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## Aluminum-induced Changes in Organic Acid and Lipid Content in Maize (*Zea mays* L.)

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**Abstract:** Aluminum is solubilized at low pH in acidic soils and is major factor limiting crop productivity in many arable lands in the world. The results indicated that the decrease in root lengths was a function of Al concentration and exposure time. Inhibition of root growth was closely associated to morphological disorders, such as stubby appearance, a large number of short laterals, swelling and deformed root apices. The results of kinetic studies during short (1, 2, 4 and 8 h) or long-term (4 days) of Al exposure (0-1000  $\mu$ M Al), showed general trend toward decreasing the malate content in roots with increasing Al concentrations and strongly suggest that malate is not involved as Al-chelating ligand in internal Al resistance mechanism. In addition, the root content of citrate was not affected by Al, indicating that citrate might contribute to internal Al detoxification. Malate dehydrogenase was significantly increased during 8 h of Al exposure, while no significant change was observed after 1, 2 and 4 h of Al exposure. By contrast, this activity was significantly decreased in roots after 4 days of Al exposure. Not significant changes were observed with Al exposure, for either roots or shoots, in the activity of NADP-isocitrate dehydrogenase.

**Key words:** Aluminum, lipids, malate dehydrogenase (MDH), NADP-isocitrate dehydrogenase (NADP-ICDH), organic acids, *Zea mays* L.

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### Introduction

Al constitutes the most abundant metal in the earth's crust. At a low soil pH (<5), Al hydrolyzes in solution, mainly in the phytotoxic form of Al<sup>3+</sup> (Delhaize and Ryan, 1995). The acid soils cover almost 40% of world's arable land and Al toxicity has been considered as the most growth limiting factor (Foy, 1988; Kochian, 1995; Matsumoto, 2000; Kochian *et al.*, 2004). The severe inhibition of root elongation is one of the most obvious symptoms of Al toxicity (Ryan *et al.*, 1993; Osawa and Matsumoto, 2001), resulting in poor nutrient acquisition and consequently leading to nutrient deficiencies and decreased crop yields (Taylor, 1990; Kochian, 1995). Two different physiological strategies that have been identified that allow plants to tolerate Al<sup>3+</sup> (i) the exclusion of toxic Al<sup>3+</sup> from the root apex by releasing Al<sup>3+</sup>-chelating ligands such as organic acids and phosphate, or by releasing OH<sup>-</sup> to increase external pH; and (ii) the detoxification of Al<sup>3+</sup> once it has entered the cytoplasm by chelation and/or sequestration to less Al<sup>3+</sup>-sensitive compartments (Taylor, 1991; Delhaize and Ryan, 1995; Kochian, 1995; Ma, 2000). The extracellular chelation by organic acids,

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such as citrate and malate has been shown to be an important mechanism of aluminum tolerance (Cobbett, 2000). Malate and citrate have been identified as important Al-chelating ligands secreted by roots (Miyasaka *et al.*, 1991; Delhaize *et al.*, 1993; Pellet *et al.*, 1995; Li *et al.*, 2000; Ma, 2000; Ma *et al.*, 2000; Matsumoto, 2000). In addition to their potential role in Al exclusion, organic acids can also play a role in internal Al detoxification. However, evidence for an Al resistance mechanism involving internal detoxification and sequestration through a complexation reaction with organic acids is starting to emerge (Pineros *et al.*, 2005).

The aim of present study is to discuss the involvement of malate and citrate in Al sequestration and their protective role in maize under Al stress. The qualitative composition of the phospholipids and glycolipids in maize roots was also investigated.

## **Materials and Methods**

### *Plant Material and Seedling Growth*

Maize (*Zea mays* L.; var. LG 23/01) seeds (purchased from Limagrain, France) were surface-sterilized with 10% (v/v) H<sub>2</sub>O<sub>2</sub> for 20 min, rinsed thoroughly with distilled water, sown on filter paper saturated with distilled water and germinated at 24°C in the dark. Three days later, seedlings selected for uniform growth were transplanted into 12-l polyethylene containers (90 seedlings per container) containing an aerated complete nutrient solution for 4 days and then grown for an additional 10 days in 6-l flat polyethylene pots (12 plants per pot). Fourteen day old maize seedlings were transferred to control or Al-containing media added as Al(NO<sub>3</sub>)<sub>3</sub>·9H<sub>2</sub>O prepared from a 0.1 M stock solution to achieve a final Al concentrations of 100, 250, 500 and 1000 µM. The pH of control and treatment solutions was adjusted initially to 4.0 with HCl before adding seedlings and was monitored daily, thus disallowing precipitation of Al(OH)<sub>3</sub> (Kinraide and Parker, 1989). Plants were exposed to Al for short-term (1, 2, 4 and 8 h) and long-term (4 days). The compositions of the control and treatment solutions were modified as described elsewhere (Chaffai *et al.*, 2005). Plants were harvested at each time point and roots were washed three times with distilled water and then blotted dry on filter papers. The fresh weight (shoot plus root) was determined and frozen in liquid nitrogen. The Root Length (RL) of the longest roots of each treatment was measured 4 days after the Al treatment.

### *Enzymatic Assays of Malate and Citrate*

The malate and citrate concentrations were assayed enzymatically in protein extracts according to Delhaize *et al.* (1993). Plant tissues were ground in liquid N<sub>2</sub> and proteins were extracted with 20 mM Tris-HCl (pH 8.0) and 5 mM ascorbic acid (2 mL g<sup>-1</sup> tissue). After centrifuging at 25 000 g, 4°C, for 20 min, the supernatant was used for the enzymatic assay of malate and citrate. For malate determination, an aliquot of the supernatant was incubated with 2 mL of buffer (0.5 M glycine, 0.4 M hydrazine, pH 9.0) and 50 µL of 40 mM NAD. The reaction mixture was preincubated for 30 min to obtain a stable A<sub>340</sub> reading before the addition of 10 units of malate dehydrogenase (MDH, Sigma). The increase in A<sub>340</sub> due to the production of NADH was monitored and is directly proportional to the amount of malate in the sample. The level of malate was followed after 1, 2, 4 and 8 h and over a period of 4 days after the shift to Al-containing media. For citrate, an aliquot of the supernatant was incubated with 2 mL of buffer (100 mM Tris-HCl, pH 7.8), 60 µL of 20 mM NADH and 5 units of lactate dehydrogenase (LDH, Fluka)/malate dehydrogenase mixture. After a stable reading was obtained, 0.5 units (100 µL) of citrate lyase (Fluka), was added and the decline in A<sub>340</sub> due to oxidation of NADH was monitored. The decrease in NADH concentration is directly proportional to the amount of citrate in the sample.

#### *Determination of Enzyme Activities*

The malate dehydrogenase (MDH, EC 1.1.1.37) activity was determined in protein extracts according to Thorne *et al.* (1963). NADP-isocitrate dehydrogenase (NADP-ICDH, EC 1.1.1.42) was assayed according to the method of Udvardi *et al.* (1993). An aliquot of the root extract was incubated in 1.5 mL of 25 mM Tris-HCl (pH 7.4) and 100  $\mu$ L of 0.1 mM NADP. To the assay solution, 50  $\mu$ L of 2 mM DL-isocitrate (Sigma) was added to start the reaction. NADP-ICDH activity was measured as the rate of reduction of NADP, monitored at 340 nm for 1-2 min. The protein concentrations in the supernatant were quantified according to the Bradford (1976) assay using Bovine Serum Albumin (BSA) as standard.

#### *Lipid Extraction and TLC Analysis*

The lipids were extracted according to the method of Folch *et al.* (1957) modified by Bligh and Dyer (1959). The root tissues (0.5 g) were washed with boiling water (10 mL) for 10 min to denature phospholipases (Douce, 1964) and then ground in 15 mL of chloroform:methanol mixture (2:1, v/v). After 24 h at 4°C, the water of fixation was added to the homogenate and centrifuged 20 min at 3000 rpm. The lower chloroformic phase was aspirated and dried at 40°C using rotary evaporator (Büchi). The residue was immediately redissolved in benzene:ethanol (4:1, v/v). The lipids were analyzed by Thin Layer Chromatography (TLC) according to the method of Phillips and Privett (1979). The phospholipids were separated from total lipids by TLC using the solvent system  $\text{CHCl}_3$ :MeOH: $\text{NH}_4\text{OH}$ , 65:35:5 (v/v/v). After drying, the plates were pulverized with molybdenum blue reagent according to Dawson *et al.* (1986). The phosphates groups of phospholipids react with the molybdenum blue giving a blue color over a white background. All lipids appeared when the plate was heated for 30 min at 100°C. The TLC was also applied to separate the glycolipids from the total lipids. The thin layer was developed by a mixture of  $\text{CHCl}_3$ :MeOH: $\text{NH}_4\text{OH}$  2.5N, 60:35:8 (v/v/v). The plate was dried and pulverized with  $\alpha$ -naphthol reagent (10.5 mL  $\alpha$ -naphthol at 15% in ethanol, 6.5 mL of concentrated  $\text{H}_2\text{SO}_4$ , 40.5 mL ethanol and 4 mL  $\text{H}_2\text{O}$ ). After heating for 5 min at 100°C, the glycolipids appeared as brown spots.

#### *Statistical Analysis*

The significance of control versus Al-treated assays was analyzed using one way ANOVA at  $p < 0.05$ .

## **Results**

#### *Al Stress Symptoms in Maize*

Based on the observations showing the morphology of roots exposed to Al, visual toxicity symptoms were observed at all Al concentrations used (100, 250, 500 and 1000  $\mu$ M). Symptoms of Al injury included stubby appearance, a large number of short laterals close to the apex of the main root axis and deformed root apices (Fig. 1). These symptoms were observed as early as 24 h after Al exposure and were most pronounced at the highest Al concentration (1000  $\mu$ M). This Al concentration caused the maximal inhibitory effect on root growth (as indicated by the root length measurement) to about 70% within 4 days of Al exposure (Fig. 2). However, concentrations lower than 100  $\mu$ M (20 and 50  $\mu$ M) also caused some growth inhibition and root swelling (data not shown).

#### *Effect of Short-term (1, 2, 4 and 8 h) Al Treatment on Malate Content and MDH Activity in Roots*

Malate content in roots was assessed in short-term (1, 2, 4 and 8 h) experiments. One hour of Al-treatment proved to have dramatic effects on malate content, but this early impact was limited to only 100 (from  $142.41 \pm 12.58$  to  $35.22 \pm 0.83$  nmol  $\text{g}^{-1}$  f. wt,  $p < 0.01$ ) and 250  $\mu$ M (from  $142.41 \pm 12.58$  to  $61.67 \pm 14.19$  nmol  $\text{g}^{-1}$  f. wt,  $p < 0.05$ ) Al treatments. In contrast, 1000  $\mu$ M Al induced significant



Fig. 1: Manifestation of aluminum stress symptoms in maize roots. Seedlings were cultivated at increasing levels of Al (0, 100, 250, 500 and 1000  $\mu\text{M}$ ) in low-ionic strength nutrient solutions (pH 4.0) for 4 days

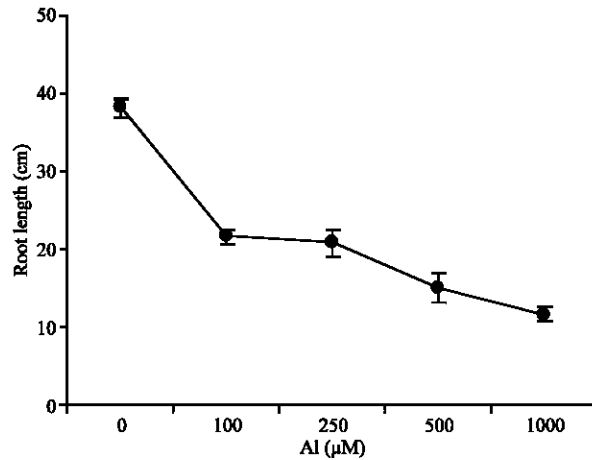


Fig. 2: Root length of main axis of maize roots measured after 4 days of Al exposure. Values are means $\pm$ SE of 6-8 plants

increase of the malate content (from  $142.41 \pm 12.58$  to  $227.50 \pm 37.74$   $\text{nmol g}^{-1}$  f. wt,  $p < 0.05$ ), but treatment with 500  $\mu\text{M}$  Al had no effect (Fig. 3A). After 2 h of Al treatment, the most prominent effect was observed at 250 and 500  $\mu\text{M}$  Al, while no significant changes were detected at 100 and 1000  $\mu\text{M}$  Al (Fig. 3A). The decreases in malate were by 58 and 65%, respectively at 250 and 500  $\mu\text{M}$  Al. As shown in Fig. 3A, at 4 h, the malate content decreased by approximately 63% ( $p < 0.05$ ) at Al concentrations of 100, 500 and 1000  $\mu\text{M}$ , whereas 250  $\mu\text{M}$  Al had no significant effect.

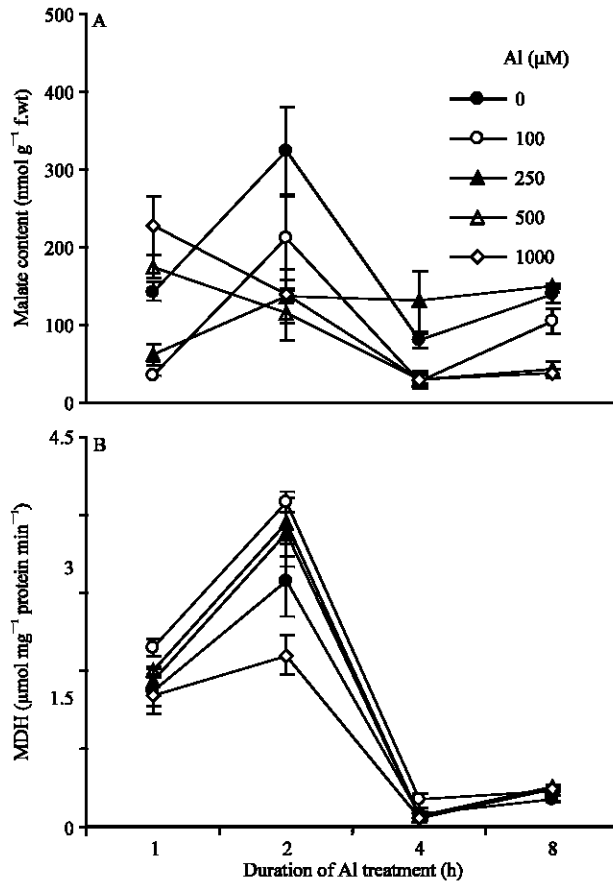


Fig. 3: Kinetic patterns of malate content (A) and activity of malate dehydrogenase (B) obtained from roots of maize. Values are means±SE of 4-5 plants

After 8 h of Al treatment, the most dramatic decrease was observed at 500 and 1000 μM Al, by approximately 71% (Fig. 3A).

In addition, during 1 h of Al exposure, MDH enzyme activity was increased by 32% at 100 μM Al, but no changes are evident at other Al treatments (Fig. 3B). However, there was significant difference between 100 and 1000 μM treatments. Examination of the MDH activity at 2-4 h of Al treatment did not reveal any changes, but differences were found between treatments (100, 250 or 500 μM Al and 1000 μM Al at 2 h; 100 and 500 or 1000 μM Al at 4 h). An effect of the short-term Al treatment was also found at 8 h. The results indicated increases in the MDH activity (Fig. 3B).

*Effect of Long-term (4-d) Al Treatment on Organic Acid Contents and Enzyme Activities*

After 4 days of Al treatment, malate levels in roots were shifted down especially at higher Al concentration (1000 μM), compared with control plants. There was a decrease in the malate content by about 34-40% at Al concentrations ranging from 100 to 500 μM Al and by 56% at 1000 μM Al (Table 1). Moreover, long-term exposure to Al stress (4 days) revealed that malate content in shoots was markedly affected when high Al concentrations (500 and 1000 μM) were applied and the decreases were by 61 and 42%, respectively (Table 1). However, the citrate content was not significantly altered in roots after Al treatment (Table 1). In shoots, the citrate levels were not

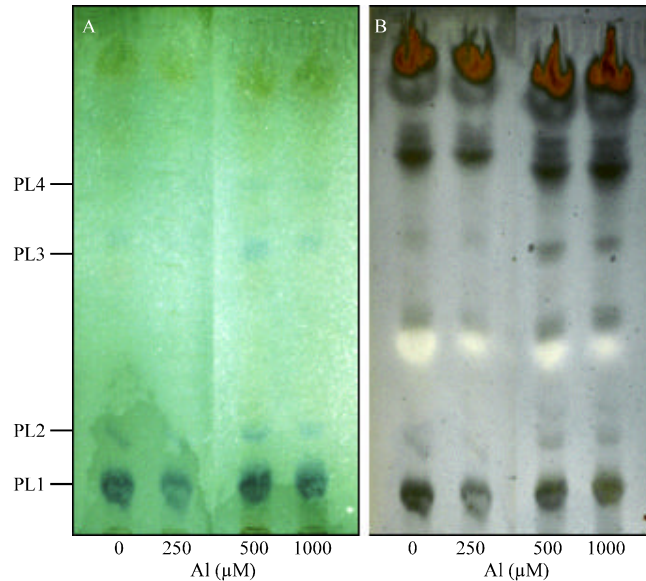


Fig. 4: Lipid composition of maize roots under Al stress. A) Phospholipids were separated from total lipids by thin layer chromatography and visualized by pulverization of the plate with molybdenum blue reagent. B) Total lipids appeared after heating the plate for 30 min at 100°C

Table 1: Root and shoot organic acid (malate and citrate) content in maize. Seedlings were cultivated at increasing Al concentrations (0-1000 μM) in low-ionic strength nutrient solutions (pH 4.0) for 4 days

Al (μM)	Root		Shoot		Root malate: citrate ratio	Shoot malate: citrate ratio
	Malate	Citrate	Malate	Citrate		
0	728.63±65.98 <sup>a</sup>	154.09±5.73 <sup>a</sup>	617.21±29.03 <sup>a</sup>	971.53±51.03 <sup>a</sup>	4.73	0.64
100	435.42±15.77 <sup>bc</sup>	141.58±6.04 <sup>a</sup>	465.09±64.46 <sup>a</sup>	863.39±51.51 <sup>a</sup>	3.08	0.54
250	503.06±30.80 <sup>b</sup>	163.94±7.08 <sup>a</sup>	525.59±52.29 <sup>a</sup>	786.03±16.54 <sup>a</sup>	3.07	0.67
500	513.48±99.43 <sup>b</sup>	153.02±0.10 <sup>a</sup>	243.39±20.81 <sup>b</sup>	771.23±35.53 <sup>a</sup>	3.36	0.32
1000	324.02±12.96 <sup>d</sup>	149.39±14.98 <sup>a</sup>	359.50±81.17 <sup>b</sup>	1074.18±75.21 <sup>b</sup>	2.17	0.33

Values are means±SE of 4-5 plants; Different letter within the same column indicate significant differences ( $p < 0.05$ ) according to one way ANOVA

significantly different from the control to the Al concentration of 500 μM. However, at 1000 μM, the citrate level increased by 23% ( $p < 0.05$ ) (Table 1). There were also significant differences between Al treatments (100, 250, or 500 and 1000 μM Al). The Al treatment also decreased the malate:citrate molar ratio in roots (from 4.73 to 2.17) and shoots (from 0.64 to 0.33) (Table 1).

Present results showed that the exposure to Al decreased by about 38% the activity of MDH in roots, but it was not significantly affected in shoots (Table 2). NADP-ICDH activity was not affected by the Al treatment in both roots and shoots (Table 2).

#### Qualitative Changes in Lipid Composition in Roots

The TLC technique was used to identify the major changes in phospholipid and glycolipid compounds induced by the Al stress. After spraying with blue molybdenum reagent, TLC detected four spots of phospholipids in root extracts, which are designated PL1, PL2, PL3 and PL4 (Fig. 4A). The lipids exhibited distinct profiles between the control and the Al-treated roots and the spot which exhibit more color intensity induced by Al is PL3 with an  $R_f$  value of 0.56. All the lipid compounds

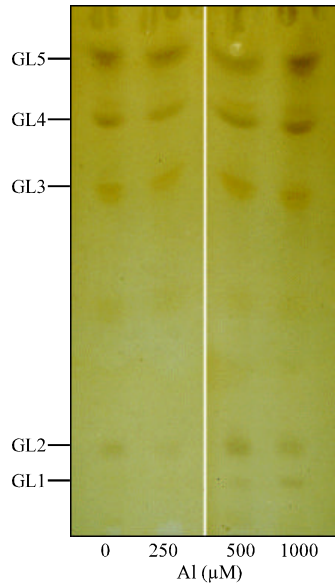


Fig. 5: Glycolipid composition of maize roots under Al stress. Glycolipids were separated from total lipids by thin layer chromatography and the plate was dried and pulverized with  $\alpha$ -naphthol reagent

Table 2: Root and shoot malate dehydrogenase (MDH) and NADP-isocitrate dehydrogenase (NADP-ICDH) activities in maize. The maize seedlings were exposed to 0, 250 and 1000  $\mu\text{M}$   $\text{Al}(\text{NO}_3)_3$  solution (pH 4.0) for 4 days. Values are means $\pm$ SE of 4-5 plants

Al ( $\mu\text{M}$ )	Root		Shoot	
	MDH	NADP <sup>+</sup> -ICDH	MDH	NADP <sup>+</sup> -ICDH
	$\mu\text{mol mg}^{-1} \text{protein min}^{-1}$			
0	0.78 $\pm$ 0.06 <sup>a</sup>	0.18 $\pm$ 0.04 <sup>a</sup>	0.16 $\pm$ 0.01 <sup>a</sup>	1.07 $\pm$ 0.32 <sup>a</sup>
250	0.46 $\pm$ 0.03 <sup>b</sup>	0.24 $\pm$ 0.08 <sup>a</sup>	0.12 $\pm$ 0.01 <sup>a</sup>	0.62 $\pm$ 0.20 <sup>a</sup>
1000	0.52 $\pm$ 0.08 <sup>b</sup>	0.19 $\pm$ 0.05 <sup>a</sup>	0.12 $\pm$ 0.02 <sup>a</sup>	0.63 $\pm$ 0.19 <sup>a</sup>

Different letter within the same column indicate significant differences ( $p < 0.05$ ) according to one way ANOVA were revealed after heating the plates at 100°C for 30 min. As shown in Fig. 4B, the intensity of spots was more prominently increased by Al when compared by the less color change in control roots. Thin layer chromatography of glycolipids revealed five spots (dark brown) after spraying with  $\alpha$ -naphthol reagent and heating the plates at 100°C for 5 min (Fig. 5). These spots were designated: GL1, GL2, GL3, GL4 and GL5. The most prominent effect of the Al treatment was observed in the spot GL2 with  $R_f$  of 0.10.

## Discussion

We first analyzed Al toxicity symptoms in maize plants in comparison of untreated plants. Most studies have focused on Al toxicity symptoms such as hematoxylin staining, lateral swelling of root apices and callose synthesis to quantify and compare the Al resistance levels (Wenzl *et al.*, 2001). It has been established that with extended Al exposure (days), the roots thicken and become stubby and darker in color (Ryan *et al.*, 1992) as shown in Fig. 1. Similar effects of Al were also reported for ruzigrass (*Brachiaria ruziziensis*) roots, particularly in root apices where major toxicity symptoms were observed (Wenzl *et al.*, 2001). This supports the hypothesis that the root apex is the primary site of Al toxicity in maize. Ryan *et al.* (1993) have shown that in maize, root elongation is inhibited



only when apices are exposed to Al, whereas exposing the remainder of the root does not inhibit elongation. A further indication of the damaging effect of Al is supported by results of experiments indicating that severe morphological distortions in the root-elongation zone, as shown by swollen cells within the inner cortex and large holes in the root surface produced by the loss of both epidermal and outer cortical groups of cells in primary roots of maize after 12 h of Al-exposure (Blancaflor *et al.*, 1998). We found that severe inhibition of root length accompanied the morphological changes in maize roots (Fig. 1 and 2). The inhibition of root elongation is an early response, which has been shown to be the most dramatic physiological symptom of Al phytotoxicity (Ryan *et al.*, 1992). The inhibition of root elongation was observed even as early as 1 h after Al exposure (Pellet *et al.*, 1996). These data suggest that Al interact actively with dividing and expanding cells in the root meristem (Yamamoto *et al.*, 2001). In addition, alterations in the cytoskeleton have been associated with Al-induced growth inhibition and morphological changes in primary roots of maize (Blancaflor *et al.*, 1998). Previous investigators have concluded that the impact of Al<sup>3+</sup> ions, the most toxic mononuclear Al species, inhibit root elongation by injuring the root apex, particularly the distal part of the transition zone (DTZ) (Ryan *et al.*, 1993; Kinraide, 1997; Sivaguru and Horst, 1998). Kollmeier *et al.* (2000) suggested that a transduction of the Al signal from the site of perception (DTZ) to its site of action, the Elongation Zone (EZ) through Al-induced alterations in indole-3-acetic acid fluxes in the root apex, finally leading to inhibited root elongation (Kollmeier *et al.*, 2000). Based on the previous characterization of Al toxicity in maize roots, the distal part of the transition zone (DTZ) have been identified as the most Al-sensitive apical root zone in the Al-sensitive maize cv Lixis (Sivaguru and Horst, 1998). A follow-up work on the same maize cultivar has been presented on this important aspect of Al rhizotoxicity (Sivaguru *et al.*, 1999). There is circumstantial evidence that the DTZ represents a potential target root zone and plays a major role in the expression of Al toxicity in the maize root apex (Sivaguru *et al.*, 1999).

In this study, we have investigated the malate and citrate content in maize and determined whether or not the level of these organic acids could be involved in the defense mechanism. The malate content was evaluated in roots over a short (1, 2, 4 and 8 h) and long time period (4 days). The exposure of the maize seedlings to Al generally caused a reduction in malate content. Similar changes have been observed in malate content from an Al-tolerant maize (cv Cateto-Colombia) plants in response to exposure to increasing levels of Al, but this effect is observed in root tips (Pineros *et al.*, 2002). In Al-resistant wheat (cv Atlas), the malate content in root apex rapidly decreased after addition of Al and the lowest level was 0.2 nmol apex<sup>-1</sup> after 2 h Al treatment (Osawa and Matsumoto, 2001). Present results contrasts with the Al-induced increase in root malate content reported for another Al-tolerant maize cultivar (SA3) (Pellet *et al.*, 1995). In addition, the internal malate content was not changed by the exposure to Al during a short time in wheat (Delhaize *et al.*, 1993) and in Al-resistant and Al-sensitive triticale seedlings (Hayes and Ma, 2003). From these results, we conclude that the strong Al-induced decrease of the concentrations of malate did not support a role for this carboxylic acid in internal detoxification and sequestration of Al. This conclusion is supported by our previous study in maize (Chaffai and El Ferjani, 2005). It also interesting that the citrate level did not change significantly in roots during Al exposure, suggesting the hypothesis that citrate production was maintained under the stress. Therefore, the citrate levels could be implicated in an internal or external Al tolerance mechanism in maize. There could be an Al exclusion tolerance mechanism based on citrate exudation and a second internal tolerance mechanism using internal levels of Al-chelating citrate. Al-activated citrate release has been documented in roots of Al-tolerant varieties of maize (*Zea mays*), rye (*Secale cereale*), soybean (*Glycine max*), *Cassia tora* and Triticale (Pellet *et al.*, 1995; Ma *et al.*, 1997c, 2000; Li *et al.*, 2000; Silva *et al.*, 2001). A significant correlation was found between the efflux

of organic anions in response to Al and the levels of organic content in roots. The rapid Al activation of citrate release was accompanied by a slower, Al-inducible increase in root citrate content (Pineros *et al.*, 2002). Moreover, increases in root tip citrate content have also been reported in Al-tolerant rye and soybean cultivars where Al stimulation of citrate release also takes place (Li *et al.*, 2000; Silva *et al.*, 2001). Earlier studies in maize from our laboratory as well as from other researchers indicated that the citrate release induced by Al was considered to be the most important tolerance mechanism in maize (Pellet *et al.*, 1995; Jorge and Arruda, 1997; Ishikawa *et al.*, 2000; Kidd *et al.*, 2001; Kollmeier *et al.*, 2001; Pineros *et al.*, 2002; Mariano and Keltjens, 2003; Chaffai and El Ferjani, 2005). However, Al-activated malate exudation was the most extensively characterized Al-exclusion mechanism in wheat (Pineros *et al.*, 2005). This role is substantiated by the fact that root apices showed enhanced malate synthesis during the initial 2 h of Al exposure which was associated with an active efflux of malate (Delhaize *et al.*, 1993b). Consequently, it is tempting to speculate that organic secretion is a principle mechanism of tolerance in maize and other crop species. It was hypothesized that crop plants have evolved resistance mechanisms that enable them to tolerate toxic levels of Al in acid soils based on chelation and exclusion of extracellular Al via Al-activated root organic acid release (Ma, 2000; Ma *et al.*, 2001; Ryan *et al.*, 2001; Kochian *et al.*, 2004). Watanabe and Osaki (2002) provide powerful arguments that for most plant species and especially crop species, Al tolerance is interpreted as the ability to exclude Al and are called Al excluders. Al exclusion in excluder species may involve some strong internal mechanisms in addition to the exudation of organic acid from roots (Watanabe and Osaki, 2002). By contrast, hyperaccumulator plants have developed mechanisms of internal Al inactivation and are called Al accumulators (Watanabe and Osaki, 2002). It is well known that some highly tolerant species such buckwheat (*Fagopyrum esculentum*) and hydrangea (*Hydrangea macrophylla*) can accumulate high concentrations of Al in leaves (Ma *et al.*, 2001) and organic acids has been implicated in internal Al tolerance mechanism in these species (Ma *et al.*, 1997a, b). This is convincingly argued by the high concentrations of citrate found in some acidophilus, Al resistant species *Fagopyrum esculentum*, *Cassia tora* and *Hydrangea* (Barceló and Poschenrieder, 2002). These species can tolerate Al by translocating it to the shoot tissue as an Al-organic acid complex and subsequently storing the Al-organic acid complex in the vacuole of leaf cells (Ma *et al.*, 1998; Ma and Hiradate, 2000; Ma *et al.*, 2001). Complexes of Al-citrate (1:1) in *Hydrangea* leaves have been identified (Ma *et al.*, 1997a).

Present study showed alterations in the phospholipid and glycolipid composition (Fig. 4 and 5), which may change membrane permeability and, consequently net ion uptake (Cumming and Taylor, 1990). Membrane lipids play a fundamental role in regulating permeability (Schuler *et al.*, 1991), fluidity (Mikami and Murata, 2003), carrier-mediated transport (Deutike and Haest, 1987) and activity of membrane-bound enzymes (Cooke and Burden, 1990). These changes in response to Al could be an adaptive response to heavy metals (Guschina and Harwood, 2002).

In conclusion, present results are not consistent with a significant role for malate in internal Al sequestration in maize. But, although root citrate release may play an important role in maize Al resistance, it is not clearly the only or the main resistance mechanism operating. Therefore, effective mechanism based on internal detoxification of Al by chelating ligands such as citrate could contribute to the tolerance to Al stress.

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