Effects of aluminum toxicity on maize (*Zea mays* L.) seedlings

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Abstract

Aluminum toxicity is one of the most deleterious factors for plant growth in acidic soils because over 50% of the world's potentially arable lands are acidic. In recent years, considerable research has been conducted to understand the mechanisms of Al toxicity and tolerance in plants. This paper reviews the effects of different concentration of Al on plant shoot, root physiological parameters such as length, fresh and dry weight, pigment content and antioxidant enzyme such as Catalase Ascorbate and Guaiacol peroxidase, malonyldialdehyde content. Maize *(Zea mays* L.*)* seeds were sterilized and these seeds were then germinated in Petri dish. The germinated seeds were allowed to grow at 27°C in darkness, and 5 ml of test solution was added to each Petri dish on the second day. The test solution contained 0, 25, 50, 75, 100mM $AlCl_3$. Aluminum toxicity caused reduction in growth in all treatments. The plants exposed to Al exhibited a substantial decline in growth, pigment content, activities of guaiacol peroxidase (GPX), catalase (CAT) and ascorbate peroxidase (APX) and leaf structure. Data show that 50 mM Al caused increased in MDA content.

Keywords: aluminum; maize *(Zea mays* L.*);* oxidative stress; root growth

Abbreviations:
Al: Aluminum; GPX: guaiacol peroxidase; APX: ascorbate peroxidase; CAT: catalase; MDA: Malondialdehyde

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Introduction

Zea mays L. (maize, corn) is one of the most economically important food crops in the world, which demands substantial water volume during plant growth and flowering. Aluminum (Al) is the third most abundant element in the Earth's crust after oxygen and silicon. Aluminum (Al) toxicity is a major factor limiting crop productivity in many acid soils throughout the tropics and subtropics. Al toxicity symptoms in plants are visible in the form of necrotic spots and lesions along the leaf margins. Heavy metal stress is known to induce many abnormalities like damage to membranes, generation of reactive oxygen species (ROS), protein denaturation and accumulation of toxic compounds etc. at various organizational levels of the cells (Nayyar et al., 2005 a, b).

These ROS interrupt normal metabolism in plants by lipid peroxidation of membrane,

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denaturation of proteins and nucleic acids. Al toxicity stress alters the critical balance between the production of ROS and the quenching activity of antioxidants, resulting in oxidative stress that causes damage to plants (Hernandez et al., 2000).

Antioxidant enzymes, namely superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione peroxidase (GPX), and non-enzymatic antioxidants, such as ascorbic acid (ASC), glutathione (GSH), α-tocopherol, carotenoids, phenolics, and proline, which play a key role in quenching ROS, are implicated in stress tolerance (Hernandez et al., 2000; Malekzadeh et al 2012).

In this paper, we researched the physiological responses and tolerance of maize seedling root and shoot to Al toxicity.

Materials and Methods

Plant materials and treatments conditions

Maize seeds were sterilized with 2.5% sodium hypochlorite for 15 min and washed thoroughly with distilled water. These seeds were then germinated in Petri dishes (20 cm) containing distilled water at 37 °C in the dark. After a 1-day incubation, uniformly germinated seeds were selected and transferred to Petri dishes (9.0 cm) containing filter paper moistened with 10 ml of distilled water. Each Petri dish contained 12 germinated seeds. The germinated seeds were allowed to grow at 27 °C in darkness, and 5 ml of test solution was added to each Petri dish on the second day. The test solution contained 0, 25, 50, 75, and 100 mM $AlCl_3$. All experiments described here were performed three times.

When the maize (*Zea mays* L.) seedlings were harvested (day 8), the root system of each seedling was separated from the shoot, and the fresh weight were measured. The length of roots and shoots were measured by ruler.

Determination of chlorophyll content

Chlorophyll content was determined by taking fresh leaf samples (0.5 g) from 3 randomly selected plants per each replicate. The samples were homogenized with 5 mL of acetone (80%, v/v) using pestle and mortar and filtered through a filter paper (Whatman, No. 2). The absorbance was measured with UV/visible spectrophotometer (HACH, USA) at 663 and 645 nm and chlorophyll contents were calculated using the equations proposed by Lichtenthaler (1987) given below. Total chlorophyll content was expressed as Chl a + Chl b.

Chl a (mg/g FW) = 11.7× A663 − 2.35 × A645
Chl b (mg/g FW) =18.61 × A645 − 3.96 × A663

Measurement of lipid peroxidation

Al-induced oxidative damage was assessed through membrane lipid peroxidation by measuring the amount of malonyldialdehyde (MDA) in plant tissues as reported by Hagége et al. (1990)**.** Buege and Aust, 1978). Fresh shoot samples were immediately crushed in 0.1% (w/v) trichloroacetic acid (TCA) at 4 °C. The homogenate was centrifuged at 15,000 g for 15 min. An aliquot (0.5 ml) of the supernatant was added to 2 ml of 0.5% thiobarbituric acid (TBA, w/v) in 20% TCA. The mixture was heated at 95 °C for 30 min and then cooled in an ice bath. The samples were centrifuged at 10,000 g for 10 min, and the absorbance of the supernatant was read at 532 and 600 nm. The malondialdehyde concentration was calculated using the following formula:

$$\mathrm{MDA}\left(\mathrm{nmol\,g^{-1}\,FW}\right) = \left[((A_{532}-A_{600})*1000*V)/ \times\left(\varepsilon*\mathrm{FW}*10^{-3}\right)\right]$$

with ε , specific extinction coefficient of MDA (155 mM^{-1} cm^{-1}); V, volume of extracting medium; FW, fresh weight of shoots; A600, absorbance at 600 nm and A532, absorbance at 532 nm.

Catalase assay

Catalase activity was assayed according to Beers and Sizer [1952]. Fresh samples (200 mg) were homogenized in 5 ml of 50 mM Tris-/NaOH buffer (pH 8.0) containing 0.5 mM EDTA, 2% (w/v) PVP and 0.5% (v/v) Triton X-100. The homogenate was centrifuged at 22000 × g for 10 min at 4 °C and after dialysis, supernatant was used for enzyme

assay. Assay mixture in a total volume of 1.5 ml contained 1000 µl of 100 mM KH_2PO_4 buffer (pH 7.0), 400 µl of 200 mM H_2O_2 and 100 µl enzyme. The decomposition of H_2O_2 was followed at 240 nm (extinction coefficient of 0.036 mM^{-1} cm^{-1}) by decrease in absorbance. Enzyme specific activity is expressed as mmol of H_2O_2 oxidized min^{-1} (mg protein)$^{-1}$.

Ascorbate peroxidase assay

About 200 mg root/shoot samples were homogenized in 5 ml of 50 mM K-phosphate buffer (pH 7.8) containing 1% PVP, 1 mM ascorbic acid and 1 mM PMSF as described by Moran et al. (1994). After centrifugation at 22000 × g for 10 min at 4 °C, the supernatant was dialyzed against the same extraction buffer and it served as enzyme. Ascorbate peroxidase was assayed according to Nakano and Asada (1981). Reaction mixture in a total volume of 1 ml contained 50 mM K-phosphate buffer (pH 7.0), 0.2 mM ascorbic acid, 0.2 mM EDTA, 20 µM H_2O_2, and enzyme. H_2O_2 was the last component to be added and the decrease in absorbance was recorded at 290 nm (extinction coefficient of 2.8 mM^{-1} cm^{-1}) using a UV-Vis spectrophotometer (HACH, USA) at 30 s intervals up to 7 min. Correction was made for the low, non enzymic oxidation of ascorbic acid by H_2O_2. The specific activity of enzyme is expressed as µmol ascorbate oxidized min^{-1} (mg protein)$^{-1}$.

Guaiacol peroxidase assay

Guaiacol peroxidase was assayed according to Egley et al. (1983). Fresh root/shoot samples weighing 200 mg were homogenized in 5 ml of cold 50 mM Na-phosphate buffer (pH 7.0). The homogenates were centrifuged at 22000 × g for 10 min and the dialyzed enzyme extracts were used for the assay. Assay mixture in a total volume of 5 ml contained 40 mM NaH_2PO_4 buffer (pH 6.1), 2 mM H_2O_2, 9 mM guaiacol and 50 µl enzymes extract. Increase in absorbance was measured at 420 nm (extinction coefficient of 26.6 mM^{-1} cm^{-1}) at 30 s intervals up to 2 min, using a spectrophotometer (HACH, USA). Enzyme specific activity is expressed as µmol of H_2O_2 reduced min^{-1} (mg protein)$^{-1}$.

Fig. I. Time course of $AlCl_3$ effect on root length of maize seedlings; vertical bars represent standard errors.

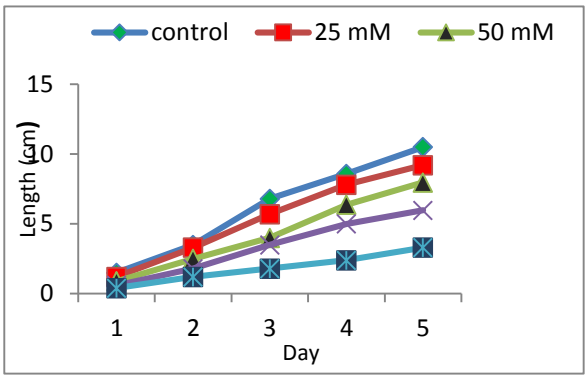
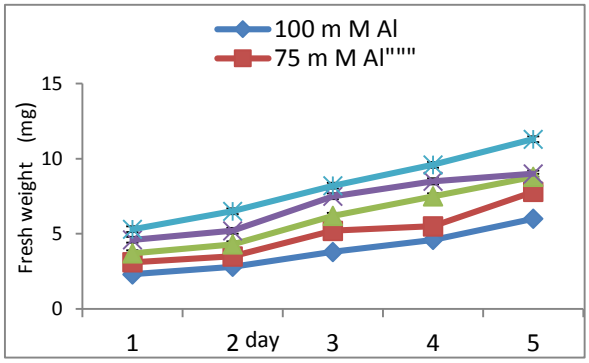


Fig. II. Time course of $AlCl_3$ effect on root fresh weight of maize seedlings; vertical bars represent standard errors.



Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test (DMRT). The values are presented as mean ± SD for 3 samples in each group. P values ≤ 0.05 were considered significant.

Results

Growth measured in terms of fresh weight and length of root declined significantly ($P < 0.05$) following Al treatments (Figs. I and II). Figure (I) shows the effect of $AlCl_3$ on the growth of maize seedlings. Increasing concentrations of $AlCl_3$ from 25 to 100 mM progressively decreased root length. However, no reduction of shoot length by $AlCl_3$ was observed. Treatment of maize seedlings with 50 and 75 mM Al resulted in 25% and 44% decline in root length, respectively.

Fig. III. Effect of $AlCl_3$ (50 mM) on the level of MDA in roots of maize seedlings; MDA level were measured after 5 days of treatment. Vertical bars represent standard errors.

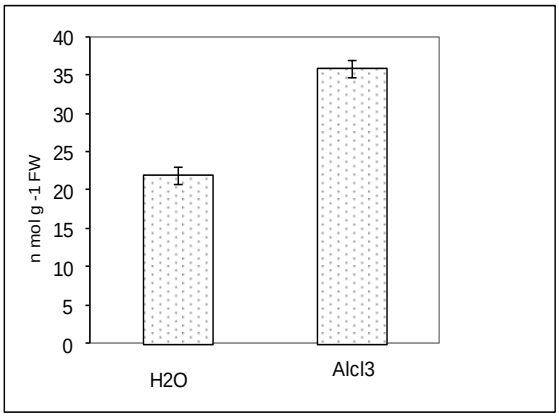


Figure (II) shows time courses of the effect of $AlCl_3$ (0, 25, 50, 75, and 100 mM) on root FW. As judged by root FW, the reduction of root growth was evident 2 days after treatment.

Data pertaining to the photosynthetic pigments i.e. chlorophyll a, chlorophyll b, and carotenoids are presented in Table 1. AL at each of the four concentrations significantly ($P < 0.05$) declined Chl a and Chl b contents. The decrease in Chl b was greater than Chl a, hence higher Chl a/Chl b ratio was observed. Al also reduced ($P < 0.05$) carotenoids content; however, impact was less than that of chlorophylls (Table 1).

Figure (III) demonstrates that $AlCl_3$ treatment resulted in a significant increase in MDA level, an indicator of lipid peroxidation. Shoot MDA levels in both provenances were comparable in control medium (Fig. III). Shoot lipid peroxidation increased markedly under Al toxicity to variable extent depending on the provenance. In maize plants, MDA concentrations were respectively 1.6 folds higher at 50 mM Al than in control plants (Fig. III).

Activity of enzymatic antioxidants is shown in Figs. (IV), (V) and (VI). Analysis of variance revealed that, the activity of enzymes such as ascorbate peroxidase (APX), catalase (CAT), and guaiacol peroxidase (GPX) in root and shoot were affected by the main effect of Al concentration. The 25, 50, 75, and 100 mM of Al stimulated ($P < 0.05$) CAT activity by 150, 240, 278, and 332%, respectively with regard to the control seedlings. Unlike, CAT activity in shoot of maize seedlings has not stimulated by Al (Fig. IV).

Fig. (V) Shows the effect of Al treatment on the activity of APX in the roots of maize plants. APX activity in roots increased significantly ($p< 0.05$) by an increase in Al concentration, but there was no significant correspondent increase in the shoots of these plants (Fig. V).

Fig. (VI) Shows that GPX activity in maize roots increased significantly ($p< 0.05$) by an increase in Al concentration in nutrient solution. However, $AlCl_3$ had no effect on the activity of GPX in shoots of maize seedlings (Fig. VI).

Table 1
Effect of Al toxicity on chlorophyll a (Chl a), chlorophyll b (Chl b), carotenoids (Car), and chlorophyll a/chlorophyll b ratio (Chl a/Chl b ratio) in maize seedlings; data are means ± standard error of 3 replicates. Values with different letters within the same column show significant differences at $P < 0.05$ significance level between treatments according to the Duncan's multiple range test.

tretments	Chl a (mg/g fresh weight)	Chl b (mg/g fresh weight)	Chl a/Chl b ratio	Car (mg/g fresh weight(
Control	2.09±0.051a	0.87±0.019a	2.4d	0.526±0.14a
25	1.85±0.06b	0.62±0.015b	2.98c	0.503±0.12a
50	1.52±0.055c	0.578±0.015b	2.92c	0.478±0.12b
75	1.38±0.04c	0.50±0.019c	3.45b	0.426±0.13c
100	1.17±0.058d	0.41±0.10c	3.77a	0.404±0.12c

Fig. IV. Effect of increasing concentration of $AlCl_3$ in the growth medium on catalase activity in roots (---) and shoots (—) of wheat seedling; antioxidative enzyme activities were measured after 5 days of treatment. Vertical bars represent standard errors.

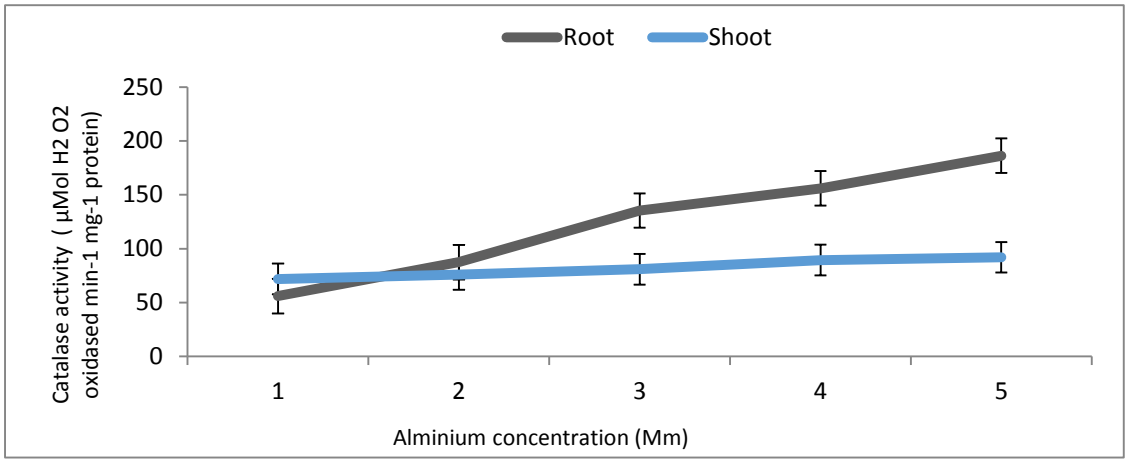
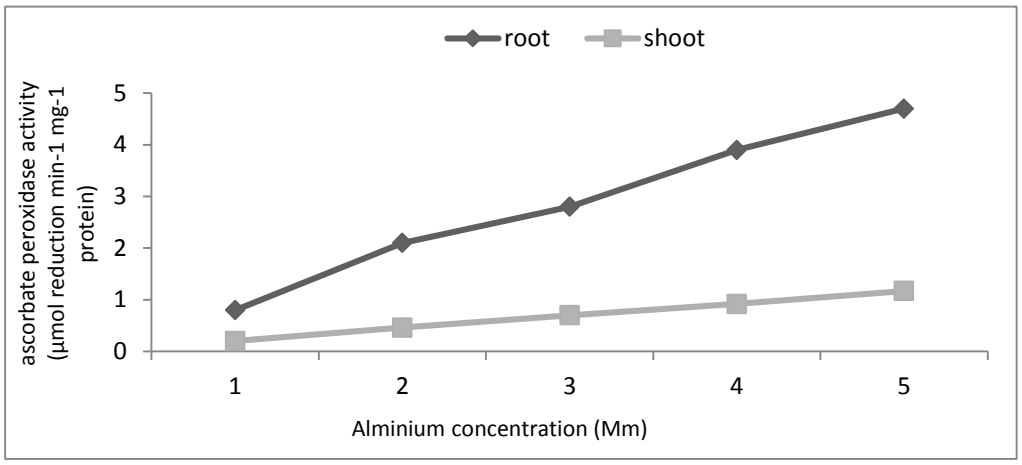


Fig. V. Effect of increasing concentration of AlCl3 in the growth medium on ascorbate peroxidase activity in roots (---) and shoots (—) of wheat seedling. Antioxidative enzyme activities were measured after 5 days of treatment. Vertical bars represent standard errors.



Discussion

The present study provides evidence for Al toxicity symptoms in maize seedlings. In the present work, when the plants were exposed to Al toxicity, root and shoot length of maize plant decreased as Al concentration in the medium increased (Fig. I). High levels of Al, may inhibit root and shoot growth directly by inhibiting cell division or cell elongation, or a combination of both, resulting in limited exploration of the soil volume for uptake and translocation of nutrients and water, inducing mineral deficiency (Hemantaranjan et al., 2000). The differential effect of Al on root and shoot growth could be accounted for by the fact that Al is accumulated mainly in roots and to a minor extent in shoots (Fernandes and Henriques, 1991).

Growth of maize seedlings was found to be adversely affected by Al toxicity stress and Al-induced impairment in growth has already been reported previously in a number of crops such as buck wheat (Shen et al., 2004), tomato (Simon et al., 1994), and citrus (Chen, 2005).

Control plants pre-treated with distilled water were considerably larger in size as indicated by higher seedling mass (Fig. I) and had significantly higher chlorophyll content (Table. 1) than those that were pre-treated with Al. Chlorophyll fluorescence has emerged as a non-invasive and powerful tool to elucidate damaging modifications in photosynthetic apparatus in

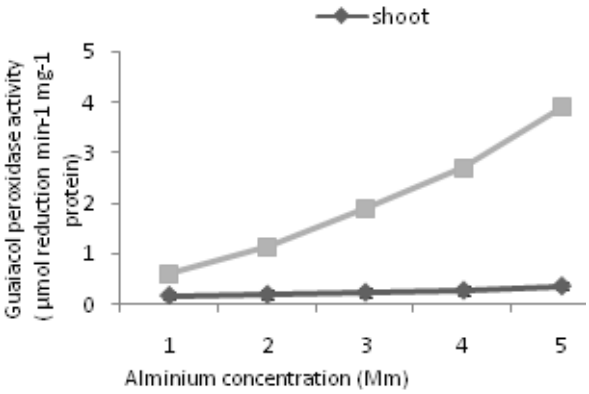
stressed plants. Photosynthetic activity of maize seedlings as determined by chlorophyll fluorescence was adversely affected by Al toxicity which may firstly be related with decline in chlorophyll contents (Table 1). The decrease in pigments content may be attributed either to Al-induced inhibition on biosynthesis or ROS-mediated degradation of pigments (Malekzadeh et al., 2014).

As shown in Fig. (III), the MDA content increased significantly ($p < 0.05$) in Al treated seedlings under stress compared with control plants. The amount of MDA in Al-stressed seedlings was significantly ($P < 0.05$) increased to 36.2 in 50 mM Al stressed seedlings compared to control plants (22.3 µmol L^{-1}). It seems that Al toxicity increased the accumulation of lipid peroxidation product, MDA, which is regarded as an indicator of the loss of structural integrity in membranes subjected to heavy meal stress (Posmyk et al., 2009; Hosseini et al., 2014).

To endure oxidative damage under conditions that contribute to enhanced oxidative stress, such as high/low temperatures, heavy metal toxicity, and salinity, plants must possess an efficient antioxidant system (Karthikeyan et al., 2007). Plants possess antioxidant systems in the form of enzymes, such as SOD, APX, PX, CAT, and metabolites, ascorbic acid, glutathione, a-tocopherol, carotenoid, flavonoids, etc. (Bowler , Van Montagu , 1992).

A uniform increase in catalase (CAT), ascorbate peroxidase (APX), and guaiacol peroxidase (GPX) activities was detected by increasing Al concentration in medium culture. The marked increase of catalase activity with increasing Al levels in maize seedlings may indicate enhanced production of reactive oxygen species (ROS) under an excess of aluminum. This enhanced activity seems to be related to increased oxidative stress tolerance (Malekzadeh et al., 2014). Among the enzymatic systems considered to play an important role in the cellular defense strategy against oxidative stress, catalase plays an important role as it decomposes H_2O_2 to water and O_2. It has been previously suggested that, accumulation of H_2O_2 caused by various environmental stresses would result in the enhanced activity of catalase in order to protect plant cells (Malekzadeh et al., 2014).

Fig. VI. Effect of increasing concentration of $AlCl_3$ in the growth medium on guaiacol peroxidase activity in roots (---) and shoots (——) of wheat seedling. Antioxidative enzyme activities were measured after 5 days of treatment. Vertical bars represent standard errors.



Overexpression of genes encoding these enzymes in several transgenic plant species conferring protection against free radicals has also been demonstrated (Allen, 1995). In the present study, Al treatment resulted in an increase in the activities of APX and GPX (Figs. V and VI), which can be considered as an indirect evidence for enhanced production of free radicals under Al stress. The increase of APX and GPX has been reported with Al (Ashraf Metwally et al., 2003; Karataglis et al., 1991; Rama Devi and Prasad, 1998; Malekzadeh et al 2012). However, Mazhoudi et al., (1997) reported that CAT activities in shoot of maize plant were not affected by Al. We also found no change in CAT activity in shoot (Fig. IV). Such a variation in response of these enzymes to Al stress could be due to the variability of plant species in producing free radicals (Mazhoudi et al., 1997). Thus, the increase in the activities of APX and GPX by Al in root of maize plant (Figs. IV- VI) suggests increased production of H_2O_2.

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