\text{M}$ , increased protein content in the root of rice seedlings at 3 and 6 hrs of treatment and then gradually declined leading to an inhibition of protein content from 48 to 96 h of application (Fig. 5a). 100  $\mu\text{M}$  Al increased protein content in the root by 44.0 and 38.0% at 3 and 6 hrs of treatment, respectively, and then it decreased that from 28.0 to 33.0% at 48 to 96 hrs of exposure (Fig. 5a).

In the shoot of rice seedlings, all the different concentrations of Al (10-150  $\mu\text{M}$ ) decreased protein content at 72 to 96 hrs of treatment except an stimulation of that from 3 to 48 hrs of treatment (Fig. 5b). 150  $\mu\text{M}$  Al increased protein content in the shoot by 28.6 to 17.0% over a period of 3 to 48 hrs leading to an inhibition of that by 27.0 and 32.5% at 72 and 96 h of application respectively (Fig. 5b). Similarly, da Cruz *et al.*<sup>(28)</sup> found that Al toxicity inhibited total soluble protein in *Sorghum bicolor*.

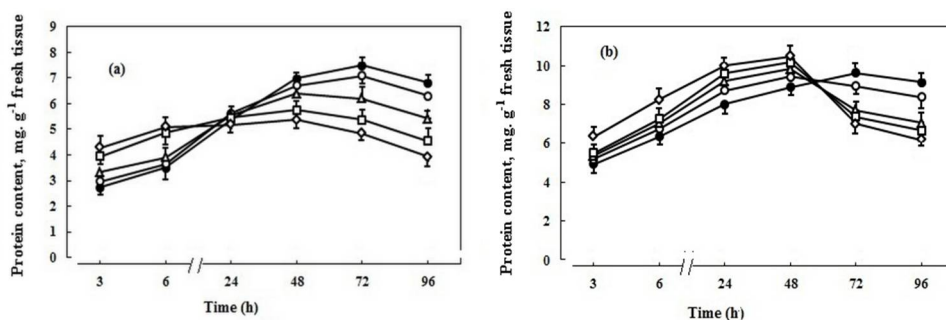


Fig. 5. The effect of different concentrations of aluminium on the protein content in the (a) root and (b) shoot of rice seedlings grown in solution culture. Otherwise as Fig. 1.

Aluminium (50  $\mu\text{M}$ ) increased accumulation of phenolic compounds in the root of rice plants by 36.0% to 2.3-folds from 7 to 28 days of treatment. 100 and 150  $\mu\text{M}$  Al caused a 48.9% to 5- and 2.6- to 6.9-folds increase in phenolic compounds, respectively, in the root of rice plants from 7 to 28 day of application (Fig. 6a).

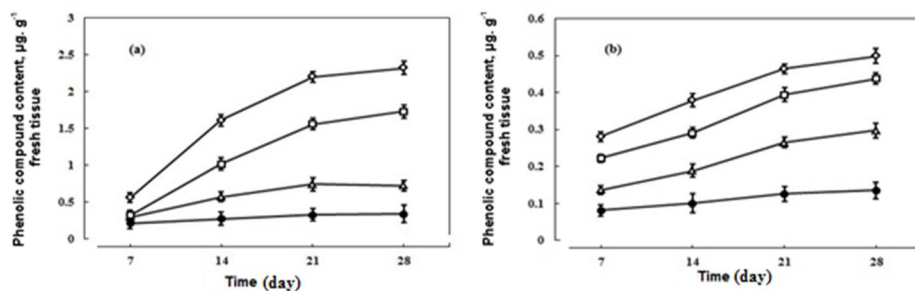


Fig. 6. The effect of different concentrations of aluminium on the accumulation of phenolic compounds in the (a) root and (b) shoot of rice seedlings grown in sand culture. ● represents control;  $\Delta$  50  $\mu\text{M}$  Al;  $\square$  100  $\mu\text{M}$  Al;  $\diamond$  150  $\mu\text{M}$  Al. Each value is the mean of three replicates  $\pm$  standard error.

In the shoot of rice, 50, 100 and 150  $\mu\text{M}$  Al caused a 67.9% to 2.2-folds, 2.7- to 3.7-folds and 3.5- to 3.7-folds increase in accumulation of phenolic compounds, respectively, from 7 to 28 day of treatment (Fig. 6b). Nevertheless, similar to present findings, Al increased total soluble phenols in the shoot of *Matricaria chamomilla* plants<sup>(29)</sup>.

A 9.0 to 22.0% inhibition of chlorophyll-a content in the leaves of rice was obtained following 100  $\mu\text{M}$  Al treatment from 14 to 28 day of exposure. 150  $\mu\text{M}$  Al caused a maximum of 11.6 to 30.0% inhibition of chlorophyll-a content in the leaves from 14 to 28 days of application (Fig. 7a).

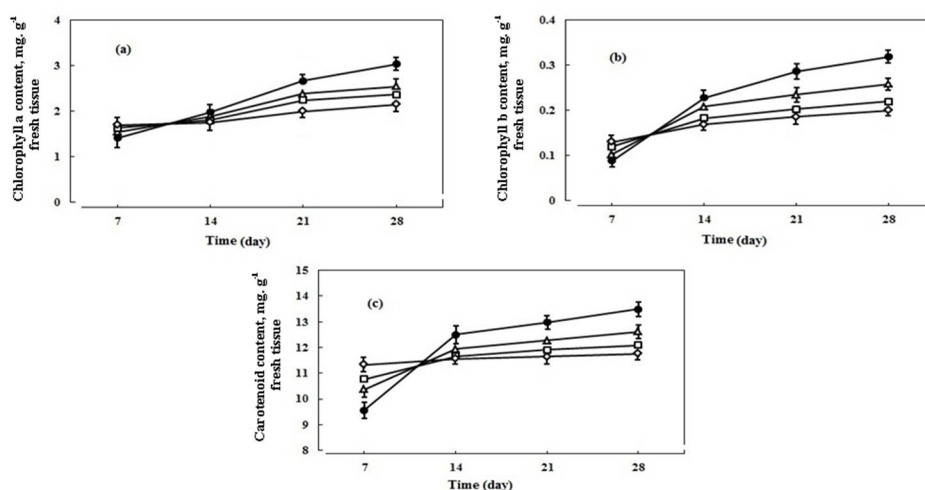


Fig. 7. The effect of different concentrations of aluminium on (a) chlorophyll-a, (b) chlorophyll-b and (c) carotenoid content in the shoot of rice plants grown in sand culture. Otherwise as Fig. 6.

Chlorophyll-b content in the leaves of rice plants was decreased by 8.8 to 19.0% from 14 to 28 days following application of 50  $\mu\text{M}$  Al. A maximum of 25.9 to 37.0% inhibition of chlorophyll-b content in the leaves of rice was observed when exposed to 150  $\mu\text{M}$  Al from 14 to 28 days of application (Fig. 7b). Similarly, Al inhibited chlorophyll-a, chlorophyll-b contents in the leaves of green melon<sup>(30)</sup>.

Al (50 - 150  $\mu\text{M}$ ) decreased carotenoid content in the leaves of rice plants from 14 to 28 days of treatment. 150  $\mu\text{M}$  Al caused 8.0 to 12.8% inhibition of carotenoids in the leaves from 14 to 28 days of application (Fig. 7c). This result was in agreement with the work of Ziaei *et al.*<sup>(31)</sup> who found that Al stress inhibited carotenoid content in sunflower.

Increase in reducing and total sugar level caused by Al might help to maintain the osmotic potential of cell sap under aluminium stress condition. Al-induced increase in proline content in rice seedlings indicates that the plant species was stressed due to exposure to aluminium. The increase in total amino acid might be related to the decrease in protein content under aluminium stress. The increase in total amino acid might be due to hydrolysis of protein which might result in observed decrease in protein content.

Aluminium toxicity increased the accumulation of phenolic compounds in rice plants. Phenolics might act as a detoxifier of aluminium toxicity. Al-induced decrease in chlorophyll a and b content might decrease photosynthesis resulting in a decrease in ion transport because ion transport is dependent on photosynthesis for energy.

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