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Alteration of the Profile of Organic Acid Content and Exudation under Aluminum Stress in Maize (*Zea mays* L.)

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Abstract: The purpose of the present study was to contribute to the literature on the role of organic acids in Al tolerance in maize (*Zea mays* L.). Treatment with increasing Al concentrations from 100 to 1000 μM for 4 days Al caused substantial inhibition of lateral roots, as well as severe alterations on root apices. In roots, the malate content was decreased significantly by Al. However, the citrate, lactate and total organic acids were not affected by Al. In shoots, the citrate, tartarate and total organic acids were decreased significantly by 100, 250 and 500 μM Al. However, the succinate was increased by 500 and 1000 μM Al, while that of lactate increased only by 1000 μM Al. Only the highest Al concentration 1000 μM induced exudation of citrate. It is shown that the enhanced citrate exudation induced by Al support the concept that organic acid exudation may be an effective strategy to cope with soil acidity and Al toxicity in maize.

Key words: Aluminum tolerance, aluminum toxicity, citrate exudation, organic acids, *Zea mays* L.

INTRODUCTION

Aluminum toxicity is one of the most important growth-inhibiting factors on acid soils. The inhibition effects induced by Al have been discussed by a large body of the literature. There is evidence that Al can reduce both root and shoot growth and consequently crop yield[1]. The roots exhibit the greater symptoms of Al toxicity. Al impairment effects occur in lateral roots that become thick and coralloid in appearance[2,3]. Aluminum affected numerous processes which result in alteration of phosphate availability[4], interference with Ca metabolism[5] and inhibition of root cell division[6-8] or/and root cell elongation[9]. The Al tolerance mechanism operates through two different and complementary: the exclusion and internal mechanisms[10]. The exclusion mechanisms prevent certain metals from crossing the plasma membrane, from entering the symplasm and reaching sensitive intracellular sites. These mechanisms include the immobilization at the cell wall, the selective permeability of the plasma membrane, the building of a plant-induced pH barrier in the rhizosphere, the exudation of chelate ligands, exudation of phosphate and Al efflux. By contrast, the internal mechanisms restrict the accumulation of metals inside the symplasm by immobilization, compartmentalization, or detoxication. They include the chelation in the cytosol by organic acids, proteins, or other organic ligands, the compartmentalization in the vacuole and the evolution of the Al tolerant enzyme activity[10]. One of strategies of plants to prevent Al toxicity is complexation of Al by organic acids in the rhizosphere or within the cell[11-16]. The organic acids assume significant functions in soils such as metal detoxification and increasing nutrients availability[15]. Organic acid exudation in the rhizosphere plays a protective role in preventing Al toxicity. These ligands are able to form stable complexes with Al, thereby reducing its activity[13,14]. This process can be efficient for Al exclusion mechanism[17]. Arp and Strucel[18] have demonstrated that Al-oxalate complex is less toxic than the inorganic forms of Al. In wheat, the Al tolerance is correlated with malate efflux from the apical zone of the root, becoming 5 to 20 times more resistant to Al[19]. Likewise, wheat cultivars that differ in Al tolerance showed differences in the efflux of malate[20]. It is also shown that the addition of citrate or succinate in the nutrient solution re-establishes the growth of *Triticum aestivum* in the presence of Al[21]. Various organic acids are excreted in response to Al stress, malate in wheat[19,22,23], citrate in maize and *Cassia tora*[24-27] and oxalate in buckwheat and taro[16,24,28,29]. Only few studies presented a comprehensive coverage on the mechanisms of aluminum tolerance and resistance in terms of changes of organic acid content in *Zea mays* L. seedlings.

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Although, however many of these were focused on the differential Al tolerance between Al-sensitive and Al-resistant genotypes. However, the role of organic acids in internal tolerance mechanism is yet not completely elucidated. The present study attempts a more understanding of the role of organic acids in Al tolerance in maize (*Zea mays* L.) and provides contribution to the literature on the central role of citrate in Al exclusion mechanism.

MATERIALS AND METHODS

Growth conditions and Al treatments: Maize (*Zea mays* L.; Var. LG 23/01) seeds were surface sterilized with 10% (v/v) H_2O_2 for 20 min, rinsed many times with tap water and germinated on filter paper moistened with distilled water in the dark at 25°C for 3 days. The germinated seedlings were transferred to 12l solutions for 4 days and subsequently placed homogenously into identical solutions in 6l plastic pots (12 plants each) for 10 days. The hydroponics cultures were continuously aerated and held in conditioned room with a 16 h light/8 h dark photoperiod, under 250 μmol m^{-2} s^{-2} irradiance provided by mercury lamps, under light/ dark temperatures 25/22°C and relative humidity (RH) 65%. The composition of nutrient solutions (pH 5.7) was as follows: (in mM) 2 KNO_3, 2.5 $Ca(NO_3)_2$, 1 KH_2PO_4, 1 $MgSO_4$; (in μM) 50 as Fe-K-EDTA complex, 30 B as H_3BO_4, 10 Mn as $MnSO_4.H_2O$, 1 Cu as Cu $SO_4.5H_2O$, 1 Zn as $ZnSO_4.7H_2O$ and 0.2 Mo as $(NH_4)_6Mo_7O_{24}.4H_2O$. For treatment purpose, the seedlings were transferred to freshly prepared nutrient solution (pH 4.0). The seedlings were treated for 4 days at various Al concentrations 100, 250, 500 and 1000 μM Al supplied as $Al(NO_3)_3.9H_2O$. At harvest, the roots were washed three times with distilled water and the fresh weight was determined and frozen in liquid nitrogen. The root cell wall was isolated by 1% (v/v) Triton X-100 according to Cathala *et al.*[30]. For the determination of Al content, the plant material was oven-dried at 70°C, then extracted by nitric acid/ perchloric acid mixture (4:1, v/v). The Al content in roots, root cell wall and shoots was determined by an atomic absorption spectrophotometer (Perkin Elmer-model 2380).

Organic acid extraction and HPLC analysis: Organic acids in roots and shoots were extracted with 0.01 N ortho-phosphoric acid (4°C). The extracts were immediately centrifuged at 20 000 g for 10 min at 4°C and the resulted supernatants were preserved at -20°C before HPLC analysis. In experiments for the determination of organic acids in root exudates, the nutrient solutions were prealably sterilized. Five milliliter aliquot of nutrient solutions that contained root exudates was evaporated. The residue was diluted in 2 mL of distilled water adjusted to pH 2.1 by perchloric acid according to Yang *et al.*[27]. The samples were subsequently diluted to 1:10 before HPLC analysis. The High Performance Liquid Chromatography (HPLC) apparatus used was a HP Model 1100 series including a 4 solvent delivery system, a 20 μL loop and a sample injector. The HPLC system is also equipped with a Variable Wavelength Detector (VWD). The signals from the detector were recorded with HP Chemstation 4.0 data which integrated peak area and was programmed with external standards. Organic acids were separated on a Dupont Zorbax SAX-5 μm column (250× 4.6 mm, HP) at 25°C at a flow rate of 1 mL min^{-1} and under constant pressure of 87 bars. The mobile phase consisted of 0.2 M potassium dihydrogen phosphate adjusted to pH 3.0 with concentrated phosphoric acid according to Billingsley *et al.*[31] and then filtered through 0.45 μm filters. The organic acids in the root and shoot extracts were detected by absorbance at 210 nm (A210 nm) specific for the carboxyl groups. The organic acids were identified by comparing their retention time (Rt) with those of authentic standards and quantified on the basis of the A210 nm standard calibration.

Determination of MDH activity: Frozen material was homogenized in 20 mM Tris-HCl buffer (pH 8.0) containing 5 mM ascorbate. The homogenate was centrifuged at 25000 g for 20 min (4°C). The malate dehydrogenase (MDH, EC 1.1.1.37) activity was determined in protein extracts according to Thorne *et al.*[32]. The assay medium consisted of 0.1 M sodium pyrophosphate (pH 9.0), 0.033 M Na-L-malate and 0.41 mM NAD^+ in a total volume of 1 mL. The reaction was initiated after addition of 100 μM of protein extract. The absorbance was monitored at 340 nm and converted to μmol using molar extinction coefficient of 6.22 103 mol^{-1} dm^3 cm^{-1}. The protein was determined by the Bradford method[33] using BSA as a standard.

RESULTS

Al toxicity symptoms and effect on growth: Aluminum toxicity symptoms occurred early on roots after Al treatment. The roots treated with the highest Al concentration (1000 μM) exhibited more damages than those treated by lower Al concentrations from 100 to 500 μM. The lateral roots showed an abnormal branching, giving rise to nodules; however, the root apices were hypertrophied, curved and brownish (Fig. 1). Plant growth, as expressed by fresh weight, showed that the impact of Al treatment was more marked in roots than in

Table 1: Effect of Al on organic acid concentrations in maize (*Zea mays* L.) roots. The seedlings were grown in nutrient solutions at increasing Al concentrations (0, 100, 250, 500 and 1000 mM) for 4 days (pH 4.0). Values are means±SE (n = 5) of five separate experiments. One Way ANOVA is performed for comparison between control and treated plants. Different letters within the same column indicate significant differences. Asteriks show statistically different means between -Al and +Al treatment: *, p<0.05; **, p<0.01

	Organic acid concentrations in roots (μmol g FW)$^{-1}$					
Al (μM)	Succinate	Malate	Citrate	Tartarate	Lactate	Total
0	8.81±1.78a	153.24±19.09a	21.65±2.39a	764.22±25.32a	1.11±0.21a	901.68±14.07a
100	12.19±1.02a	68.01±15.81b*	26.49±2.97a	579.95±88.67b*	1.67±0.16a	754.63±85.92a
250	13.55±1.95a	63.09±10.59b**	24.51±0.42a	689.47±28.18a	1.22±0.22a	783.32±49.31a
500	18.80±6.10a	53.47±7.90b**	20.51±3.59a	643.30±10.59	2.11±0.11a	740.57±15.49a
1000	21.17±2.62b*	54.14±11.78b**	22.48±1.09a	646.90±35.84	1.55±0.33a	783.58±25.08a

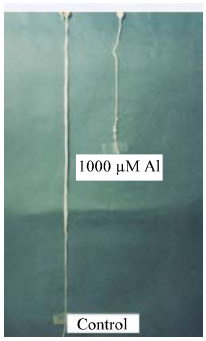

Fig. 1: Aluminum injuries at lateral roots and root apice of maize seedling treated for 4 days with 1000 μM Al

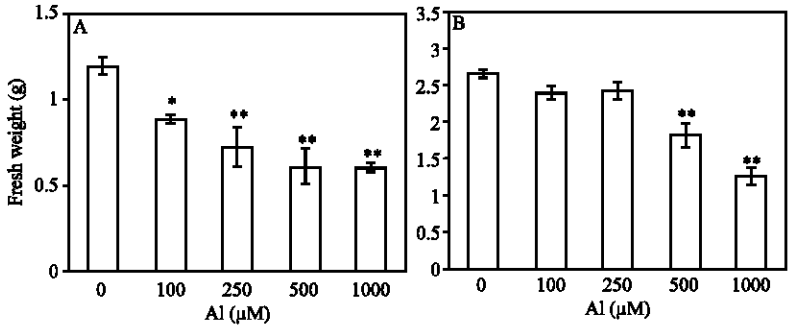

Fig. 2: Effect of aluminum on (A) root and (B) shoot fresh weight in maize (*Zea mays* L.). The seedlings were grown in nutrient solutions at increasing Al concentrations 0, 1 00, 250, 500 and 1000 μM for 4 days (pH 4.0). Values represent means±SE (n = 6) of six separate experiments. One Way ANOVA was performed for comparison between control and treated plants. The histogram bars represent the SEM. Asteriks show statistically different means between -Al and +Al treatment: *, p<0.05; **, p<0.01

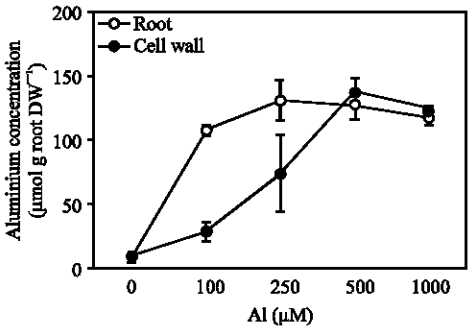

Fig. 3: Aluminum (Al) content in roots and root cell wall of maize (*Zea mays* L.). The seedlings were grown in nutrient solutions at increasing Al concentrations 0, 100, 250, 500 and 1000 μM for 4 days (pH 4.0). Values represent means±SE (n = 6) of six separate experiments

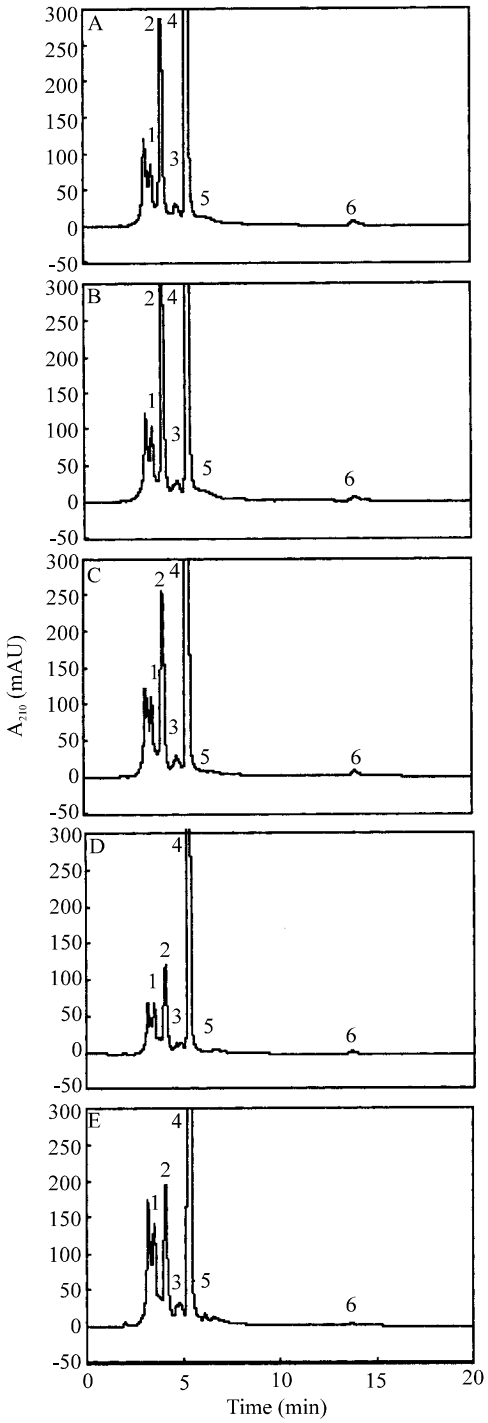

Fig. 4: HPLC chromatograms of organic acids in root extracts of maize (*Zea mays* L.). The seedlings were grown in nutrient solutions at increasing Al concentrations (A) 0; (B) 100; © 250; (D) 500 and (E) 1000 μM for 4 days (pH 4.0). The organic acids were separated on a 250 x 4.6 mm i.d. HP Zorbax SAX a strong anion exchange column. The mobile phase consisted of 0.2 M KH_2PO_4 (pH 3.0) at a flow rate of 1 mL min^{-1} and at 25°C. The elution peaks: 1, succinate; 2, malate; 3, citrate; 4, tartarate; 5, lactate; 6, unknown

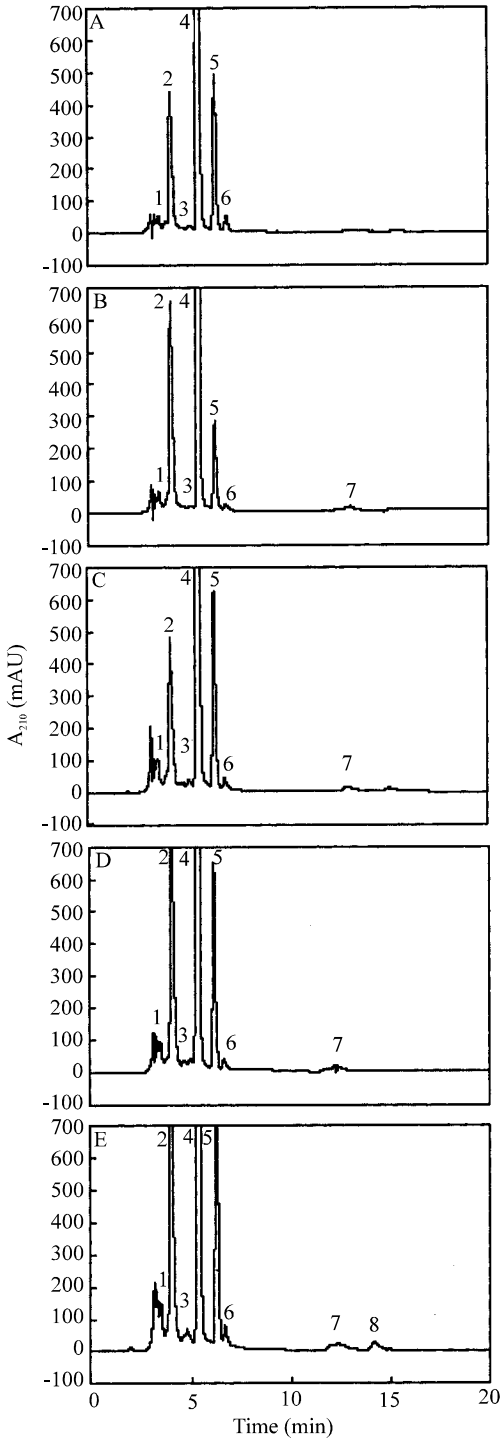

Fig. 5: HPLC chromatograms of organic acids in shoot extracts of maize (*Zea mays* L.). The seedlings are grown in nutrient solutions at increasing Al concentrations (A) 0; (B) 100; © 250; (D) 500 and (E) 1000 μM for 4 days (pH 4.0). The organic acids separation was isocratic on a 250 x 4.6 mm i.d. HP Zorbax SAX, a strong anion exchange column. The mobile phase consisted of 0.2 M KH_2PO_4 (pH 3.0) at a flow rate of 1mL min^{-1} and at 25°C. The elution peaks: 1, succinate; 2, malate; 3, citrate; 4, tartarate; 5, lactate; 6, unknown; 7, salicylate; 8, unknown

Table 2: Effects of Al on organic acid concentrations in maize (*Zea mays* L.) shoots. The seedlings were grown in nutrient solutions at increasing Al concentrations (0, 100, 250, 500 and 1000 mM) for 4 days (pH 4.0). Values represent means±SE (n = 5) of five separate experiments. One Way ANOVA is performed for comparison between control and treated plants. Different letters within the same column indicate significant differences. Asteriks show statistically different means between -Al and +Al treatment: *, p<0.05; **, p<0.01; ***, p<0.001

	Organic acid concentrations in roots (μmol g FW)$^{-1}$					
Al (μM)	Succinate	Malate	Citrate	Tartarate	Lactate	Total
0	2.96±0.68a	246.46±38.03a	34.04±1.67a	742.70±7.73a	197.82±17.10a	1200.64±26.09a
100	8.98±1.19ab	181.58±11.41ab	29.04±0.52b*	637.84±31.65b*	143.21±29.75a	951.78±76.72bc*
250	10.92±1.61ab	127.89±5.89b*	21.81±0.36c***	591.34±21.39b**	174.18±34.30a	839.42±38.40b**
500	12.70±3.81bc*	275.54±36.17ac	24.98±0.68c**	637.84±21.05b*	158.64±31.19a	849.04±52.24b*
1000	19.64±1.61c**	282.55±25.88ac	30.39±1.04ab	622.12±4.53b*	320.94±11.32b*	1065.65±55.63ac

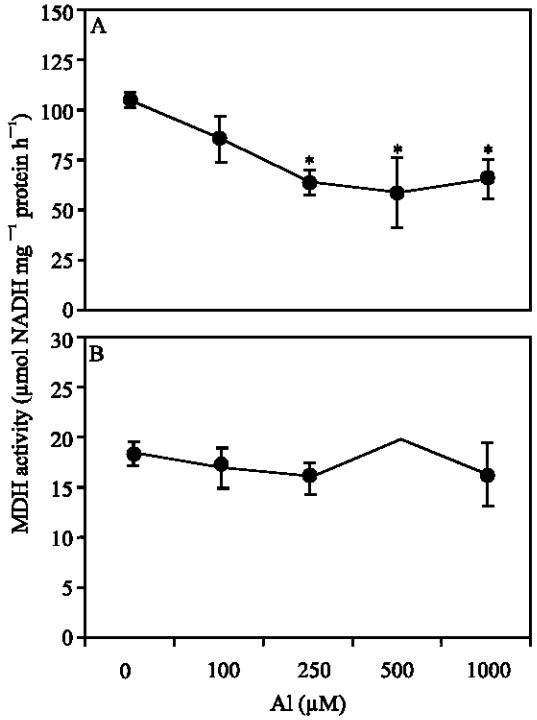

Fig. 6: Effect of Al treatment on MDH activity in (A) roots and (B) shoots of maize (*Zea mays* L.). Seedlings were treated with increasing Al concentrations: 0, 100, 250, 500 and 1000 μM (pH 4.0) for 4 days. Values are means±SE (n = 6) of six independent experiments. Asteriks show statistically different means between - Al and +Al treatment: *, p<0.05

shoots (Fig. 2). The root fresh weight was substantially decreased by Al treatments. The percentage of reduction varied from 25.8 to 50%. However, treatment by 500 and 1000 μM Al decreased significantly shoot fresh weight, respectively by 31.6 and 50% (Fig. 2).

Al content in roots and shoots: The total Al content in the roots of treated seedlings did not differ significantly after 4 day of Al treatment (Fig. 3). However, the Al content in the cell wall which showed different pattern of accumulation, increased substantially only in seedlings treated with 500 and 1000 μM Al (Fig. 3). No significant amount of Al was detected in shoots.

Effects of Al on organic acid concentrations in roots: The HPLC chromatograms of organic acids in control roots showed 5 peaks identified by comparing their retention times (Rt) to those of known standards: succinate (Rt 3.487), malate (Rt 4.002), citrate (Rt 4.757), tartarate (Rt 5.298) and lactate (Rt 6.121) (Fig. 4). An unidentified organic acid was detected with the a Rt of 13.961 (peak 6). The chromatograms of Al-treated roots show the same peaks as in control. The root content of total organic acids, citrate and lactate were not affected by Al stress (Table 1). By contrast, Al stress affected the malate content in roots causing a substantial decrease. The percentage of reduction showed similar values, on average 60%. However, the tartarate content decreased significantly only by 100 μM Al. The Al treatment did not induce a significant change in succinate content in seedlings treated with Al ranging from 100 to 500 μM. However, treatment with 1000 μM Al caused a 2.4-fold increase in this content compared to the control (Table 1).

Effect of Al on organic acid concentrations in shoots: Chromatogram of organic acids in control shoots showed 6 peaks, among which 5 were identified to represent succinate (Rt 3.651), malate (Rt 4.054), citrate (Rt 4.630), tartarate (Rt 5.346) and lactate (Rt 6.201) (Fig. 5). A non identified peak was detected (peaks 6) with Rt of 6.891. However, chromatograms of treated shoots showed the same peaks as in the control but exhibited two other peaks, peak 7 corresponding to salicylic acid (Rt 12.342) and a non identified compound (peak 8, Rt 15.463). The latter was more pronounced in the chromatogram of organic acids seedlings treated with 1000 μM Al. The shoot content of total organic acids, citrate and tartarate was significantly decreased in seedlings treated with Al ranging from 100 to 500 μM Al. However, the tartarate content was only affected with 1000 μM Al (Table 2). Furthermore, the Al treatment decreased malate content only at 250 μM Al without any significant effect for other

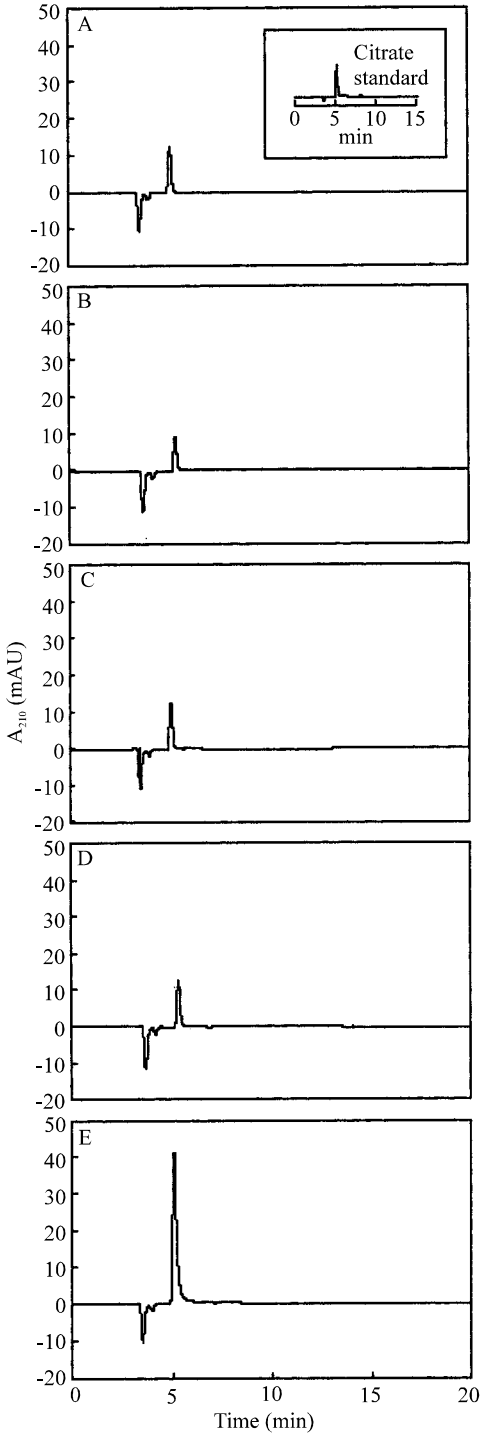

Fig. 7: HPLC chromatograms of organic acids in root exudates of maize (*Zea mays* L.). The seedlings were grown in nutrient solutions at increasing Al concentrations (A) 0; (B) 100; © 250; (D) 500 and (E) 1000 μM for 4 days (pH 4.0). The organic acids were separated on a 250 x 4.6 mm i.d. HP Zorbax SAX, a strong anion exchange column, using 0.2 M KH_2PO_4 (pH 3.0) as mobile phase at a flow rate of 1 mL min^{-1} and at 25°C

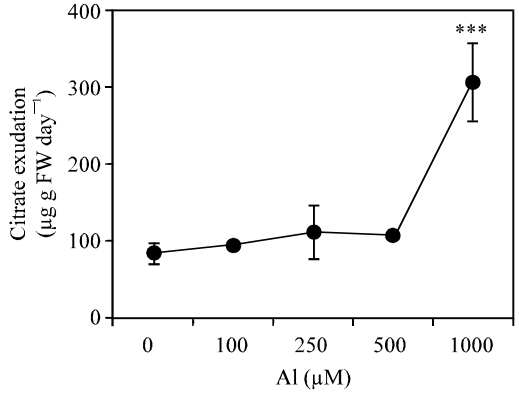

Fig. 8: Effect of Al treatment on citrate exudation. Maize (*Zea mays* L.) seedlings were treated with increasing Al concentrations: 0, 100, 250, 500 and 1000 μM (pH 4.0) for 4 days. Root exudates were collected than analyzed by HPLC. Values are means±SE (n = 3) of three independent experiments

Al treatments (Table 2). Moreover, the succinate content increased by 1000 μM Al, whereas that of lactate increased by 500 and 1000 μM Al (Table 2).

Effect of Al on MDH activity: In roots Al treatments with 250 to 1000 μM affected significantly the MDH activity by about 37% (from 105.3 to 66.2 μmol of NADH mg^{-1} protein h^{-1}, respectively for control and treated seedlings with 1000 μM). However, in shoots, the Al had no significant effect on the MDH activity (Fig. 6).

Al induced citrate exudation: The HPLC chromatograms of organic acids in the root exudates showed an only one peak corresponding to citrate with a retention time of 5.202 (Fig. 7). This peak remained unchanged in chromatograms of organic exudates from roots treated with 100 to 500 μM Al, but it increased substantially in the chromatogram of root exudates from roots treated with 1000 μM Al. The amount of citrate exuded was 3.7 fold higher in root exudates of treated seedlings with 1000 μM than that in control (Fig. 8).

Enhancing effect of citrate on plant growth: The addition of citrate to nutrient medium containing 500 μM Al had an ameliorative effect on plant growth, particularly on roots. The roots of seedlings treated with Al:citrate ratio of 1:2 showed the most ameliorative effect (Fig. 9). The treatment with exclusively 500 μM Al decreased significantly the root and shoot dry weight by, respectively 47 and 58.2% compared to control (Fig. 10). The addition of citrate was correlated to an increase in

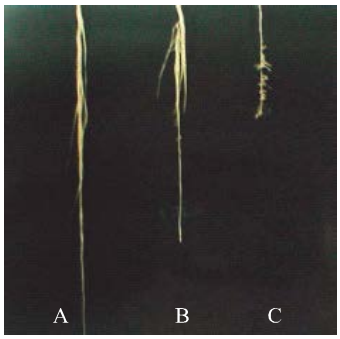

Fig. 9: Ameliorative effects of citrate on root growth of maize (*Zea mays* L.). (A) Control roots, (B) Roots treated with 500 μM Al + 1000 μM citrate; © Roots treated with 500 μM Al

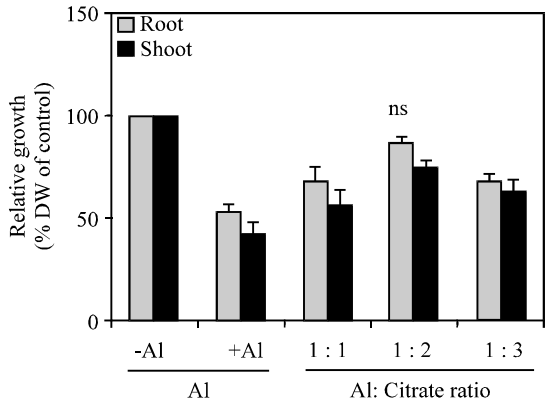

Fig. 10: Effect of various molar ratios of Al:citrate on root and shoot dry weight of maize (*Zea mays* L.). Seedlings were grown in nutrient solution containing no Al (-Al), 500 μM Al (+Al) or 500 μM Al combined with citrate at various ratios for 4 days (pH 4.0). Bars represent±SE of six independent experiments (n = 6). ns, not significant

root and shoot dry weight. Thus, an increase by, respectively 33.8 and 32.7% in root and shoot dry weight was observed in seedlings treated by Al:citrate ratio of 1:2 (Fig. 10).

DISCUSSION

Al toxicity symptoms and effect on growth: The Al induced inhibition of secondary roots and alteration of root apices, represent common Al toxicity symptoms (Fig. 1). These results confirm the previous study by Rengel[34] that Al can induce substantial root damage even when it occurs only during short periods. The non significant effects on above- ground agree well with previous studies, suggesting that toxicity symptoms occur first on the roots before foliage symptoms are detected and that root growth must usually be severe before significant effects can be seen on the aerial parts of the plants. The browning and callus-like nodules (Fig. 1) were a normal wound response likely resulting from an extensive period of Al exposure[35-37]. Many authors reported similar morphologic changes induced by Al at the roots level[13,14,38,39]. Factors such as the Al concentration in the nutrient solution, the plant age, the plant species, as well as the growth conditions can all affect the timing and extent of Al toxicity symptoms[40]. The Al induced inhibition of root growth (Fig. 2) may result from direct inhibition of nutrient uptake or the disturbance of root cell functions[15]. It is recognized that the root tips are the primary sites of Al-induced damage in plants[41]. The Al can affect the meristematic cells and has the effect of diminishing or even disrupting the root elongation process[37].

Al content in roots and shoots: The pattern of Al accumulation (Fig. 3) suggests that the cell wall may represent a potential barrier to ion uptake, particularly for high Al concentrations of 500 and 1000 μM. The strong biding of Al to the cell wall might provide an extracellular sink for Al[10]. Accumulation of Al in the cell walls could result from Al precipitation as suggested by Wagatsuma and Ezoe[42] who shown that precipitation of hydroxyl-Al polymers would contribute more to root Al levels than Al bound to CEC sites. The Al complexation with chelating root exudates or binding to mucilage may also be implied in Al exclusion[43]. At 100 and 250 μM Al, a fraction of Al probably was supposed to be accumulated in the cytosol (Fig. 3) and the cell wall represents only a step for Al absorption[10]. The restricted transport of Al from roots to shoots suggests that the primary action of Al appeared to be on the root system with a secondary effect on the shoot.

Mechanism of Al tolerance: The exudation of organic acids from the roots is considered one of the most important strategies by which Al is excluded[8]. The literature on the central role played by organic acid exudation in the Al-tolerance mechanism is growing. Previous studies have shown that under Al conditions, the amount of organic acids exuded by wheat and maize plant roots is more significant in the Al-resistant cultivars than in the sensitive ones[22,26]. Studies by De la Fuente *et al.*[44] have shown an increase in citrate synthase (CS) activity and citrate exudation when they introduced a *Pseudomonas aureginosa* CS gene into tobacco and papaya. Similarly, Koyama *et al.*[45] introduce

a mitochondrial CS gene isolated from *Daucus carota* into *Arabidopsis thaliana*, causing the same effect in transgenic plants. There is evidence that citrate has the ability to form more stable complex than succinate or malate, which are considered to be less efficient than citrate[21]. The malate is exuded instead of citrate in other plant species and malate efflux is considered as a general Al tolerance mechanism in wheat[46]. Alternatively, the citrate exudation may be induced by phosphate deficiency and not a response to Al stress. This is supported by earlier work demonstrating that selected cell lines of *Daucus carota* in the presence of insoluble Al-phosphate exuded more citrate in nutrient medium than the wild ones[47]. When grown in the absence of insoluble phosphate, the selected lines do not exude citrate and become consequently more sensible to ionic Al than the wild types[48]. Present results confirm previous studies that citrate has an alleviating effect on Al toxicity (Fig. 9 and 10). The introduction of citrate in medium containing Al ameliorates the cell culture of *Nicotiana plumbaginifolia*[49]. Likewise, Suhayda and Haug[50] have reported a similar protective effect of citrate on the membrane ATPase in maize. The reduction of Al toxicity by citrate was also reported with respect to *Trifolium pratense*[51]. Moreover, citrate, oxalate and tartarate ameliorated the toxic effect of Al on root growth in *Gossypium hirsutum* L.[52]. There is evidence that the complex of Al with citrate, the EDTA, or the extracted organic matter of the soil have less toxicity than the ionic Al in maize[53]. Organic acids seemed to be less affected in roots than in shoots by Al (Table 1 and 2), suggesting that tolerance mechanisms could operate to prevent Al induced disruptions of organic acid synthesis and degradation. The maintenance of normal level of organic acids in the roots might be essential for tolerance to Al stress[10]. Similarly to our results, Pellet *et al.*[26] have reported that Al had no effect on citrate content in roots of Al-sensitive Tuxpeño and Al-tolerant SA3 maize cultivars. Unlike Pellet *et al.*[26], however, the results point out to an increase in malate and total organic acid content in roots of Al treated plants for both cultivars. It is also observed elsewhere that Al reduces the root's content in citrate and malate more in sensible than in tolerant cultivars of *Hordeum* vulgare, *Phaseolus vulgaris*, *Pisum* sativum, *Triticum aestivum* and *Zea mays*[54,55]. It has been clearly demonstrated that organic acid concentrations in roots and leaves decline under Al stress[50,55,56]. Present results demonstrated that Al stress reduced the concentrations of some organic acids, in particular in shoots, verifying in part this general tendency. Foy *et al.*[57] reported that Al affected the organic acids concentrations in roots of five wheat cultivars differing in Al tolerance. Foy *et al.*[57] have shown that there is no evidence that tolerance to Al was correlated to change in organic acid concentrations in roots or in shoots. However, numerous studies have demonstrated that organic acid exudation was well correlated to enhanced Al tolerance. The salicylic acid was increased in shoots of Al treated plants with high Al concentration 1000 μM. Salicylic acid is thought to play essential role in inducing the systemic acquired resistance in plants infected by pathogenesis agents[58].

These results contributed to our knowledge about the Al tolerance mechanism in *Zea mays* L. This research introduces a new perspective into studying the Al tolerance mechanisms by extending this study to other maize cultivars differing in Al tolerance.

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