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Aluminum Toxicity in Maize Seedlings (*Zea mays* L.): Effect on Growth and Lipid Content

[1]Radhouane Chaffai, [2]Ali Tekitek and [1]Ezzedine El Ferjani
[1]Laboratoire de Biologie et Physiologie Cellulaires Végétales,
[2]Laboratoire de Biochimie et de Biologie Moléculaire,
Faculté des Sciences de Bizerte, 7021 Zarzouna, Tunisie

Abstract: Maize seedlings were grown in hydroponic nutrient solutions containing 0, 20, 50, 100, 250, 500 and 1000 μM $Al(NO_3)_3 \cdot 9H_2O$. The effect of aluminum on growth parameters and lipid composition of maize organs are studied. The results showed that Al inhibited dramatically root growth. Morphological symptoms characteristic of Al toxicity were observed in roots. The Al reduced significantly the fresh and dry matter production. Analyze of the lipid content showed a decrease in phospholipids in roots and shoots particularly at 1000 μM. Moreover, Al affected glycolipids in roots without any changes in shoots. The steryl lipids did not undergo variations. The increase of SL/PL ratio in roots and shoots point out elevated lipid membrane fluidity under Al stress. All the changes in lipid content were essential to restore optimal membrane properties for continuous growth under Al stress.

Key words: Aluminum toxicity, lipids, lipid peroxidation, *Zea mays* L.

INTRODUCTION

Aluminum is the third most abundant element in the earth's crust. Solubilization of Al-containing minerals is enhanced in acidic environments. In many acid soils throughout the world Al is the most growth limiting factor[1], possibly affecting up to 70% of the world's arable land that is potentially usable for food and biomass production[2]. Toxicity concerns however only some of its soluble forms, where the most toxic monomer species Al^{3+} prevails in acidic conditions[3-5].

It's largely recognized that root tips are the primary site of Al-induced injury in plants[6]. The best-known effect is the reduction in the growth of plant roots[7]. This effect is present even with respect to micro molar concentrations[5]. The characteristic symptom of toxicity is the apparition of nodosities in the apical region of the roots[8]. The Al can affect many metabolic processes in particular those related to the phosphorylation of sugars[9].

Phospholipids are recognized as major component of biological membranes. These molecules play a central role in cellular activities by controlling membrane permeability and activity of attached enzymes[10]. Galactolipids are a specific component of chloroplastic membranes. A large body of literature has described changes on phospholipids in response to temperature[11], salinity[12] water-deficit and pathogen infection. The effects of Al on lipid composition however are less common studied. It has been suggested that cells tend to respond to membrane-perturbing environmental factors by altering membrane lipid composition and such changes are thought to restore optimal physical properties[13].

Only few reports have mentioned the interaction of Al with membrane lipids.

The aim of this study was to examine the effect of aluminum on growth and lipid content of maize seedlings.

MATERIALS AND METHODS

Plant material and growth conditions: The corn (*Zea mays* L. Cv. LG 23/01) seeds were disinfected with 10% (v/v) H_2O_2 for 20 min then rinsed many times with distilled water and germinated in darkness at 25°C. The seedlings were transferred to plastic beakers filled with continuously aerated basal nutrient solution. For treatment purposes, fourteen-days-old seedlings are transferred to fresh solution. Varying Al concentrations were added as $Al(NO_3)_3 \cdot 9H_2O$ (0, 20, 50, 50, 100, 250, 500 and 1000 μM). The pH of the solutions was adjusted to 4.0.

The composition of the basal nutrient solution was as follows: (in mM) 2 KNO_3, 2.5 $Ca(NO_3)_2$, 1 KH_2PO_4, 1 $MgSO_4$; (in μM) 50 Fe as Fe-K-EDTA complex, 30 B as H_3BO_3, 10 Mn as $MnSO_4.H_2O$, 1 Cu as $CuSO_4.5H_2O$, 1 Zn as $ZnSO_4.7H_2O$ and 0.2 Mo as $(NH_4)_6Mo_7O_{24}.4H_2O$.

Corresponding Author: Radhouane Chaffai, Laboratoire de Biologie et Physiologie Cellulaires Végétales, Faculté des Sciences de Bizerte, 7021 Zarzouna, Tunisie
Tel: +216 22 822 523 Fax: +216 72 590 566 E-mail: radhouanec2002@yahoo.fr

Cultures are performed in conditioned room with 16 h photoperiod, using mercury lamps providing a light intensity of 150 μmol m^{-2} s^{-2}, a day/night temperatures of 25/22°C and 65%, relative humidity.

Lipid extraction: The lipids were extracted according to the method of Folch *et al.*[14] modified by Bligh and Dyer[15]. The plant tissues were fixed in boiling water for 5 min to denature phospholipases[16] and then homogenized in chloroform:methanol mixture (2 : 1, v/v). The homogenate was centrifuged at 3000 rpm for 20 min. The lower chloroformic phase containing lipids was aspirated and evaporated at 40°C using rotary evaporator (Büchi). The residue was immediately redissolved in toluene:ethanol (4:1, v/v).

Total lipid determination: The total lipids were quantified by the absorbance at 215 nm. An aliquot of lipid extract (200 μL) was evaporated. The dry residue was dissolved in 3 mL ethanol. Total lipids were quantified using standard calibration curve of corn oil.

Steryl lipids determination: Steryl lipids in total lipids were determined according to Huang *et al.*[17]. An aliquot of lipid extract (200 μL) was evaporated in glass tubes. After addition of 1 mL of acetic acid, the tubes were vortexed and 2 mL of Liebermann-burchard reagent (1 mL of concentrated H_2SO_4 was added to 20 mL of acetic anhydride) were added. The tubes were incubated at room temperature in darkness for 1 h and the absorbance was measured at 525 nm. Cholesterol (Sigma) was used as a standard.

Glycolipids determination: The glycolipids were quantified in total lipid extracts by measuring sugar content according to Roughan and Batt[18]. An aliquot of lipid extracts (200 μL) was placed in glass tubes to evaporate. After addition of 0.5 mL 2% (v/v) phenol and 2 mL of concentrated H_2SO_4, the tubes were vortexed and incubated for 10 min. After centrifugation at 2-000 g for 5 min, the supernatants were used for the determination of the sugars. Absorbance was measured at 480 nm. Galactose (Sigma) was used as standard.

Phospholipids determination: The phospholipids were quantified by measuring the inorganic phosphorous content in lipid extracts according to Bartlett[19].

A lipid sample (200 μL) was evaporated in glass tubes, heated on flame and 0.5 mL of concentrated H_2SO_4 was added. After the appearance of a white smoke, few drops of H_2O_2 were added. The tubes were cooled and the volume was adjusted to 2 mL with distilled water. Six milliliter of acetate buffer containing copper sulfate 0.01 M, sodium acetate 0.33 M and acetic acid 2M (pH 4.0), 1 mL 5% (w/v) ammonium molybdate and 1 mL reducing agent (2 g of paramethylaminophenol sulfate in 100 mL 10% (w/v) sodium sulfite) were added consecutively. The absorbance of the resulted colored solution was measured at 880 nm. A standard calibration curve was prepared using KH_2PO_4.

MDA determination: The extent of lipid peroxidation was estimated according to Heath and Packer[20]. The plant materials were ground in 0.25% (w/v) TBA 10% TCA. The mixture was then heated at 95°C for 30 min and immediately cooled inside ice beaker and centrifuged at 1000 g for 10 min.

The absorbance of the supernatant was measured at 532 nm and corrected by subtracting the non-specific absorbance at 600 nm. The MDA concentration was calculated using the extinction coefficient (155 mM^{-1}_cm^{-1}).

RESULTS AND DISCUSSION

Symptoms of aluminum toxicity: Toxicity symptoms has appeared for all Al-treatments. The Al has triggers morphologic changes (Fig. 1). Among the earliest symptoms of Al-toxicity, inhibition of secondary roots, thickness and browning in root apices. Plants treated with the highest concentration (1000 μM) were found to be severely damaged compared to control plants (Fig. 2). The apical zone becomes curved and brown-colored. The growth of lateral roots was severely inhibited and the unusual development gives rise to structures resembling nodosities (Fig. 2).

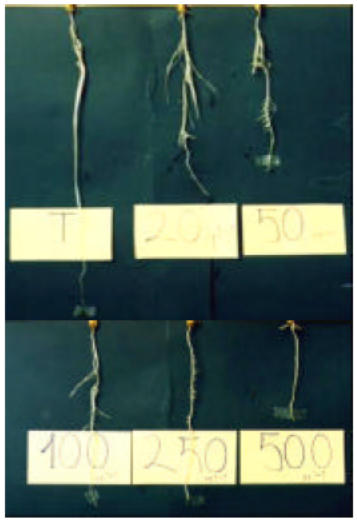

Fig. 1: Morphological symptoms of Al toxicity in maize seedling grown in hydroponics for 14 days then treated for 4 days

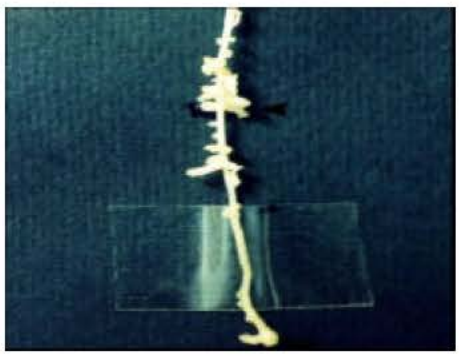

Fig. 2: Terminal zone of maize root showing characteristic morphological changes due to Al exposure (1000 μM Al)

Many researcher[21-24] reported similar morphological changes induced by Al. Foy[1] indicated that the changes affected the apical zone of the roots, which become shorter, harder and brown. It's largely recognized that root tips are the primary sites of Al-induced injury in plants[6].

Al-stressed plants expressed cumulative Al toxicity damage. A number of different toxicity symptoms expressed during different developmental stages point to a various levels and degrees of adaptation to the stressful environment[25]. Generally, some symptoms of the Al toxicity syndrome appear after a short term-exposure measurable within minutes or even seconds followed by the long-term responses that are measured hours or even days after the addition of Al. Since long-term responses

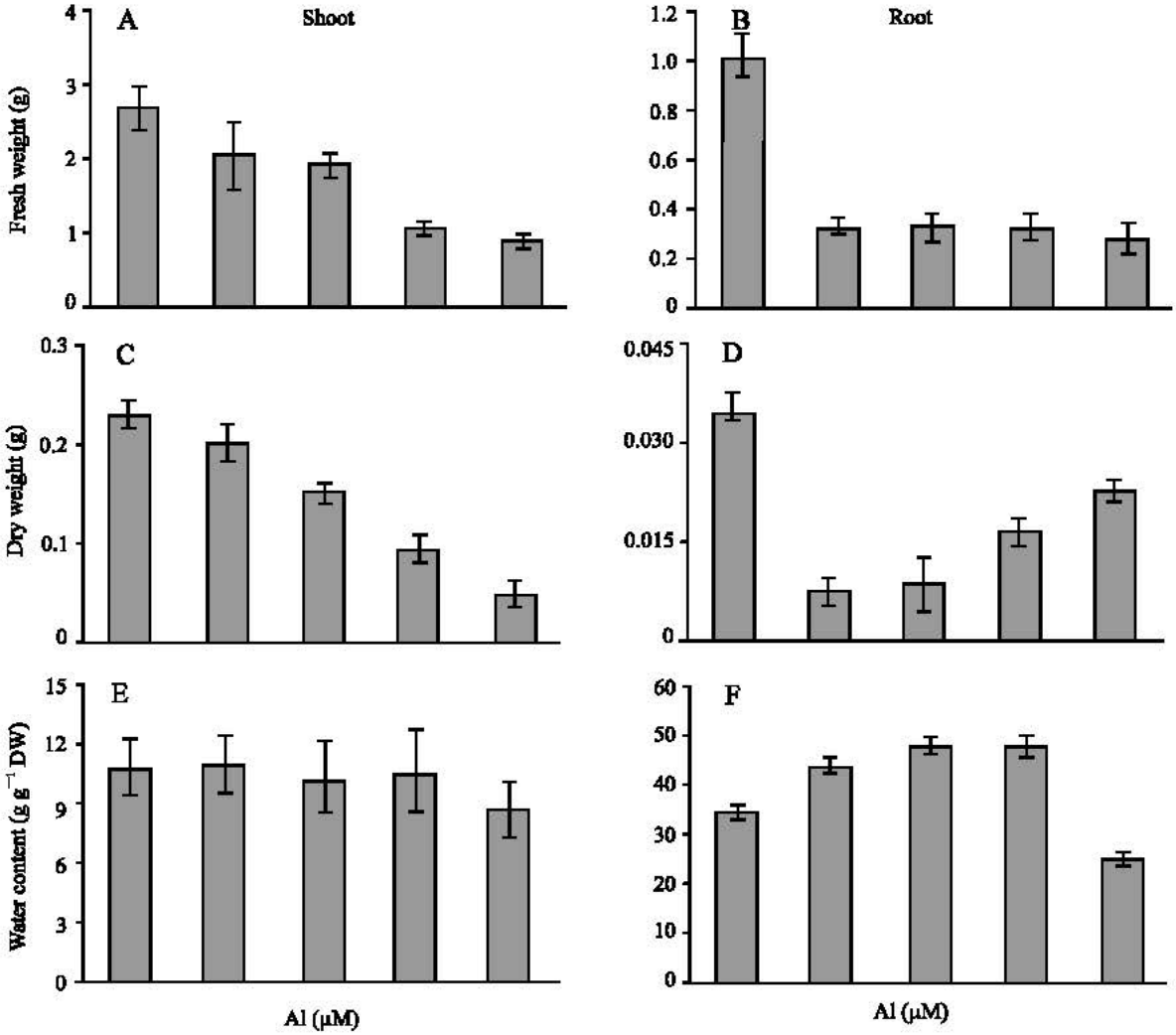

Fig. 3: Effect of Al on fresh weight (A, B), dry weight (C, D) and water content (E, F) of maize seedlings grown in hydroponics for 14 days then treated for 4 days. The results were given as the mean±standard error of at least five independent experiments. Significant differences between treated and control plants were determined using Student's t test ($p<0.05$)

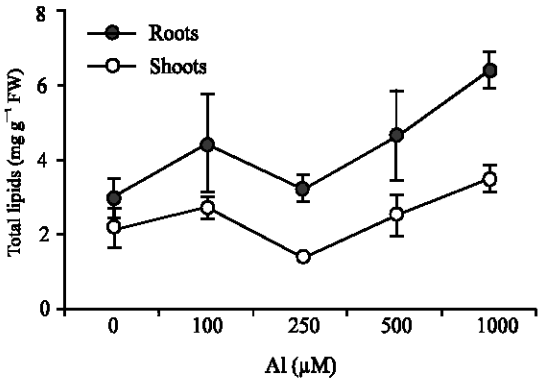

Fig. 4: Effects of aluminum on total lipids in roots (A) and shoots (B) of maize seedlings grown in hydroponics for 14 days then treated for 4 days. The results presented were the mean values±standard error obtained from at least five independent experiments. Significant differences between treated and control plants were determined using Student's t test ($p<0.05$)

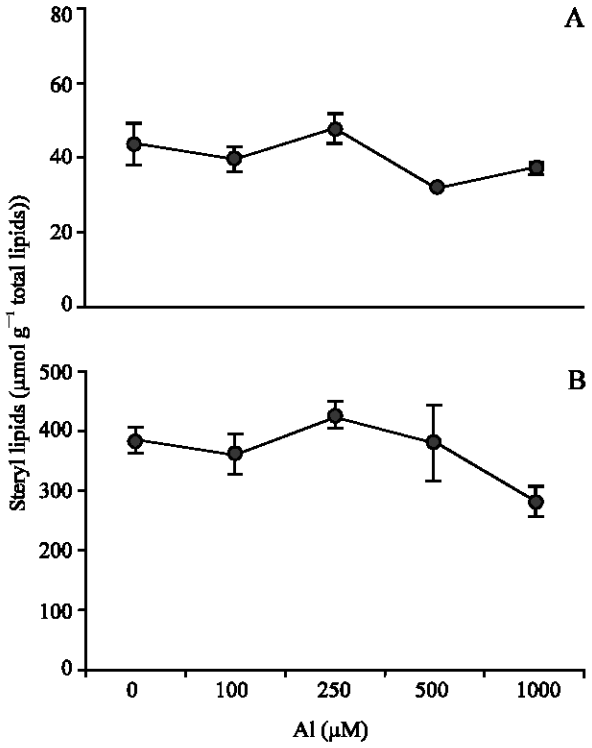

Fig. 5: Effects of aluminum on steryl lipids in roots (A) and shoots (B) of maize seedlings grown in hydroponics for 14 days then treated for 4 days. The results presented were the mean values±standard error obtained from at least five independent experiments. Significant differences between treated and control plants were determined using Student's t test ($p<0.05$)

are not necessarily caused by Al directly, but might rather be a consequence of Al-related impairment of numerous other biochemical and physiological processes[26]. Elsewhere, Rasmussen[27], observed the same symptoms of toxicity in maize roots. Regarding the aerial parts, the symptom of toxicity takes place only after a long exposure to Al[28]. Clark[29] suggests that the red color developed in aerial part of plant is indicative of phosphorus deficiency.

Effect on growth: Aluminum had toxic effects on maize growth. Exposure to varying concentrations of Al resulted in inhibition of root biomass production. The fresh weight of Al treated roots decreased intensively with increasing Al concentrations. At 1000 µM Al, the reduction was about 66% compared to control plants. In shoots, Al caused a decrease by 80% in fresh weight production (Fig. 3 A and B). Moreover, a decline in dry weight production was recorded in the both organs (Fig. 3 C and D). The water content in roots did not undergo variations for all Al treatments. In shoots however, this content increases significantly to 500 µM Al then it decreased with 1000 µM Al (Fig. 3 E and F).

The inhibition of the root growth may be due to the disturbances in cellular divisions of meristematic apical zone[30]. According to Foy[1], the most recognized effect of Al toxicity is inhibition of root growth. Reduction in root growth reduces the absorption of nutrients and water and consequently, crop yield[3]. The more important effects by excess Al: interference with cell division in root and lateral root[31], increased cell wall rigidity by cross linking pectins[9], altered root membrane structure and functions[32], inhibition of the uptake and the utilization of most of essential[21,33], reduced root respiration which consequently reduced uptake of water and nutrient[31]. Trivalent Al coordination forms complex with carboxyl and sulfhydryl groups of proteins producing cross linkage[34].

We have proposed to study the effect of Al stress on lipid composition in maize as related to growth inhibition.

Lipid analysis: After four days of Al treatment, lipids were analyzed in roots and shoots of maize seedlings. Increasing Al concentrations from 100 to 500 µM did not have any effect on total lipids in roots. For the highest Al concentration (1000 µM), total lipids increased significantly by 115% compared to the control (Fig. 4 A). Nevertheless, no variations were found for total lipids in shoots (Fig. 4 B).

The steryl lipids analyzed in total lipids showed no significant variation, neither in roots nor in shoots for all Al concentrations (Fig. 5 A and B).

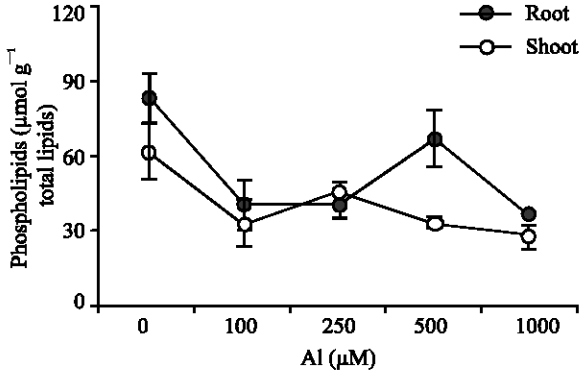

Fig. 6: Effects of aluminum on phospholipids in roots (A) and shoots (B) of maize seedlings grown in hydroponics for 14 days then treated for 4 days. The results presented were the mean values±standard error obtained from at least five independent experiments. Significant differences between treated and control plants were determined using Student's t test ($p<0.05$)

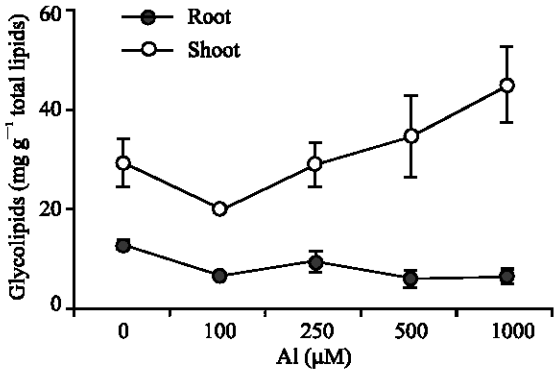

Fig. 7: Effects of aluminum on glycolipids in roots (A) and shoots (B) of maize seedlings grown in hydroponics for 14 days then treated for 4 days. The results presented were the mean values±standard error obtained from at least five independent experiments. Significant differences between treated and control plants were determined using Student's t test ($p<0.05$)

Moreover, the total PL content decreased significantly in roots at 100 and 250 µM, increased at 500 µM and decreased again at 1000 µM Al. The observed reduction in PL was approximately 50% compared to control (Fig. 6 A).

The analysis of phospholipids in shoots showed a decrease at 500 and 1000 µM Al concentrations. The reduction was about 55% and almost the same to that found in roots (Fig. 6 B).

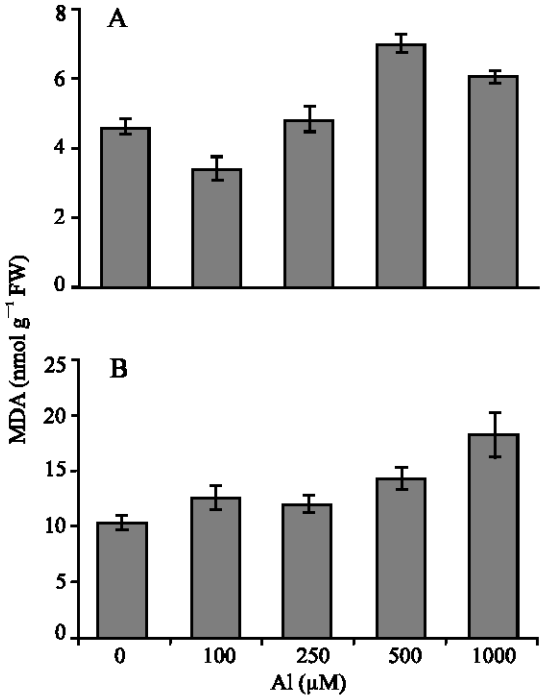

Fig. 8: Effects of Al on MDA content in maize seedlings grown in hydroponics for 14 days then treated for 4 days. The results presented were the mean values±standard error obtained from at least five independent experiments. Significant differences between treated and control plants were determined using Student's t test ($p<0.05$)

Table 1: The SL/PL ratio in roots and shoots of maize seedlings grown in hydroponics and treated by varying Al concentrations for 4 days.

	SL/PL				
Al (µM)	0	100	250	500	1000
Root	0.55±0.12	0.73±0.24	1.23±0.20	0.36±0.10	0.99±0.00
Shoot	6.40±1.23	12.19±2.24	9.29±1.66	11.34±1.45	10.97±2.38

The data represented a mean±standard error of at least five independent experiment

Treatment with Al resulted in significant decrease in total GL in roots by 47% while no significant change was observed in shoots (Fig. 7 A and B).

The SL/PL ratio was determined in roots and shoots of maize seedlings. The results showed that Al at 250 and 1000 µM decreased the SL/PL ratio in roots while no change was observed at 100 and 500 µM Al concentrations. The SL/PL ratio increased approximately by 123 and 80% in roots, respectively with 250 and 1000 µM Al concentrations (Table 1).

However, the SL/PL increased in shoot for all Al concentrations.

A previous study of Zhang *et al.*[45] showed a 32% increase in SL of microsomal membranes isolated from 5 mm root tips of an Al-sensitive wheat genotype Katepwa, while no change was found in Al-resistant PT741 after prolonged exposure (3 days) to 50 μM Al. In a next study, they found a decrease in the SL in plasma membrane obtained from root of PT-741 while no change was observed in Katepwa at 20 μM Al[36].

Zhang *et al.*[35] have studied on purified plasma membrane fraction of root apices, whereas we were interested in the study of lipids in the whole root system. Thus, the lipid composition of plasma membranes from entire root differed from that of microsomal membranes from root apices. Thus the changes in lipid composition of the plasma membrane induced by Al may not affect microsomal membranes.

Zhang *et al.*[35] found a decrease in phospholipids of the two wheat genotypes with prolonged exposure (3 days) to 50 μM Al. Al treatment had no effect in total PL in plasma membranes isolated from roots exposed to 20 μM Al for 3 days[36].

The decrease in PL content in response to Al could be attributed either to a degradation processes or to reduced PL synthesis.

The SL/PL ratio decreased in PT-741, but not affected in Katepwa[36]. In another result, Al induced an increase in the SL/PL ratio in both genotypes after longer exposure, due mainly to the reduction in PL[35]. Thus, the increased SL/PL ratio in the present study is consistent with previous observation of an Al-induced change in membrane fluidity[37,38].

Enhanced SL/PL ratios are known to reduce bilayer fluidity of the plasma membrane[39-41].

Aluminum decreased lipid fluidity in plasma membranes of an Al-sensitive mycorrhizal fungus *Amanita muscaria*[38] and in *Thermoplasma acidofilum*[37]. Likely, the Ca binding to the negative charged head groups phospholipids determine the optimal plasma membrane fluidity[42]. Al ions bind more tightly to these sites displacing Ca ions and reducing membrane fluidity[43]. Aluminum can modify lipid arrangement in the membrane facilitating lipid peroxidation by iron (II)[44].

Previous studies have shown that Al binds to PL, decreasing membrane lipid fluidity[37,45-47]. An increase in membrane fluidity was observed in an Al-resistant fungus, *Lactarius piperatus*[48].

Increases in membrane lipid fluidity are one of the toxic lesions induced by Al, changes in membrane composition may constitute a response which contributes to the restoration of optimal physical properties in the face of Al stress.

The changes in lipid content were crucial to allow plant growth under Al induced stress.

Effect of Aluminum on lipid peroxidation: The MDA content, product of lipid peroxidation was analyzed in roots and shoots. The TBA assay can be regarded as a reliable method for evaluating the degree of lipoperoxidation. The TBA in roots and shoots is analyzed on basis of fresh weight. In roots, concentration of MDA was significantly increased by Al at the 500 and 1000 μM Al (Fig. 8). Changes in MDA was also recorded in shoots, showing a little decrease for all concentrations used. Lipid peroxidation started in metal-exposed plants, due to increased production of toxic oxygen free radicals. Al treatment decreased linolenic acid in purified plasma membrane fraction of root apices of Al-sensitive *Sorghum* which is indicative of lipid peroxidation[43]. Plasma membranes obtained from entire root system of the Al-sensitive *Sorghum* cultivar showed higher concentrations of malondialdehyde than the Al-tolerant cultivar, independent of the Al treatment which reflects a higher production of reactive oxygen species in this cultivar. Addition of Al to the nutrient solution did not have any effect on the production of MDA in both cultivars. Similar results were obtained by other researchers[20,40,49] using different plant species.

Lipid peroxidation can be started by redox active metal ions themselves, such as copper[50].

Fraction of plasma membranes obtained from root apices showed increased concentration of MDA in the presence of Al in the Al-sensitive cultivar with no change in the Al-tolerant cultivar.

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