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Full Length Research Paper

Behavior of aluminum adsorption on cell wall of pineapple root apices

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Pineapple (*Ananas comosus* (L.) Merrill) is commonly grown in strongly acid soil, in which high aluminum (Al) concentration is often toxic to the roots of plants. The root apices of plants are most sensitive to Al toxicity. This paper is to evaluate the effect of Al on the growth of roots of four pineapple cultivars: Cayenne, Tainung No.6, Tainung No.13 and Tainung No.17 in Taiwan. The differences in the amount of callose and malondialdehyde (MDA) in root apices (1 cm in length) between Al-resistant and Al-sensitive pineapple treated with 0 and 300 µM $AlCl_3$ were determined. The role that the cell wall of root apices plays in Al-resistant characteristics is also discussed. After treating with 300 µM $AlCl_3$ in hydroponic solution (pH 4.5) for 72 h, the root elongation of Cayenne, Tainung No. 6, Tainung No. 13 and Tainung No. 17 was 115, 85, 93 and 73% of the values obtained compared to that without Al treatment, respectively. $AlCl_3$ treatment did not increase callose and MDA contents for Cayenne, but caused significant increase for Tainung No.17. Upon exposure to Al, Al adsorption on cell walls of root apices increased with time and $AlCl_3$ concentrations for Cayenne and Tainung No.17, but relatively greater in Tainung No.17. It reflects the fact that, Cayenne is Al-resistant and Tainung No.17 is Al-sensitive. When root apices were pretreated with 1 and 10 mM of malic acid, the Al adsorption of the cell walls of Cayenne's root apex was lower by 18 and 31%, respectively, relative to the values obtained without the malic acid treatment. It indicates that root apex of Cayenne may secrete more malic acid that is capable of Al complexation, as well as reducing the Al binding to cell walls in order to better resist Al toxicity.

Key words: Aluminum, pineapple, root apices, cell wall, adsorption.

INTRODUCTION

Aluminum (Al) is the most abundant insoluble metallic chemical element in soil. However, in strong acid soil (pH<5.5), Al may be in an ionic state with toxicity. Al toxicity is becoming a serious problem to plants in acid soil (Foy, 1978; Roy et al., 1988). It may inhibit root growth of crops, resulting in poor crop quality and production loss (Foy, 1992). The initial symptom of Al toxicity is the inhibition of root growth (Delhaize and Ryan, 1995). Nutrient absorption and cell function will be disrupted after exposure to high Al concentrations for 1 to2 h (Kochian, 1995). Till now, the responses of the parts of the root to Al exposure are rarely discussed.

Root apex is the site where the Al and the root interact; root cell walls have a role in preventing Al from entering the cells. So, root cell walls play an important role in Al resistance characteristics (Clarkson, 1967; Ryan et al., 1993; Horst, 1995; MacFarlane et al., 2000; Hall, 2002; Blamey et al., 1995). Cell walls are composed of pectin with high negative charges, which are the main substance for binding of cations (Blamey et al., 1995; Franco et al., 2002; Polec-Pawlak et al., 2007). When

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root apex is subjected to Al, nutrients such as K^+, Ca^{2+}, Mg^{2+} and NO_3^- will be reduced in entering into the cells (Cakmak and Horst, 1991; Olivetti et al., 1995; Macdiarmid and Gardner, 1998). If excessive binding occurs between Al and cell walls of root apex, root growth will be inhibited (Goldbold and Jentschke, 1998; Schmohl and Horst , 2000). As root apex is intoxicated by Al, the content of callose (1, 3-β-glucans) serves as an index to the damage of pineapple root apex (Le Van et al., 2004). Malondialdehyde (MDA) is an index for oxidative stress of plants. Lower activities of callose and MDA display a higher anti-oxidative ability, reflecting a higher stress resistance (Zhang et al., 1994). Besides, organic acids secreted by root apex are the most important mechanism for Al resistance of crops (Delhaize et al., 1993; Ryan et al., 1995; Le Van et al., 2004). Ma and collaborators (1999) conducted experiments on okra (*Abelmoschus esculentus* Moench) hypocotyls and found that the root could grow normally again, if half of the Al bounded with the cell walls of root apex was desorbed by malic acid. Thus, organic acids may reduce Al toxicity caused by adsorption of Al onto the cell walls (Mariano and Keltjens, 2003).

Zhang and Taylor (1989) conducted a complex experiment on Al adsorption onto cell walls of intact root apex. They found that the adsorption was rapid and linear at the earlier stage but became slow and non-linear at the later stage. As a result of this experiment on intact root apex, the early rapid Al adsorption was involved in the entrance of Al into symplast or Al-binding that consumed energy in apoplast (Pettersson and Strid, 1989; Zhang and Taylor, 1989), it was difficult to explain the Al adsorption of cell walls under such complicated mechanisms (Matsumoto, 2000). Zeng et al. (2004) extracted the cell walls of root apex from a rye cultivar (*Triticum aestivum* L.) and treated them with malic acid. They found that the amount of Al adsorption onto the cell walls was reduced to about 60%. Their experiment involved only one Al-resistant rye cultivar (that is Atlas 66).

Le Van and Masuda (2004) evaluated the Al-resistant characteristics of different pineapple cultivars, and found that the Al-resistant pineapple (*Ananas comosus* (L.) Merr.) was inhibited when treated with 300 µM $AlCl_3$. After exposure to such high Al concentration for 72 h, root apex of Al-resistant pineapple was apparently more Al repellent than Al-sensitive one. Al-resistant pineapple (that is Cayenne) secreted more malic acid than Al-sensitive one (that is Tainung No.17) did. The concentration of organic acids is higher at apoplast of root apex than at the rhizosphere. Thus, organic acids may interact with the components of cell walls, affecting surficial physiological and biochemical characteristics of the cell walls, and in turn enhancing Al-resistant capacity (Pellet et al., 1995). Pineapple is an important fruit in Taiwan, cultivated generally in acid soil.

However, the Al toxicity is often the limiting factor for crops grown in such kind of soils (Von Uexkull et al., 1995). The purposes of this study are:

(1) To test and discuss Al-resistant characteristics of root apices of four pineapple cultivars (Cayenne, Tainung No.6, Tainung No.13 and Tainung No.17) based on root elongation;
(2) To evaluate the difference in callose and MDA contents in root apices between Al-resistant and Al-sensitive pineapple cultivars treated with 300 µM $AlCl_3$;
(3) To isolate cell walls of root apices from Al-resistant and Al-sensitive pineapple cultivars, so that the difference in their Al adsorption onto cell walls of root apices was evaluated. The effect of malic acid on cell walls of Al-resistant pineapple root apices was evaluated as a reference for further improvement of pineapple grown in strongly acid soil.

MATERIALS AND METHODS

Experimental design

Seedlings of Cayenne, Tainung No.6, Tainung No.13 and Tainung No.17, each weighing about 81± 8 g, were selected and cleaned with deionized water. Each seedling was planted in 10 L hydroponic solution contained in a rounded plastic pot (25 cm in inner radius, 30 cm in height). The components of the hydroponic solution were that mentioned by Konish (1985) as follows: 1.10 mM $(NH_4)_2SO_4$, 0.35 mM $Ca(NO_3)_2$, 1.0 mM Na_2HPO_4 , 0.51 mM K_2SO_4, 0.35 mM $CaCl_2$, 1.00 mM $MgSO_4$; 6.3 µM Fe-EDTA, 9.3 µM H_3BO_3, 18.0 µM $MnSO_4$, 1.5 µM $ZnSO_4$, 0.4 µM $CuSO_4$, 0.5 µM Na_2MoO_4.

Seedlings were transferred to an aerated growth chamber with 27 and 23°C, and humidity at 65 and 85%, respectively, for day and night. The hydroponic solution was replaced by a new one every 5 days. Then, seedlings were transferred after 4 weeks to other hydroponic solution (pH 4.5) for 5 to 6 h.

Estimation of root length

Five healthy roots were selected from each seedling and marked with oil marker at root base. The length of each root was measured with a plastic ruler (Digi Kanon, EMS-8, Taiwan). Each plant was then planted in a 10 L hydroponic solution that contained either no Al or 300 µM $AlCl_3$. Triplicate samples were prepared for this study. The hydroponic solution was continuously aerated; its pH was adjusted daily with 0.1 M HCl or 0.1 M NaOH. After 72 h, the root length was measured and compared with the initial value. The experiments were repeated 3 times. These root apices were used for the determination of the contents of callose and MDA.

Determination of callose contents in root apex

Callose in 10 mm root tips was employed as a marker for Al-induced injury using the spectrofluorometric procedure described by Zhang et al. (1994). The root tip segments were immediately fixed with absolute ethanol (boiled in a water bath at 70°C for 10 min). The ethanol was then decanted. The samples were kept at –40°C until analysis. Root tip segments stored in freezer were thawed, rinsed in deionized water, and then homogenized in 1 ml of 1 M NaOH, ultrasonicated for 2 min. After placing in a water bath at 80°C for 30 min, they were centrifuged for 15 min at 10,000 × *g*,

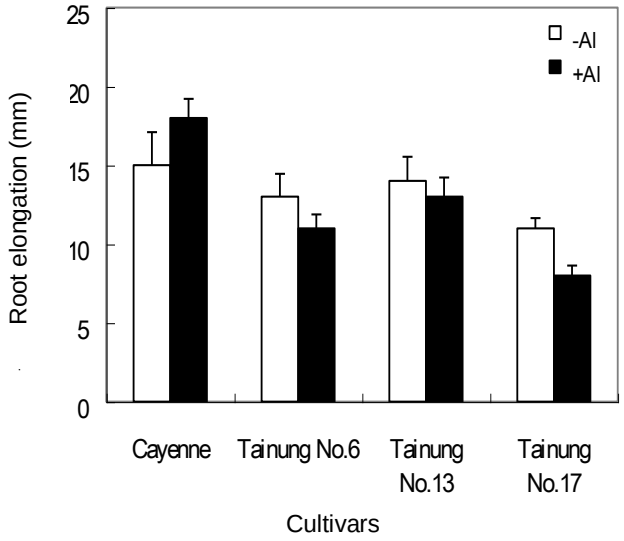

Figure 1. The root elongation of four pineapple cultivars cultivated in the hydroponic solution that contained 0 and 300 µM $AlCl_3$ for 72 h.

and the supernatant was collected. Callose content was determined by using a Shimadzu fluorescence spectrophotometer (Shimadzu, Tokyo, Japan) with excitation at 350 nm and emission at 500 nm. Laminarin (LE) was used as an external standard and callose content was expressed as mg laminarin.

Determination of MDA in root apex

Following the method by Heath and Packer (1968), about 0.1 g root apex was grounded together with 4 ml of 5% (w/v) trichloroacetic acid (TCA). The sample was centrifuged at 10,000 × *g* for 5 min at 20°C. One ml of the supernatant was added with 4 ml thiobarbaturic acid (0.5% (w/v), in 20% (w/v) TCA) and put in 95°C water bath for 30 min, then immediately immersed in ice to chill. After supersonicating to remove bubbles, it was centrifuged at 5,000 × *g* for 10 min and then measured for absorbance difference between 532 and 600 nm using UV-Vis spectrophotometer (Hitachi, U-2001, Japan).

Isolation of root apex cell walls

Under the same cultivation conditions described earlier, Cayenne and Tainung No.17 were cultivated for 4 weeks, and then the root apices (1 cm) were collected. According to Zhong and Lauchli (1993), root apices of 5 g were ground and then put in 50 ml tube for centrifugation at 1,000 rpm for 10 min. After removing the supernatant, the residue was immersed in acetone and mixture of methyl-methyl chloroform (1:1, v/v) at 1:7 (root weight (g) : vol (ml)) and in methyl each for 1 h. The supernatant was discarded after centrifugation at 1,000 rpm. The final residue was ground to powder in liquid nitrogen as cell wall, which was stored in 4°C for further experiments.

Adsorption of Al onto cell wall

The solution for Al adsorption experiments was prepared according to the revised method of Zheng et al. (2004). This solutioncontained and 300 µM $AlCl_3$. Coarse cell wall samples, each at 0.3 g, were treated with solution of different Al concentrations. Each cell wall sample held in a 2 ml column, served as a filter to one of the solutions flowing at 2 ml per 10 min using a peristaltic pump. The filtrate was collected at 20 min intervals, until its Al concentration reached that of the initial solution. The amount of Al adsorbed onto the sample at each interval was calculated and plotted against the adsorption time. All the adsorption experiments were conducted in triplicates and the results were presented as adsorption curves.

Malic acid treatment

Samples of coarse cell wall at 0.3 g were treated with malic acid (1 and 10 mM) and Al adsorption experiments were conducted on these samples with the procedure as previously described. Al content in collected solution was measured. The Al adsorbed at different time intervals was calculated and plotted against time as adsorption curves.

Measurement of Al content

Al measurements were conducted with the aluminon method (Hsu, 1963). The collected solution with Al was put in a 100 ml glass beaker and heated to dryness in a hot bath. After adding 10 ml deionized water and 1 ml of 0.5% ascorbic acid, the sample in the beaker was heated at 80 to 90°C water bath for 30 min. The sample, after cooling, was transferred into a 50 ml graded cylinder and added with deionized water till 35 ml. Then 10 ml of aluminon-acetate buffer solution was added and adjusted the volume to 50 ml by deionized water. After thorough mixing, the sample was settled for 2 h before measurement of its absorbance at 530 nm wavelength with a UV-VIS spectrophotometer (Hitachi, U-2001, Japan). Measurements of standard solutions were conducted with the same procedure. A standard Al solution at 5 mg L^{-1} was drawn at 2, 4, 6, 8, 10 and 12 ml separately into a 100 ml beaker and proceeded by the same way for their absorbances.

The Al concentration of each sample was determined from the calibration curve of the standards. The Al concentration in the coarse cell wall sample was presented in mg kg^{-1} equals C × D × V/w, where C is Al concentration in sample solution, D is dilution factor, V the volume and W the weight of the coarse cell wall sample (g).

Statistical analysis

The Windows SPSS 10.0 statistical software was used to carry out Analysis of Variation, (ANOVA) analyses. The least significant difference and the Duncan's test were used to distinguish the difference of various treatments. Significant differences are identified when $P < 0.05$.

RESULTS

Effect of Al on pineapple root growth

Figure 1 showed root elongation for four pineapple cultivars treated with 300 µM $AlCl_3$. For Cayenne, it was 115% of that without Al treatment. For Tainung No.6, Tainung No.13 and Tainung No.17, it was 85, 93 and 73%, respectively, relative to the control, without Al treatment. Cayenne was the most Al-resistant cultivar while Tainung No.17 was the most Al-sensitive one.

Table 1. The content of callose and malondialdehyde at 0 and 300 µM $AlCl_3$.

	Cayenne		Tainung No.17	
	0 µM $AlCl_3$	**300 µM $AlCl_3$**	**0 µM $AlCl_3$**	**300 µM $AlCl_3$**
Callose (µg LE/g FW)	N.D.	1.1±0.4	N.D.	4.9±1.2
MDA (µmole/g FW)	3.2±1.0	8.5±2.3	3.4±0.7	28±3.2

Callose: 1,3-β-glucan. MDA: malondialdehyde.

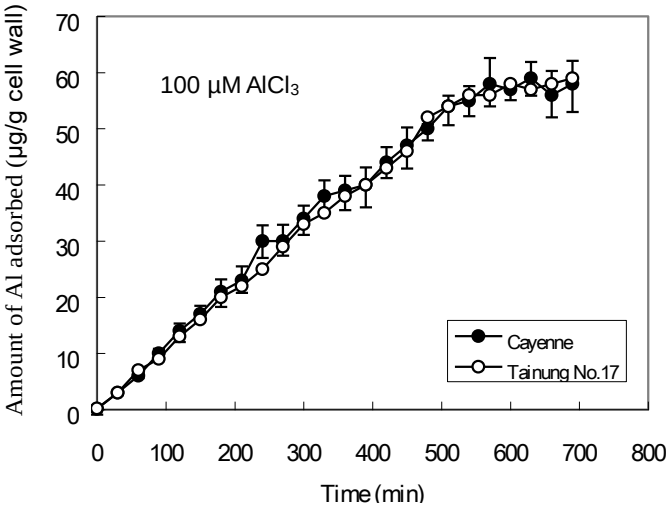

Figure 2. Al adsorption as a function of time for Cayenne and Tainung No.17 when treated with 100 µM $AlCl_3$ in hydroponic solution.

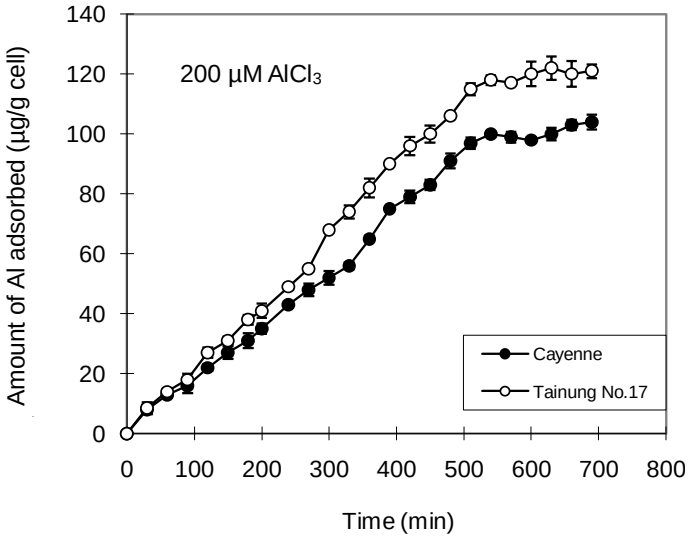

Figure 3. Al adsorption as a function of time for Cayenne and Tainung No.17 when treated with 200 µM $AlCl_3$ in hydroponic solution.

Effect of Al on callose and MDA contents

Table 1 showed the contents of callose and MDA in Cayenne and Tainung No.17 at 0 and 300 µM $AlCl_3$. No callose was detected in the root apex of Cayenne or Tainung No.17 when hydroponic solution was not supplemented with Al. When treated with 300 µM $AlCl_3$, the callose contents in Cayenne was 1.1 mg LE/g fresh weight (FW) while that in Tainung No.17 was 4.9 mg LE/g FW. Without Al treatment, the content of MDA in the root apex was approximately the same, 3.2 and 3.4 µmol/g FW, respectively, for Cayenne and Tainung No.17. After Al treatment, the MDA content increased to 8.5 µmol/g FW for Cayenne, and 28 µmol/g FW for Tainung No.17. The damage of root apex to Al toxicity was greater for Tainung No.17 than Cayenne. .

Al adsorption on cell wall

Figure 2 showed the Al adsorption curves for cell walls of both Cayenne and Tainung No. 17. With 100 µM $AlCl_3$ solution, the curves indicate a linear increase with time at the same concentrations until 500 min. The curves then reached the plateau after 600 min. Thus, their cell wall adsorption of Al are quite similar, reaching saturation at 60 µmole/g cell wall. Figure 3 showed the Al adsorption curves for both Cayenne and Tainung No.17, after they were treated with 200 µM $AlCl_3$. Both curves have increased with time but adsorption for Tainung No.17 becomes greater than for Cayenne after 240 min. Both curves reach saturation after 500 min with 100 and 120 µmole/g cell wall, respectively for Cayenne and Tainuug No.17. Treated with 300 µM $AlCl_3$, both Al adsorption curves increase similarly before 180 min but Tainung No.17 shows greater than Cayenne after 180 min (Figure 4). Both curves indicate saturation after 500 min (160 and 210 µmol/g wall, respectively).

Effect of malic acid pretreatment on the adsorption of Al in the cell wall

After treating with 1 and 10 mM malic acid, the Al adsorption of cell walls of Cayenne root apex was lower by 18 and 31% respectively, relative to that without malic acid treatment. The maximum Al adsorption without malic acid treatment was 55 µg/g cell wall; that with 1 mM malic acid treatment was 45 µg/g cell walls and that with 10 mM malic acid was decreased to 38 µg/g cell wall (Figure 5).

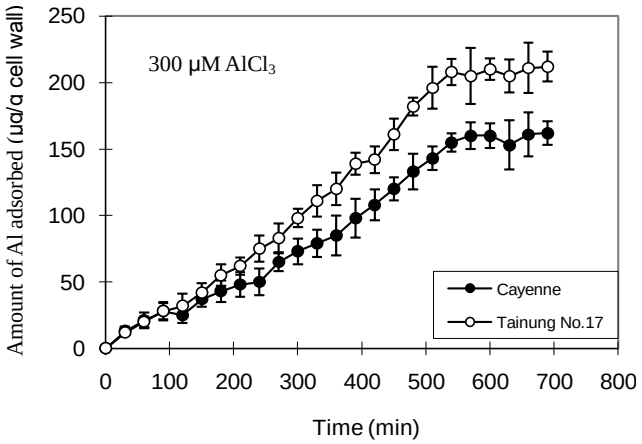

Figure 4. Al adsorption as a function of time for Cayenne and Tainung No.17 when treated with 300 μM $AlCl_3$ in hydroponic solution.

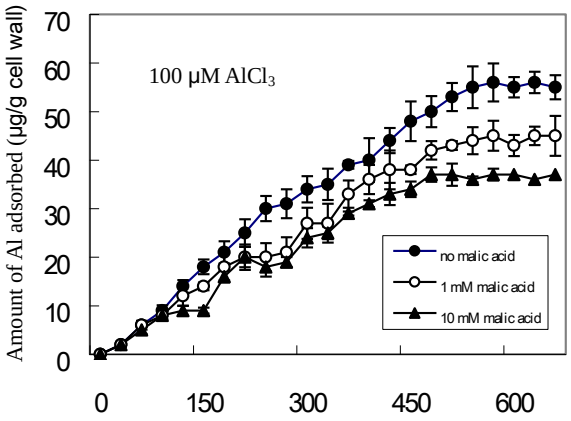

Figure 5. Al adsorption of cell walls as a function of time after treating with 1 and 10 mM malic acid, and then leaching with solution of 100 μM $AlCl_3$.

DISCUSSION

The Al adsorption on cell wall of pineapple

At earlier stage, all Al adsorptions increase linearly with time but the increase slow down at a later stage. Treated with 100 μM $AlCl_3$, both Cayenne and Tainung No.17 display similar curves with similar amount of Al adsorption, indicating no difference between Al-resistant and Al-sensitive cultivars at this Al concentration level (Figure 2). This is because low Al concentration may effectively alleviate the H^+ damage in acid environment (Kinraide, 1993). Le Van and Masuda (2004) treated 7 pineapple cultivars with 100 μM $AlCl_3$ and found root elongation for each cultivar. This is consistent with our experimental results. Treated with 200 μM $AlCl_3$, the cell wall Al adsorption of root apex was similar within 240 min. After that, the Al adsorption is greater for Tainung No.17 than Cayenne and the difference increased with time (Figure 3). Le Van and Masuda (2004) found that Al accumulation on root apex increased significantly, when Al-sensitive cultivars were treated with 200 μM $AlCl_3$, but root elongation was better than those with no Al treatment. With $AlCl_3$ concentration increased to 300 μM, Tainung No.17 showed greater Al adsorption than Cayenne after 180 min, indicating the cell wall of Cayenne could repel Al much better than that of Tainung No.17. Thus, the root apex of Cayenne can reduce its damage by Al and continue to grow, while that of Tainung No.17 is damaged by high Al accumulation at its root apex, affecting its growth.

The effect of Al on the damage of pineapple root apices

In a normal physiological condition, cellular synthase on plant cells will continue to help cells form cellulose. However, under poor environmental conditions, the function of cellular synthase would be inhibited and the function of callose synthase would be activated in order to defend against the poor environmental conditions (Delmer, 1987). In our experiment, both callose and MDA were markedly increased when treated with 300 μM $AlCl_3$ relative to no Al treatment. Although, callose was produced in large quantity by Tainung No.17 treated with high Al, it was unable to resist its root apex damage (as MDA indicated). As Al accumulation at cell walls of root apex was greater for Tainung No.17 than Cayenne, the damage to root apex was greater for Tainung No.17 than Cayenne.

The effect of malic acid on the Al adsorption of pineapple root cell wall

Al accumulation in root apoplast is mainly accomplished by bonding with negative charges of pectin (Roxova and Markovic, 1976). The other possibility is that xyloglucan, enzyme and phosphorus in the cell walls of plant root apex may bond with Al (Horst, 1995; Millard et al., 1990). Recent studies indicated that Al resistance of crops is due to secretion of ligands by root apex in response to Al in poor environment. These ligands then bond with Al and so reduce Al toxicity (Matsumoto, 2002). Secretion of malic acid was mainly the process for Al-resistant pineapple (Ryan et al., 1995; Zheng et al., 1998a, b). After secretion of organic acid anions, they have to go through the cell walls before entering into the Donna free space and then into the rhizosphere. Delhaize et al. (1993) suggested that the secretion of organic acid by rye root apex not only acts for Al detoxification by forming complex ions with Al, but also reduce the binding of Al with materials on the cell walls.

In this study, we found that Al adsorption decreased when malic acid concentration increased. Besides, Al

adsorption could reach its plateau level faster, after the root apex was treated with malic acid (Figure 5). Thus, secretion of organic acids may provide an explanation for repelling Al by root apex of Al-resistant pineapple. Zhang et al. (2004) suggested that about one fifth of malic acid was in the form of anions, and about 75% was in neutral form when pretreated with groundmass of 1 or 10 mM malic acid, 0.5 mM $CaCl_2$ at pH 4.5. Thus, organic acids with Van der Waals force and H^+ adsorption onto the cell walls are dominant, forming a layer of organic acids and reducing negative charges for Al adsorption on the surface of cells. In order to find out which components are responsible for organic acids adsorption, the effects of organic acids on the components of cell walls of pineapple need to be further studied. The components may include pectin, cellulose and semi-cellulose.

Conclusion

This study further confirms that Al adsorption onto cell walls of root apex is different between Cayenne and Tainung No.17 when treated with different Al concentrations. This may explain their Al-resistant characteristics. Malic acid may form complex ions with Al detoxification or interact with substances on cell walls of pineapple root apex, thus reducing the adsorption of Al. It is the most important mechanism for Al-resistance of pineapple.

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