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ALUMINIUM TOXICITY ON THE ACTIVITIES OF ANTIOXIDANT ENZYMES IN RICE (*ORYZA SATIVA* L.) AND CHICKPEA (*CICER ARIETINUM* L.) SEEDLINGS GROWN IN HYDROPONIC CULTURE

RIFAT SAMAD*, PARVEEN RASHID AND JL KARMOKER

Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh

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Abstract

Effects of four different concentrations of aluminium (10, 50, 100 and 150 µM) on some antioxidant enzyme activities like peroxidase, catalase and superoxide dismutase of the seedlings of rice and chickpea grown in hydroponic culture showed aluminium toxicity which caused a dramatic increase in peroxidase and catalase activities in the root and shoot of rice seedlings. Al (150 µM) caused eight to nine-folds increase in peroxidase and catalase activity, respectively, in the root of rice seedlings. On the contrary, Al stress decreased superoxide dismutase (SOD) activity in the root and shoot of rice seedlings. In chickpea seedlings, Al stress caused a few-folds increases in peroxidase, catalase and SOD activities in the roots and leaves. A dramatic 14.8 and 14.6-folds increase in SOD activity was recorded in the roots and leaves of chickpea seedlings, respectively. It is noted that there is a generic difference between rice and chickpea with respect to the effect of Al stress on SOD activity.

Introduction

In the earth crust aluminium is the most abundant metallic element and third most abundant of all elements. At mildly acidic or neutral soil pH values, it occurs primarily as insoluble deposits and essentially is biologically inactive (Nasr 2013). Solubolization of Al-containing minerals is enhanced by low pH in acidic environment in many acid soils throughout the world and soluble Al^{3+} is the most growth-limiting factor (Foy 1988), possibly affecting 70% of world's arable land which is potentially usable for food and biomass production (Haug and Caldwell 1985). Acid soils occupy 30 - 40% of the arable lands (von Uexküll and Mutert 1995) and are consequence of a rapid industrial development and environmental pollution, this area increases from year to year especially in developing countries.

The examination of antioxidant enzyme activities often serves as a key biochemical indicator to assess the sensitivity of plants under stress conditions (Sharma *et al.* 2012). Peroxidase, catalase and superoxide dismutase (SOD) are the key enzymes in antioxidative defense system. Reactive oxygen species (ROS) is closely related to the response of plants to heavy metals (Nagajyoti *et al.* 2010). Production of reactive oxygen species (ROS, 0.5 O^- and H_2O_2) is increased under various environmental stresses. These ROS cause oxidative damage to cellular organelles and biomolecules, and thus lead to several metabolic alteration (Ma 2007, Jain *et al.* 2008). Free radical scavenging enzymes such as peroxidase, catalase and SOD keep the cellular level of ROS under control and help to avoid oxidative damage (Ribeiro *et al.* 2012). Al toxicity-induced increase in peroxidase, catalase and SOD plays a vital role in aluminium stress tolerance.

*Author for correspondence: <rifatsamad@gmail.com>.

Aluminium toxicity increased the activities of superoxide dismutase (SOD) and peroxide (POD) and glutathione reductase in greengram. On the contrary, it decreased catalase activity in the same plant (Panda *et al.* 2003). Al stimulated SOD activity but decreased that of catalase in pearl millet (Suresh Babu *et al*. 2013). In the present study, an attempt was taken to investigate the effect of aluminium toxicity on the activities of peroxidase, catalase and superoxide dismutase of rice and chickpea seedlings grown in hydroponic culture.

Materials and Methods

Rice (*Oryza sativa* L. var. BRRI Dhan-53), most widely consumed cereal grain of the family Poaceae and chickpea (*Cicer arietinum* L. var. BARI chhola-7), the third most extensively planted grain legume of the family Fabaceae were used as experimental plant materials. Seeds of rice were collected from Bangladesh Rice Research Institute (BRRI) and that of chickpea were procured from Bangladesh Agricultural Research Institute (BARI). The experiments were conducted at the Department of Botany, University of Dhaka, Dhaka, Bangladesh.

The seeds were surface sterilized according to Samad and Karmoker (2013). 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 transferred to modified half-strength Hoagland solution (Hoagland and Arnon 1950) and the beakers with the seedlings were placed in a light bank. Rice seedlings were grown at a day/night temperature of 30 ± 1°C/25 ± 1°C and day/night length of 14 hrs/10 hrs. Chickpea seedlings were grown at a day/night temperature of 25 ± 1°C/18 ± 1°C and day/night length of 10 hrs/14 hrs. Light intensity was 160 µ-einstein/m^2s. The solution was continuously aerated through bubbler with the help of air compressor (Rockyvac 320). The solution was replenished every 48 hrs. Seven-day-old seedlings were transferred to half strength Hoagland solution as control and 10, 50, 100 and 150 µM $AlCl_3$ solution made in half strength Hoagland solution were used as treatments. The pH of all solutions including control were adjusted to 4.2 with 0.2N H_2SO_4.

For the determination of the activities of antioxidant enzymes, 0.5 g of plant sample was homogenized in 0.05 M phosphate buffer (pH 7.8). Homogenate was centrifuged at 10,000 rpm for 10 min at 4ºC. Supernatant was separated and used for specific enzyme assay.

The activity of peroxidase was determined by the method as described by Zhang *et al.* (1995). The reaction mixture consisted of 0.1 M phosphate buffer (pH 7.8), 30% H_2O_2 and 0.05 M guaiacol (freshly prepared). The supernatant (0.2) ml was added to the reaction mixture. The change of optical density (O.D) was measured at a wavelength of 470 nm with a spectrophotometer for every 30 sec for 2 min. Peroxidase activity was expressed as µmol/min/mg/ protein.

The catalase activity was assayed according to the method of Barber (1980). Catalase activity was measured by using assay solution containing 0.05 M phosphate buffer (pH 7.8), 0.1 M H_2O_2 and 0.2 ml extract. Decrease in absorbance of H_2O_2 was recorded within 2 min at 240 nm. One unit of catalase activity was defined as the amount of enzyme required to reduce 1 µmol of H_2O_2 per min. Catalase activity was expressed as µmol/min/mg/protein.

The superoxide dismutase (SOD) activity was determined according to the modified method of Zhang *et al.* (2005). The SOD activity was determined by measuring the inhibition of the photochemical reduction of nitroblue tetrazolium chloride (NBT). The reagents used for assay included 50 mM phosphate buffer (pH 7.8), 130 mmol/l methionine, 750 µmol/l NBT, 100 µmol/l Na_2-EDTA and 20 µmol/l riboflavin. After adding 1 ml of supernatant (extract) to the reaction mixture, the test tubes were exposed for 10 mins to fluorescent light (13 W). Then the change in

absorbance was followed up to 2 min at a wavelength of 560 nm using a spectrophotometer (Shimadzu, Model: UV-1800, Japan). One unit of SOD was defined as the amount of enzyme required to inhibit NBT reduction by 50% under specified conditions of the assay. SOD activity was expressed as unit min/mg/protein.

Results and Discussion

Peroxidase activity was found to increase in the root of rice by 4 - 7.9-folds following 96 hrs of exposure to 10 to 150 µM Al (Fig. 1a). In the shoot of rice, a 1.8 - 2.9-folds stimulation of peroxidase activity was recorded at 10 to 150 µM Al application, respectively (Fig. 1b).

Similarly, Al (10 µM) increased peroxidase activity in the root of chickpea seedlings by 29.5% at 96 hrs of treatment. The 50 to 150 µM Al caused a 3.7 - 4.8 times stimulation of peroxidase activity in the root (Fig. 2a). In the leaves of chickpea, 10 to 150 µM Al increased peroxidase activity by 2 - 13-folds at 96 hrs of application (Fig. 2b).

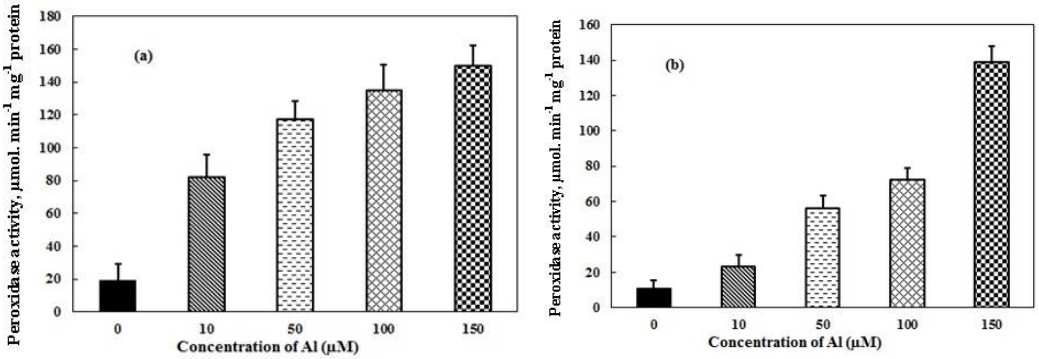

Fig. 1. Effects of different concentrations of aluminium on the activity of peroxidase in : (a) root and (b) shoot of rice seedlings grown in solution culture at 96 hrs of treatment. ■ represents control; ▨ 10 µM Al, ⊟ 50µM Al, ◇ 100µM Al and ▩ 150µM Al. Each value is the mean of three replicates ± standard error.

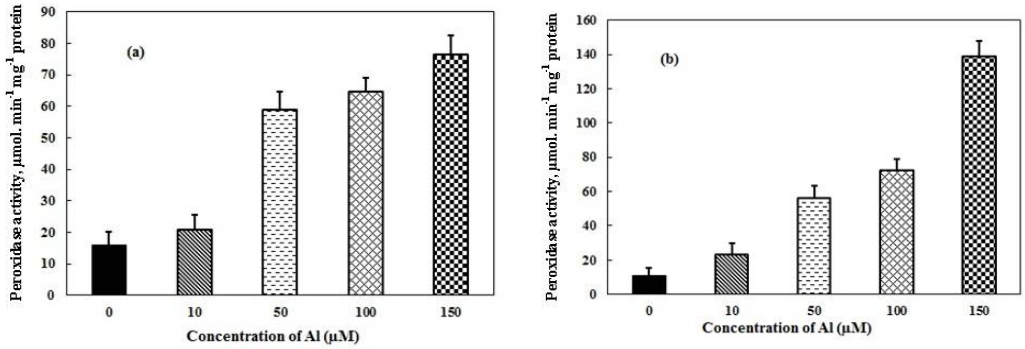

Fig. 2. Effects of different concentrations of aluminium on the activity of peroxidase in : (a) root and (b) leaves of chickpea seedlings grown in solution culture at 96 hrs of treatment. Otherwise as Fig. 1.

Catalase activity in the root of rice was stimulated by Al stress. The 50, 100 and 150 µM Al caused a 2.8, 5 and 9-folds increase in catalase activity in the root, respectively, at 96 hrs of exposure (Fig. 3a). In the shoot of rice, 10 to 150 µM Al increased the activity of catalase by 38.4% to 3.5-folds at 96 hrs of treatment (Fig. 3b).

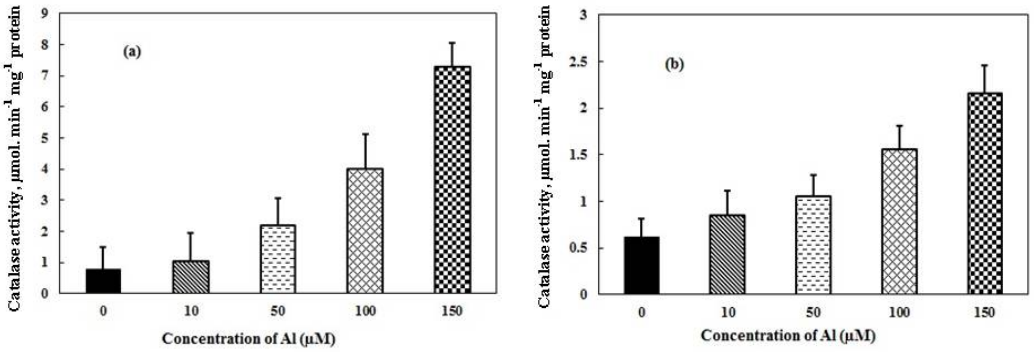

Fig. 3. Effects of different concentrations of aluminium on the activity of catalase in : (a) root and (b) shoot of rice seedlings grown in solution culture at 96 hrs of treatment. Otherwise as Fig. 1.

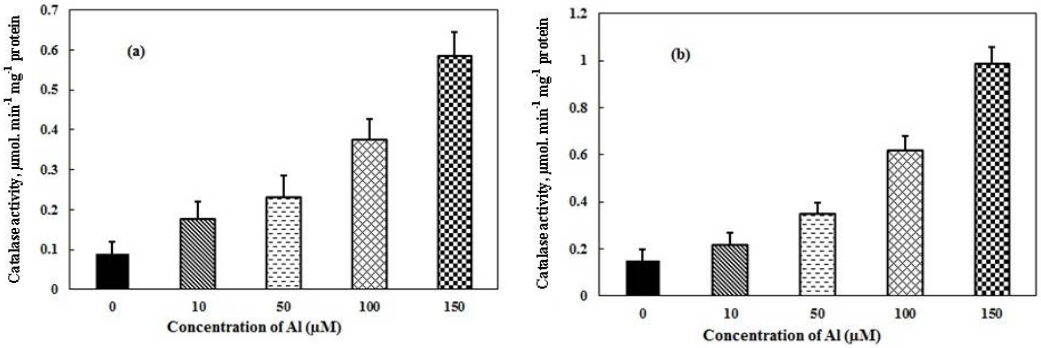

Fig. 4. Effects of different concentrations of aluminium on the activity of catalase in : (a) root and (b) leaves of chickpea seedlings grown in solution culture at 96 hrs of treatment. Otherwise as Fig. 1.

Similar stimulatory effect on catalase activity was also observed in the root and leaves of chickpea. Al (10 to 150 µM) progressively increased the activity of catalase from 98.9% to 6.6-folds in the root of chickpea at 96 hrs of exposure (Fig. 4a). In the leaves of chickpea, all the concentrations of Al (10 to 150 µM) increased catalase activity. The increase of catalase activity in the leaves was 2.3 - 6.5 times following exposure to 50 - 150 µM Al (Fig. 4b).

Al stress decreased SOD activity in the root of rice. Aluminium (10 -150 µM) inhibited SOD activity by 14.8 - 50.0% in the root of rice at 96 hrs of exposure (Fig. 5a). In the shoot of rice, Al (10 - 150 µM) decreased SOD activity by 15.7 - 47.0% at 96 hrs of treatment (Fig. 5b).

On the contrary, Al (10 µM) increased SOD activity in the root of chickpea by 48.7% at 96 hrs of exposure. SOD activity increased with the increase in Al concentration from 50 - 150 µM. A dramatic 14.8 times increase in SOD activity was recorded in the root following 150 µM Al treatment (Fig. 6a). Similarly, SOD activity was found to increase in the leaves following 10 to 150 µM Al application. One hundred and 150 µM Al caused a dramatic 9.5 - 14.6-folds stimulation of SOD activity, respectively in the leaves, at 96 hrs of exposure (Fig. 6b).

Aluminium stress caused a maximum increase in the activity of peroxidase and catalase but decreased that of SOD in the root and the shoot of rice seedlings (Figs 1, 3 and 5). However, Al treatment caused a few-folds stimulation of the activities of peroxidase, catalase and SOD in the root and leaves of chickpea seedlings (Figs. 2, 4 and 6). Similar aluminium toxicity-induced stimulation of the activities of peroxidase, and SOD was found in tomato (Surapu *et al.* 2014). On the contrary, Al caused an inhibition of catalase activity in tomato (Surapu *et al.* 2014). Giannakoula *et al.* (2010) and Ma *et al.* (2012) working with two maize and rice cultivars with different tolerance to Al, respectively showed that the improvement in protection against Al toxicity was obtained by an increase in the activity of the antioxidant enzymes. Lee *et al.* (2001) suggested that SOD might function in signaling of oxidative stress which might lead to the induction of antioxidant enzymes associated with a 1/2 O_2 scavenging system.

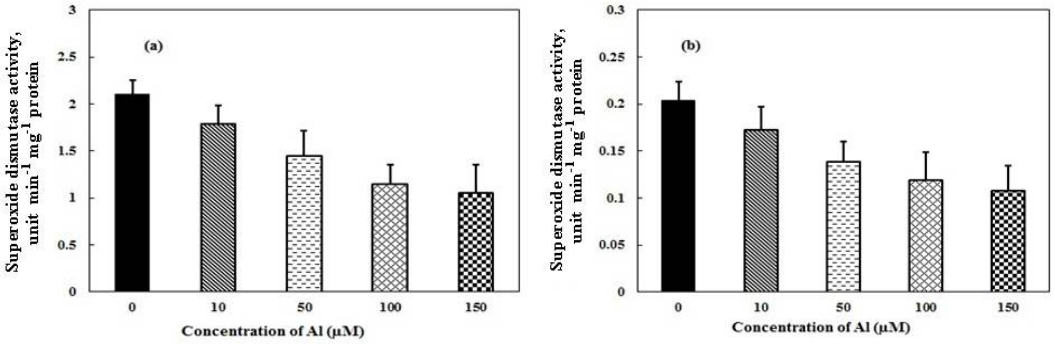

Fig. 5. Effects of different concentrations of aluminium on the activity of superoxide dismutase in: (a) root and (b) shoot of rice seedlings grown in solution culture at 96 hrs of treatment. Otherwise as Fig. 1.

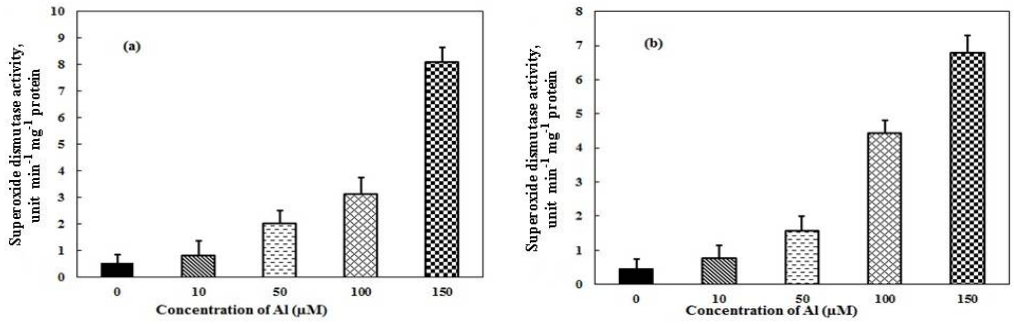

Fig. 6. Effects of different concentrations of aluminium on the activity of superoxide dismutase in : (a) root and (b) leaves of chickpea seedlings grown in solution culture at 96 hrs of treatment. Otherwise as Fig. 1.

Antioxidant enzyme activities serve as a key biochemical indicator to assess the sensitivity of plants under stress condition. Al triggers an increased production of ROS which includes singlet oxygen (0.5 O_2^-), superoxide radical (O_2), hydroxyl radical (OH) and H_2O_2 in the tissue. ROS causes damage to lipid, protein and nucleic acid in the cells. The most effective antioxidant enzymes preventing ROS-induced cellular oxidative damage is SOD which catalyzes the conversion of 0.5 O_2^- to H_2O_2 whereas catalase scavenges H_2O_2 and peroxidase use H_2O_2 for oxidation of various organic substrates. Thus, Al-induced increase in activities of antioxidant enzymes peroxidase, catalase and SOD play a vital role in tolerance of plant under Al-stress.

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