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Association of Al^{3+} Toxicity with Disruption of Photosynthetic Pigments and Oxidative Metabolism of *Zea mays* L. Plants

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The present study was undertaken to assess the toxicity of Al^{3+} on photosynthetic pigments, total soluble protein and MDA contents as well as the activities of SOD, CAT and POD in *Zea mays* leaves after 6-days of supplying aluminum in the nutrient solution. The results indicated that aluminum stress treatment adversely affected plant growth and disturbed the cell metabolism seriously. The development of toxic symptoms, corresponded to a high accumulation of Al^{3+}, were due to the increase in MDA content, to the decrease in soluble protein contents and to the much elevated SOD and POD activities in leaves. In addition, the results demonstrated that exposure to high concentration of aluminum (Al^{3+} > 20 ppm) could result the disintegration of antioxidant system in maize seedlings. Also, the significant decrease in the contents of photosynthetic pigments was related to high-level metal stress. The outcome of this study corroborate that *Zea mays* is a suitable candidate for the phytoremediation of low-level aluminum contaminated soil.

Key words: Aluminum; Antioxidant; Protein; Photosynthetic pigments; phytoremediation.

Acid soils are found throughout the world; it is estimated that about 40% of the world's arable soils and 12% of the land in crop production have a pH below 5.5 (von Uexkull and Mutert,1995). Moreover, soil acidification is increasing in world-wide. Increasing soil acidity of soil solution lead generally to increased availability of heavy metals (Punz and Sieghardt, 1993). Plants growing in acid soils suffer aluminum toxicity stress, where crop production is markedly reduced. Aluminum accumulating plants grow well in strongly acidic soils experiencing cumulative Al^{3+} toxicity damage (Godbold, 1994). The toxicity of Al^{3+} has been recognized as a major factor that limits plant growth in acidic soil (Taylor, 1991).

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Disturbance of the metabolism by excessive Al^{3+} or heavy metal appears to happen in multiple ways including, reduction of chlorophyll content, inhibiting plant growth and respiration, changing the ultrastructure of the cell organelles, and altering the activity of the many enzymes of various metabolic pathways (Frankart *et al*., 2002; Yamamoto *et al*., 2003). It has been proposed that heavy metals can lead to oxidative stress resulting in inhibition of photosynthesis, respiration rate and other metabolic processes in plants. Many evidences suggested that these metabolic disturbances were closely related to the accumulation of heavy metals and the subsequent excessive production of reactive oxygen species (ROS) in plants, including superoxide radical ($O2^{\cdot-}$), hydroxyl radical ($^{\cdot}OH$) and hydrogen peroxide (H_2O_2) (Hall, 2002; Gardea-Torresdey *et al*., 2004; Ibrahim, 2006; Hou *et al*., 2007).

High concentrations of heavy metals in plant tissues can induce the disorder in nutrient metabolism, as a consequence leading to abnormal growth. While, ROS can cause oxidative damage to the bio-molecules such as lipid that leading to cell membrane peroxidation, loss of ions, protein hydrolysis, and even DNA strand breakage. To mitigate the oxidative damage initiated by ROS, plants have developed a complex defense antioxidative system, including low-molecular mass antioxidants, as well as antioxidative enzymes, such as superoxide dismutase (SOD) and peroxidases (POD) (Halliwell and Gutteridge, 1984; Han, 1999). SOD is the most effective antioxidative enzyme in preventing cellular damage, that catalyzes the conversion of the superoxide anion to H_2O_2, while POD utilizes H_2O_2 in the oxidation of various inorganic and organic substrates. Many evidences suggest that high levels of Al can affect protein metabolism and induce oxidative stress in plants (Guo *et al*., 2007) On the other hand, the great differences in Al^{3+} tolerance have been found among plant species and genotypes within a species (Guo *et al*., 2007). Therefore, it is very essential to identify the difference in antioxidative defense system, the adaptive capacity and toxicity of maize plant exposed to aluminum.

In the present study, concentrations of Al^{3+} were chosen according to preliminary test in order to examine the inhibitory effect of acid soil on maize seedlings with reference to: (1) changes in soluble protein content; (2) changes in the activities of chlorophyll a, chlorophyll b and carotenoids; and (3) changes in the contents of antioxidant enzymes such as, peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD), as well as the content of malondialdehyde (MDA). So this can determine the concentration extent to which *Zea mays* is suitable for remediation of polluted area by aluminum.

MATERIAL AND METHODS

Plant materials

Grains of maize from 5 cultivars were screened for their germination response to Al^{3+}. *Zea mays* L. was the most tolerant variety. Maize grains were surface-sterilized (3% hydrogen peroxide and 95% ethanol for 5 and 2 min, respectively), extensively washed with tap water for three times and germinated in limed (pH 5.5–6.0) peat for 3 days at 25 °C. Germination was conducted under controlled climatic conditions with 28/18 °C (days/ night), 18/6 h (light/dark) cycles, 70% relative air humidity, and a photon flux density of 230 m mol m^{-2} s^{-1} photosynthetic active radiation at plant height. The plastic pots (15 cm diameter x 20cm height) were furnished with a hole at the bottom and this was covered with filter paper before filling with sand. Pots were irrigated with distilled water every two days for 15 days under the condition previously described. Homogenous seedlings were taken carefully and washed thoroughly from adhering sand. Seedlings were transferred to wide mouth bottles containing nutrient solution (1/10 Hoagland solution) alone as control or supplemented with 5, 20 and 50 ppm Al^{3+} (aluminum chloride).

After six days exposure to Al^{3+}, soluble protein, photosynthetic pigments, POD, CAT, SOD, and MDA of leaves were determined. A minimum of three replicates were performed in each treatment.

Photosynthetic pigments and soluble protein determination

Approximately 150 mg weight was homogenized on ice with mortar and pestle in 3 ml of 66 mM phosphate buffer, pH 7.2 with 10 mM KCl. The homogenate was then extracted with cold acetone (80%). The absorbance of pigment extract was measured at wavelength of 470, 626, 645, 663 and 730 nm with spectrophotometer (Jenway 6305 UV/Vis, UK). The contents of Chl a, Chl b and carotenoids were calculated in accordance with experimental equations as described by Lichtenthaler (1987).

Total protein content of the phosphate extract buffer used for the analysis was determined according to Bradford (1976) using bovine serum albumin as the standard.

Antioxidant enzymes extraction

Leaves were powdered in liquid nitrogen. The powder (150 mg) was extracted 15 min at 40°C in 5 ml of cold extracted buffer (100 m M potassium phosphate) pH 7.8, 300 mg polyvinylpyrrolidone, 1% (v/v) Triton X-100 and 5mM ascorbate (Schwanz *et al*., 1996). The extract was centrifuged (30 min 20,000 g, 4°C and the supernatant passed through a Sephadex G-25 column (PD-10 column, Pharmacia, Germany) and equilibrated with 100mM

potassium phosphate, pH 7.8. The supernatant was used for the enzyme assays.

Enzymes assay

SOD(EC1.15.1.1) activity was assayed using the photochemical nitrobluetetrazolium (NBT) method according to Stewart and Bewely (1980). The samples (0.5 g) were homogenized in 5mL extraction buffer consisting of 50mM phosphate, (pH 7.8), 0.1% (w/v) bovin serium albumin, 0.1% (w/v) ascorbate, 0.05% (w/v) β-mercaptoethanol. The assay mixture in 3mL contained 50mM phosphate buffer, pH(7.8), 9.9mM methionine, 57mM NBT, 0.025% (w/v) Triton X-100, and 0.005% (w/v) riboflavin. The photo-reduction of NBT (formation of purple formazan) was measured at 560 nm and an inhibition curve was made against different volumes of extract. One unit of SOD is defined as being present in the volume of extract that causes inhibition of the photo-reduction of NBT by 50%.

CAT (EC 1.11.1.6) activity was determined by measuring the rate of H2O2 conversion to O_2 spectrophotometrically at 240 nm (Aebi, 1983). One unit of enzyme activity is equal to 1 mmol of H_2O_2 decomposed per min.

POD (EC 1.11.1.7) activity was estimated according to Adam *et al*.(1995) and measured with guaiacol as the substrate in a total volume of 3 ml. The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 6.1), 1% guaiacol, 0.4% H_2O_2 and enzyme extract. Increase in the absorbance due to oxidation of guaiacol (E = 25.5mM^{-1} cm^{-1}) was measured at 470 nm. Enzyme activity was calculated in terms of mmol of guaiacol oxidized min^{-1} g^{-1} fresh weight, at 25±2°C.

Determination of MDA content

The level of lipid peroxidation was expressed as MDA content and was assayed by the method of Hodgson and Raison (1991) as 2-thiobarbituric acid (TBA) reactive metabolites. Plant fresh tissues (0.2 g) were homogenized and extracted in 10mL of 0.25% TBA made in 10% trichloroacetic acid. Extract was heated at 95°C for 30 min and then quickly cooled on ice. After centrifugation at 10,000×g for 10 min, the absorbance of the supernatant was measured at 532 nm. Correction of non-specific turbidity was made by subtracting the absorbance value taken at 600 nm. The content of MDA was expressed as mmol g^{-1} fresh weight by using an extinction coefficient of 155$mM^{-1}cm^{-1}$.

Statistical analysis

All data presented in the paper are the means of at least five replicates. Significance of differences of samples was calculated by Student's t-test. Results of testing were considered significant if calculated P-values were ≤0.05.

RESULTS

Effects of aluminum on photosynthetic pigments in *Zea mays*

The amounts of photosynthetic pigments in the tissue of *Zea mays* grown under different concentrations of aluminum are shown in Table(1). A concentration-dependent decline of Chl a occurred as a consequence of exposure to Al^{3+} concentrations of 0- 50 ppm, where a minimum decrease of 44% from the control was reached at the highest Al^{3+}concentration (Table 1). However,

Table 1. Changes in Chl a, b and total carotenoids contents in leaves of Zea mays leaves treated with various concentrations of Al^{3+} for 6 days. Values in parentheses were expressed as the percent of reduction relative to the control (100%). Each value is the mean of three replicates (± SD). Values carrying asterisk are significantly different at P ≤ 0.05.

Pigmentmg g^{-1}	Al^{3+} ppm			
D.W.	Control	5	20	50
Chl *a*	0.457±0.049 (100)	0.381±0.02^{m} (83)	0.291±0.021^{m} (64)	0.202±0.013^{m} (44)
Chl *b*	0.263±0.014 (100)	0.257±0.017 (98)	0.221±0.025^{m} (76)	0.163±0.019^{m} (62)
Carotenoids	0.195±0.022 (100)	0.181±0.020 (93)	0.121±0.014^{m} (62)	.095±0.014^{m} (49)

the content of Chl b decreased at a relatively slower rate where its value was 62% of the control level at the highest Al^{3+} concentration (Table 1). Fig. (1B) showed that the total chlorophyll content steadily declined with the increase of Al^{3+} in growth medium. Moreover, the ratio of Chl a/b under Al^{3+} exposure declined regularly with the increasing Al^{3+} concentration (Fig. 1C). The changing trends of carotenoids content in *Zea mays* leaves exposed to a range concentration of Al^{3+} were similar to those of Chl a (Table 1), where its content reduced steadily with the increased Al^{3+} concentration.

Effects of aluminum on soluble protein in *Zea mays*

Changes of soluble protein content in *Zea mays* treated with aluminum were shown Fig.(1A). Exposure to 5 ppm of Al^{3+} slightly decreased soluble protein content after 6 days of treatment, while 20 ppm of Al^{3+} decreased protein content significantly. The presence of 50 ppm Al^{3+} in growth medium resulted in a significant reduction of protein content, reaching about 27% relative to control.

Effects of aluminum on antioxidant enzymes and MDA in *Zea mays*

The lowest Al^{3+}concentration (5 ppm) produced a significant stimulation (42%) in the POD activity (Fig. 2A). This activity increased up to a maximum value of 1498 U mg protein^{-1} min^{-1} asthe concentration of Al^{3+} reaching to 20 ppm. However, the POD activity decreased with highest Al^{3+} concentration to reach 15% lower than that of the control.

The presence of aluminum in growth medium stimulated the SOD activity, and reached to maximum value 167% over the corresponding control at the 50 ppm Al^{3+} concentration (Fig. 2B).

As the concentration of Al^{3+} increased to 20 ppm, the CAT activity reached a maximum value of 94 U mg protein^{-1} min^{-1}, however the CAT activity decreased with the increment of Al^{3+} concentration (Fig. 2C).

MDA content increased gradually with the increased concentration of aluminum. Significant stimulation (225%) of MDA appeared after 6 days at 50 ppm Al^{3+} treatment (Fig.3).

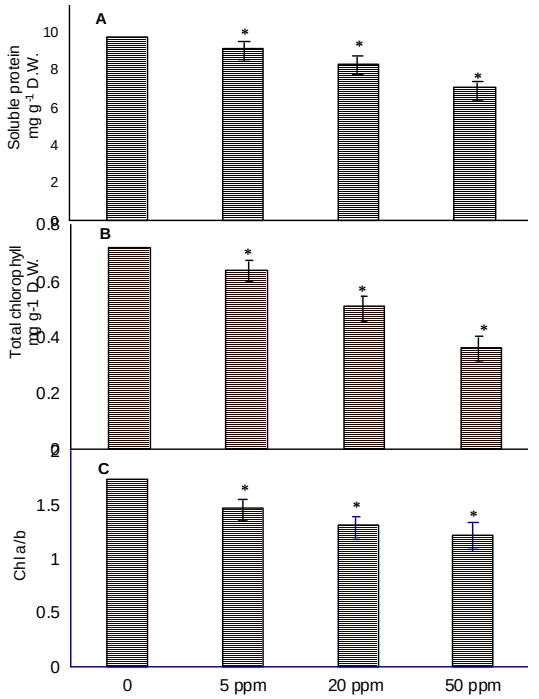

Fig. 1. Soluble protein (A), total chlorophyll (B) and chl a/b ratio (C) in maize leaves subjected to various concentrations of Al^{3+} for 6 days. Each value represents the mean ±SE of five replicates. Significant differences (P<0.05) between treatments according to LSD test are shown by an asterisk

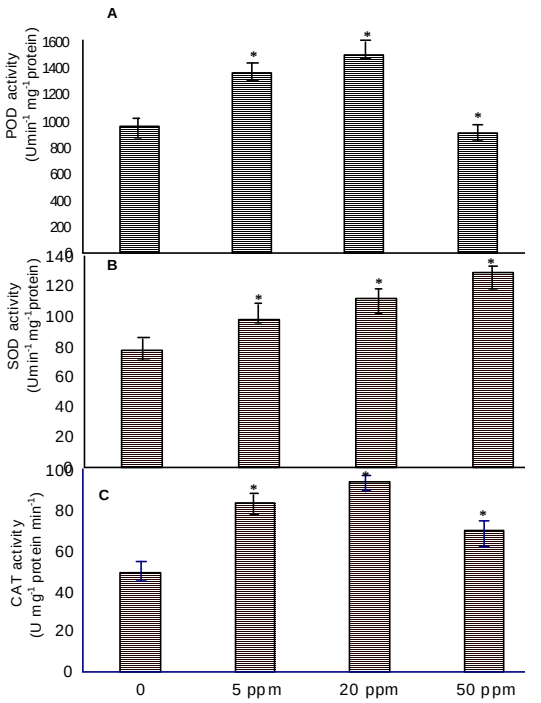

Fig. 2. Antioxidant enzymes activities POD (A), SOD (B) and CAT (C) of maize leaves subjected to various concentrations of Al^{3+} for 6 days. Each value represents the mean ±SE of five replicates. Significant differences (P<0.05) between treatments according to LSD test are shown by an asterisk

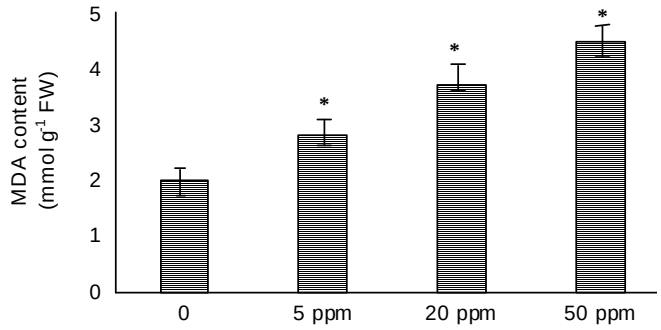

Fig. 3. MDA content in *Zea mays* subjected to different concentration Al^{3+} concentrations. Each value represents the mean ±SE of five replicates. Significant differences ($P<0.05$) between treatments according to LSD test are shown by an asterisk

DISCUSSION

Effects of aluminum on soluble protein and photosynthetic pigments

The present results clearly indicate that the presence of aluminum ion brought about the toxicity to maize leaves, due to its strong inhibitory effects on the contents of soluble protein and photosynthetic pigments. It was reported that Al^{3+}or Cd^{2+}resulted in a significant inhibition of protein level in *Brassica juncea* L and root tips of barley seedlings (Singh and Tewari, 2003; Liu *et al*., 2005). Similarly, changes in soluble protein content of maize leaves exhibited irrverse relationships with increased Al^{3+} concentrations, where higher concentration of Al^{3+} significantly reduced the soluble protein content more than lower Al^{3+}concentration did. The inability of maize leaves to synthesize protein after aluminum treatment may be due to acute oxidative stress induced by excess Al^{3+} in plant cells.

The lower Al^{3+} concentration(5 ppm) þhad a slight inhibition of photosynthetic pigments, but the 20 and 50 ppm Al^{3+} were sufficient to induce a decrease of pigments, indicating that aluminum could be a strong inhibitor of photosynthesis when present in excess (Frankart *et al*., 2002).The loss in chlorophyll content could be due to peroxidation of chloroplast membranes mediated or replacing magnesium in chlorophyll molecule by copper (Sandmann and Boger,1980; Mal *et al*., 2002). Numerous sites were identified as targets of aluminum action in chloroplast and therefore excess of Al^{3+} ions may result in decrease in the electron transfer rates consequent to its binding to the sites (Gaume *et al*., 2001).

It has been reported by Vieira *et al*.(2000) that Al^{3+} reduced the activity of delta-aminolevulinic acid dehydratase in mouse blood. Thus, aluminum may inhibit the formation of chlorophyll by interfering with proto chlorophyllide and the synthesis of aminolevulinic acid. Such effect may affect different steps of Calvin cycle, resulting in the inhibition of photosynthetic CO_2 fixation. Also, aluminum could do great harm to chloroplast envelope and thylakoid via increased production of free radicals (Kochian *et al*., 2004). Moreover, the degradation rate of Chl b under aluminum stress slower than that of Chl a and carotenoids, suggesting that suggest that the damage of aluminum on Chl a is greater than that on Chl b. It is well known that Chl a is one of the most important center pigments in photosynthesis and therefore the decrease of Chl a can inhibit the photosynthesis greatly. Carotenoids also play a role to guard of chlorophyll, and serve as an antioxidant to quench or scavenge the free radicals and reduce the damage of cell, cell membrane, and its main genetic composition (Gaume *et al*., 2001).

Effects of aluminum on antioxidant enzymes and MDA

The activity of POD and CAT of *Zea mays* treated with aluminum mainly displayed biphasic responses due to increase Al^{3+} concentration. At low-level metal stress, leaves could activate POD and CAT activities, which led to a strengthening of plants, to scavenge ROS responsible for lipid peroxidation (Dark´o *et al*., 2004). However, the activities of both enzymes decreased distinctly under too acute stress which overloaded cellular

defense system of plants (Guecheva *et al*., 2003). Also, the decline in the activities of POD and CAT might be due to the formation of protein complex with metals that change the structure integrity of proteins (Kollmeier *et al*., 2000). Similar results were obtained in effects of cadmium on CAT activity on maize plant (Ibrahim, 2006). For plants treated with increased Al^{3+} concentration, POD and CAT activities appeared to be inhibited exposed to 50 ppm (Fig 2 A and C). The results suggested that aluminum was toxic to *Zea mays* for the reason that at relatively high concentrations, antioxidant system appeared to be in disorder.

In the experiment, SOD activity under elevated aluminum stress was steadily stimulated with the increasing metal ion value in medium up to 50 ppm. The results showed that, under high metal stress, POD and CAT activities were inhibited, while SOD activity stimulated, indicating that those enzymes were located at different cellular sites, which had different resistances to heavy metals. Thus, the deterioration of cellular system functions by high metal stress might result in the inactivation of enzyme activity (Mittler, 2002; Dewez *et al*., 2005).

MDA is the decomposition product of polyunsaturated fatty acids of biomembranes that increased under high-level oxidative stress. Cell membranes stability has been widely used to differentiate stress tolerant and susceptible cultivars of many crops and in some cases higher membrane stability could be correlated with better performance (Premachandra *et al*., 1991). The present result showed that increasing aluminum concentration increased the MDA content of leaves due to the enhancement of lipid peroxidation (Fig. 3). Similar results were obtained in the effects of Al^{3+} on *Zea mays* (Gaume *et al*., 2001).

Finally, the present study showed that *Zea mays* could tolerate low level aluminum stress less than 20 ppm. Also, the low-level toxic Al^{3+} soil was quite efficient for corn growth. Therefore, the bioremediation of low-level polluted soil by aluminum is available.

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