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Full Length Research Paper

Effects of aluminum on root growth and absorption of nutrients by two pineapple cultivars [*Ananas comosus* (L.) Merr.]

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Aluminum (Al) is a biotoxic which often influences the absorption of nutrients by plants in strongly acidic soils. In this experiment, the effect of Al on root growth, absorption of macronutrients; phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and micronutrients; iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn) by two pineapple cultivars (ig. Al-resistant Cayenne and Al-sensitive Tainung No.17) were studied. Four levels of Al concentration treatments were imposed to the hydroponic solutions containing 0, 100, 200 and 300 μM $AlCl_3$. After treatments with Al for four weeks, the root elongation of Cayeen was increased with Al concentration, however, Tainung No.17 was decreased. The dry weight of Cayenne and Tainung No.17 was increased and decreased with Al concentration, respectively. The absorptions of macronutrients and micronutrients were not affected in Al-resistant Cayenne. However, the absorption of Ca, Mg and K were inhibited when $AlCl_3$ was 200 μM, and Fe, Mn and Cu absorption were inhibited significantly when $AlCl_3$ was 300 μM in the Al-sensitive Tainung No.17. On the other hand, Ca, Mg and K absorption increased significantly in Cayenne when $AlCl_3$ was 200 μM. It is possible that Ca, Mg and K uptake was an important clue for Al-resistant pineapple to resist Al in root.

Key words: Acid soils, Al toxicity, macronutrients, micronutrients, absorption.

INTRODUCTION

Acid soils occupy nearly 40% farmland in the world. They are mainly distributed in the tropical and subtropical area (Kochian, 1995). Al toxicity in strongly acidic soils is one of the main factors which reduce crop growth (Uexküll and Mutert, 1995). Al in the strongly acidic soil (pH <5.5) has toxic effect and limits the root elongation of crops (Foy, 1992). When the pH of soil is lower than 5.0, Al mostly exists in soluble Al^{3+} and influences the growth of plants. Hence, the toxicity of Al on the crop production need be noticed (Kochian, 1995; Matsumoto, 2000).

The initial symptom of Al toxicity is the inhibition of root elongation (Delhaize and Ryan, 1995). After exposure to Al for a long time, the function of cell of root apice was altered (Kochian, 1995) and absorption of nutrients such as Ca^{2+}, Mg^{2+}, K^+, NH_4^+, NO_3^- and $H_2PO_4^-$ was inhibited (Durieux et al., 1995; Miyasaka et al.,1989; Nichol et al., 1993; Rengel and Robinson, 1989; Rengel and Elliott, 1992). Some studies suggested that the toxic symptom of Al resembles that of the physiological disease of P, Ca and Mg deficiency (Larsen et al., 1997).

Pineapple [*Ananas comosus* (L.) Merr] is cultivated in many tropical and subtropical countries. In Taiwan, pineapple (*Ananas comosus* (L.) Merr) is one of important fruits in sacrifice and daily consumption (Chang, 1995). Most of the pineapples were cultivated on strongly acidic soils. However, Al toxicity is the limited factor for crops (Uexkull et al., 1995). Hydroponic cultivation is the main method to understand the absorption of nutrients of crops (Campbell and Carter, 1990; Horst et al., 1997). Hence, this study was conducted to explore the effect of Al on the root elongation, the accumulation of Al on root tips and nutrients absorption of Al-resistant Cayenne and Al-sensitive Tainung No.17.

MATERIALS AND METHODS

Determination of toxic aluminum in hydroponic solution

To understand the resistance of the pineapple cultivars to Al^{3+} concentration, it was necessary to examine root growth in hydroponic solution supplemented with physiologically relevant concentrations of $AlCl_3$. The prepared hydroponic solutions (pH 4.5) with 0, 100, 200 and 300 μM $AlCl_3$ were each filtered through 0.45 μm filter. Inorganic monomeric Al was determined using the aluminon method (Kerven et al., 1989). After mixing with aluminon/acetate buffer and 0.5% ascorbic acid for 30 s the absorbent of the filtrate was analyzed with a spectrophotometer (Hitachi, 2001) at 530 nm.

Determination of pineapple root elongation and dried weight

Seedlings (81 ± 8 g) of Cayenne and Tainung No.17 were selected from the Chiayi Branch of Taiwan Agricultural Experimental Station. They were cleaned with deionized water. Each seedling was planted in 10 L hydroponic solution contained in a circular plastic pot (25 cm inner diameter and 30 cm height). The hydroponic solution was prepared using the recipe of Konishi et al., (1985) with modifications. The ingredients included 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 $CuSO_4$ and 0.5 μM Na_2MoO_4. Plants were grown in a growth chamber at 27℃ in day time (14 h, 65% RH) and 23℃ at night (10 h, 85% RH) with an illumination of 280 mol/s.m^2 and the roots were aerated continuously. The hydroponic solution was replaced every five days. After four weeks, the sprouts with roots were moved to pH 4.5 hydroponic solutions to grow for 5 – 6 hours. Five healthy roots were selected and marked with permanent markers. Each root length was measured with a plastic ruler (Digi Kanon, EMS-8). Each plant was then planted in a 10 L hydroponic solution that contained 0, 100, 200 and 300 μM $AlCl_3$, respectively. Triplicate samples were prepared for this study. The pH of hydroponic solutions were adjusted daily with 0.1 N HCl or 0.1N NaOH. After four weeks, the root length was measured and compared with the original root length. The plants before and after Al treatments were dried in an oven (65℃ for two days), weighed and the variations of dried weight were calculated.

Extraction of aluminum from root apices

Triplicates of ten root apices for the two cultivars (Cayenne and Tainung No.17) grown in 0, 100, 200 and 300 μM $AlCl_3$ were separately collected. The root apices were transferred to Eppendorf tubes with 2mL of 2 N HCl; they were kept at room temperature for 48 h and then stored at –20° C until Al analyses.

Determination of Al content

Al content was determined by an inductively coupled plasma spectrometer (ICP, Jobin Yvon Ultima 2). Briefly, the samples in 2 N HCl solution were carried by argon gas into the apparatus to be nebulized and then sent to high temperature plasma to be evaporated; the organic substances contained in the sample were also decomposed into atoms and ions that were then excited. After they returned to the normal states, some spectra were emitted. The spectra lines emitted by Al included atomic and ionic spectra and these were used for qualitative and quantitative Al analyses. The Al concentrations were computed based on the integration of peak areas. The stock Al standard with 1000 ppm (Nacalai Tesque, Inc., Kyoto, Japan) was used for the preparation of diluted standard solution.

Statistical analysis

The windows SPSS 10.0 statistical software was used to carry out the 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 were identified when $P < 0.05$.

RESULTS AND DISCUSSION

Aluminum toxicity in hydroponic solution

Concentrations of the Al contained in the prepared hydroponic solution with $AlCl_3$ addition was analyzed by the aluminon method. The actual tocxic Al concentrations are 38.9 ± 1.56 μM, 65.5 ± 1.35 μM, and 80.8 ± 1.29 μM for the prepared 100 μM, 200 μM and 300 μM $AlCl_3$ solution, respectively.

Al toxicity symptoms and plant growth

Visible Al toxicity symptoms on Al-sensitive Tainung No.17 was chlorosis on young leaves and roots were short and coarse when grown for 25-28 days in hydroponic solution with treatments above 200 μM $AlCl_3$. Toxicity symptoms disappeared gradually in the cultivar grown in treatments below the 200 μM $AlCl_3$. The dry weight decreased with Al concentration increase as described. On the other hand, roots growth of Al-resistant Cayenne was unaffected by $AlCl_3$ concentration up to 300 μM. It was the same as description of Le Van and Masuda (2004).

The influence of Al concentration on root elongation and Al accumulation in root apices of the two pineapple cultivars

Before Al treatments, the seedlings of Cayenne and Tainung No.17 were cultivated in hydroponic solution for four weeks. Although, the number of roots of Tainung No.17 was higher than Cayenne however, the fresh weight of Cayenne root was higher than Tainung No.17 because the length and diameter of Cayenne root were higher than Tainung No.17 (Table 1). In strongly acidic environment, the hydrogen ion (H^+) in high concentration was toxic for crop root apices. Kinraide (1993) showed that Al^{3+} can reduce the toxicity of H^+. In our experiment, root elongation was inhibited when Cayenne and Tainung No.17 was cultivated in hydroponic solution containing no Al. It may be due to the H^+ toxicity on root when root was grown in pH 4.5. The growths of roots were excellent in both cultivars treated with 100 μM $AlCl_3$ in hydroponic solution (Figure 1a). There were no Al accumulation in root apices of the two cultivars in no Al treatment but however, the accumulation of Al by 1 g fresh weight was nearly 50 μg (50 μg/g FW) when treated with 100 μM $AlCl_3$ (Figure 1b). The root elongation of Cayenne was increased with increased Al concentration. However, the root elongation of Tainung No.17 was inhibited when the $AlCl_3$ concentration was 200 μM (Figure 2a). The

Table 1. Root growth of Cayenne and Tainung No.17 pineapple before seedlings were put into Al treatment.

Cultivars	Root number	Fresh weight (g)	Length (mm)	Diameter (mm)
Cayenne	28 ± 4	8.61 ± 1.23	83.12 ± 5.30	1.12 ± 0.04
Tainung No.17	38 ± 5	7.23 ± 0.89	71.33 ± 4.11	0.89 ± 0.03

Root growth of Cayenne and Tainung No.17 pineapple before seedlings were put into Al treatment. Seedlings (young shoots) were grown hydroponically in nutrient solution for four weeks. Values are the means ± s.e. ($n \geq 10$).

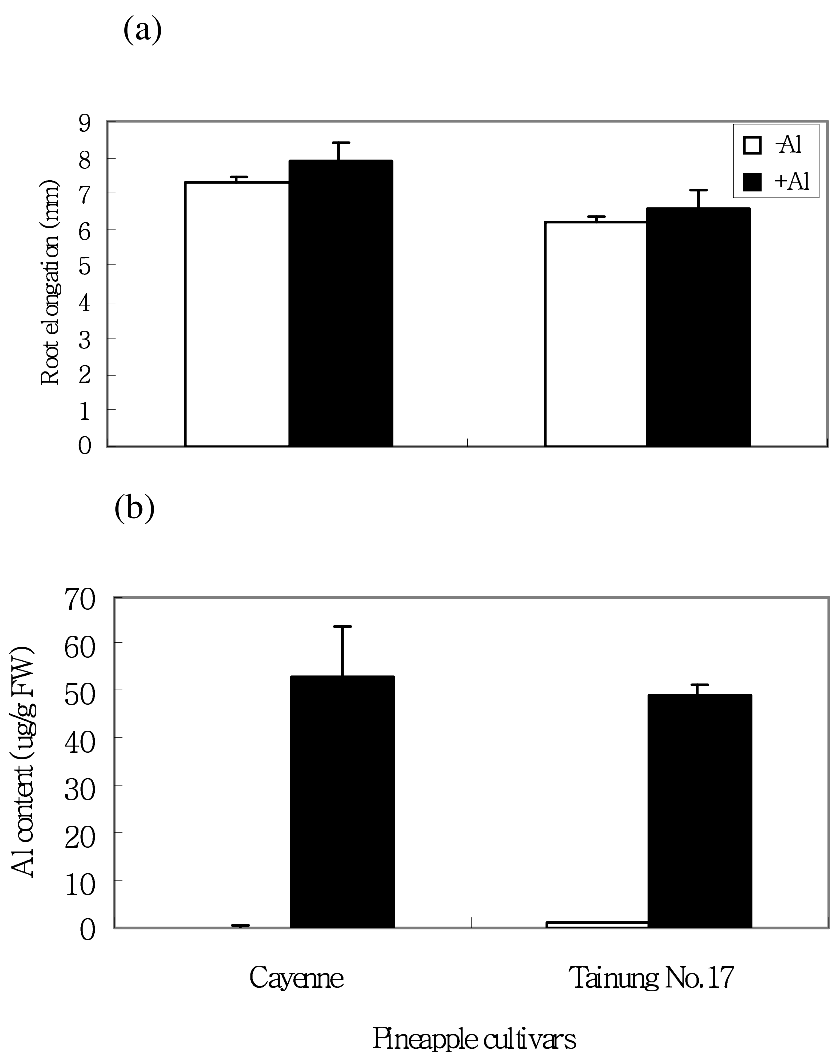

Figure 1. Effect of $AlCl_3$ concentration (0, 100 μM) on (a) root elongation and (b) Al content in 1 cm root apices of Cayenne and Tainung No.17 after planting in the hydroponic solution for four weeks.

was only 116 μg/g FW (fresh weight) at the 200 μM Al concentration and the accumulation amount of Tainung No.17 was 141 μg/g FW (Figure 2b). In the treatment of 300 μM $AlCl_3$, the root elongation was consistently increased, however, Tainung No.17 was seriously inhibited (Figure 3a). The Al accumulation of Cayenne and Tainung No.17 was 193 and 241 μg/g FW, respectively (Figure 3b). They were significantly different. The accumulation of Al in Tainung No.17 was higher than Cayenne in high Al concentration. This may be the

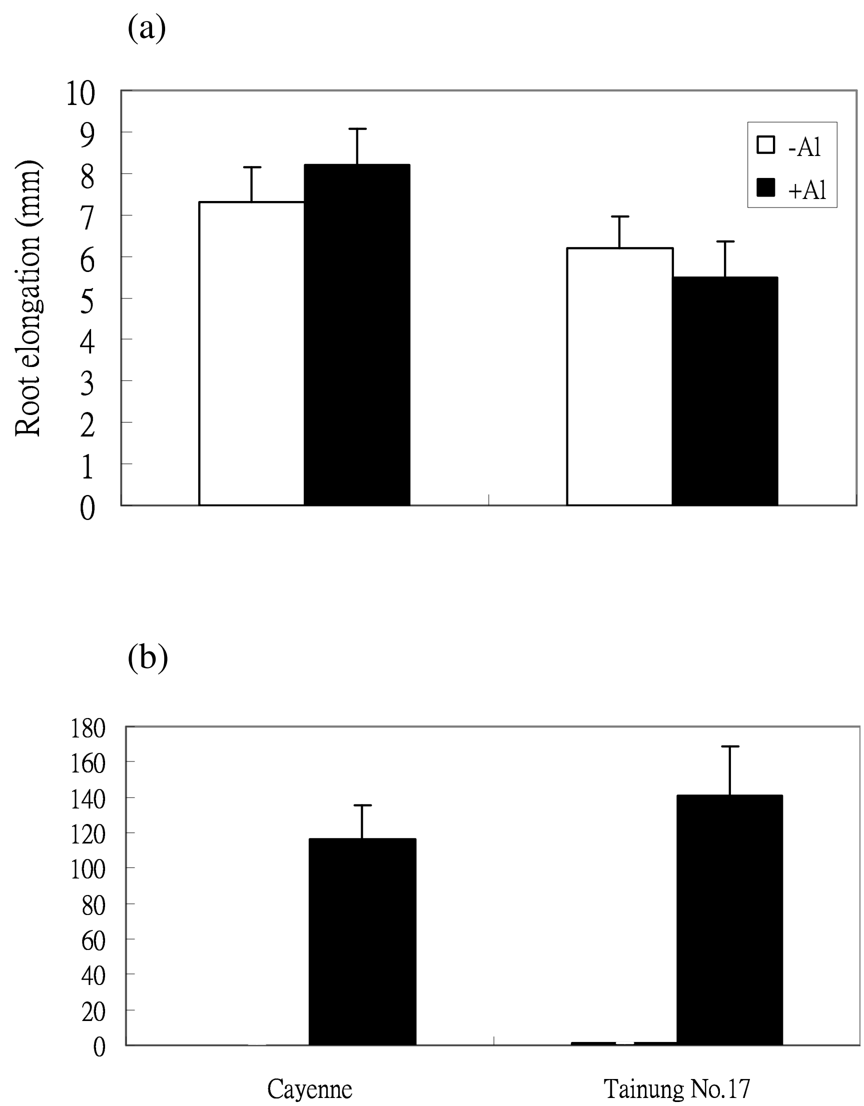

Figure 2. Effect of $AlCl_3$ concentration (0, 200 μM) on (a) root elongation and (b) Al content in 1 cm root apices of Cayenne and Tainung No.17 after planting in the hydroponic solution for four weeks.

reason for the root elongation inhibition in Tainung No.17. The secretion of organic acids and Al-resistant proteins may reduce Al toxicity on root apice (Le Van and Masuada, 2004).

The influence of Al concentration on dry weight and absorption of nutrients of two pineapple cultivars

The dry weight of Cayenne was increased, however, Tainung No.17 was decreased with increased $AlCl_3$ concentration and this may be involved in the absorption of nutrients.

Figure 4 shows the concentrations of P, K, Ca and Mg in Cayenne and Tainung No.17 under various Al treatments in the hydroponic solution. The absorption of P, K, Ca and Mg were similar in the treatments of 0 and 100μM $AlCl_3$, however, the absorption of the four elements were apparently higher in Cayenne than in Tainung No.17. For Cayenne, K, Ca and Mg contents in the plant showed similar trends with increased Al concentration before treatment of 100 μM $AlCl_3$. However, the absorption of the three elements was sharply increased in the treatments of 200 and 300 μM $AlCl_3$. On the other hand, the absorption amount of K, Ca and Mg in Tainung No.17 were steady before the treatment of 100 μM $AlCl_3$; however, they were sharply increased in the treatments of 200 and 300 μM $AlCl_3$. K, Ca and Mg absorption decreased significantly in Tainung No.17 when $AlCl_3$ was added at 200 μM $AlCl_3$ in hydroponic solution, and this was almost the same point at which root growth was inhibited.

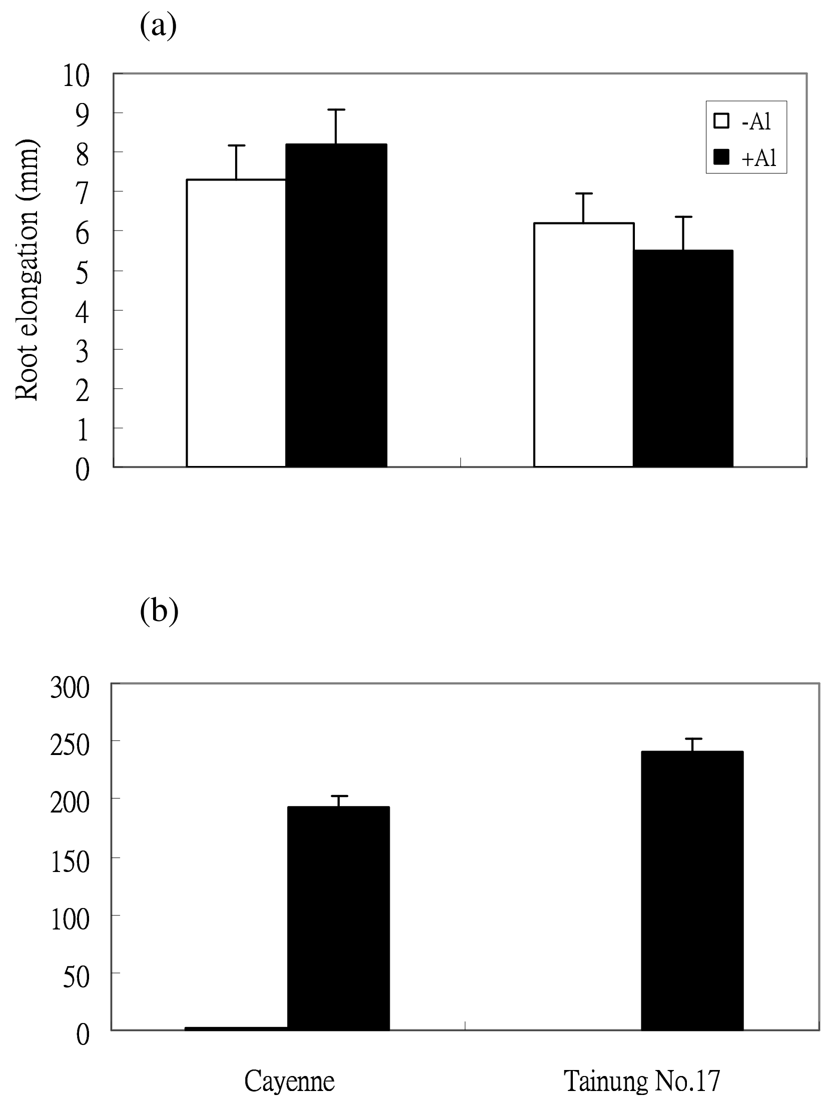

Figure 3. Effect of $AlCl_3$ concentration (0, 300 μM) on (a) root elongation and (b) Al content in 1 cm root apices of Cayenne and Tainung No.17 after planting in the hydroponic solution for four weeks.

Figure 5 shows the concentrations of Fe, Mn, Cu and Zn in the roots under various Al levels. Fe, Mn, Cu and Zn concentrations in Cayenne and Tainung No.17 were not affected as Al increase from 0 to 200 μM $AlCl_3$. However, it sharply decreased at 300 μM $AlCl_3$ treatment ($p < 0.001$). Gussarsson (1994) suggested that toxic metals including Al may interfere with nutrient uptake by altering the plasma membrane permeability and by affecting element transport processes across the membrane. Brune and Dietz (1995) reported no or little change in the P content of barley under heavy metal stress. Our results suggest that the plasma membrane of root cells of Cayenne was protected but was damaged in Tainung No.17 up to 200 μM $AlCl_3$ exposures and K, Ca and Mg uptake by roots play important roles. Especially, Ca is largely bound to the cell wall and to the exterior surface of the plasma membrane. It provides intermolecular linkages and is thought to play a crucial role in cell wall and membrane stabilization. Wang et al. (1992) suggested that a strong interaction between Ca and cell wall constituents may be important in providing sufficient Ca to the plasma membrane to maintain its integrity. An increase of Ca absorption under Al stress would be a possible mechanism for reducing the toxic effects of Al and a decrease of Ca concentration under Al toxicity may be a symptom of a damaged intercellular defense system. Similar results have been reported by Brune and Dietz (1995) that the Ca contents first increased and then decreased at very high Al concentrations. This result indicates that among the micronutrients, Zn absorption was not affected by the Al concentrations. This result agrees well with previously

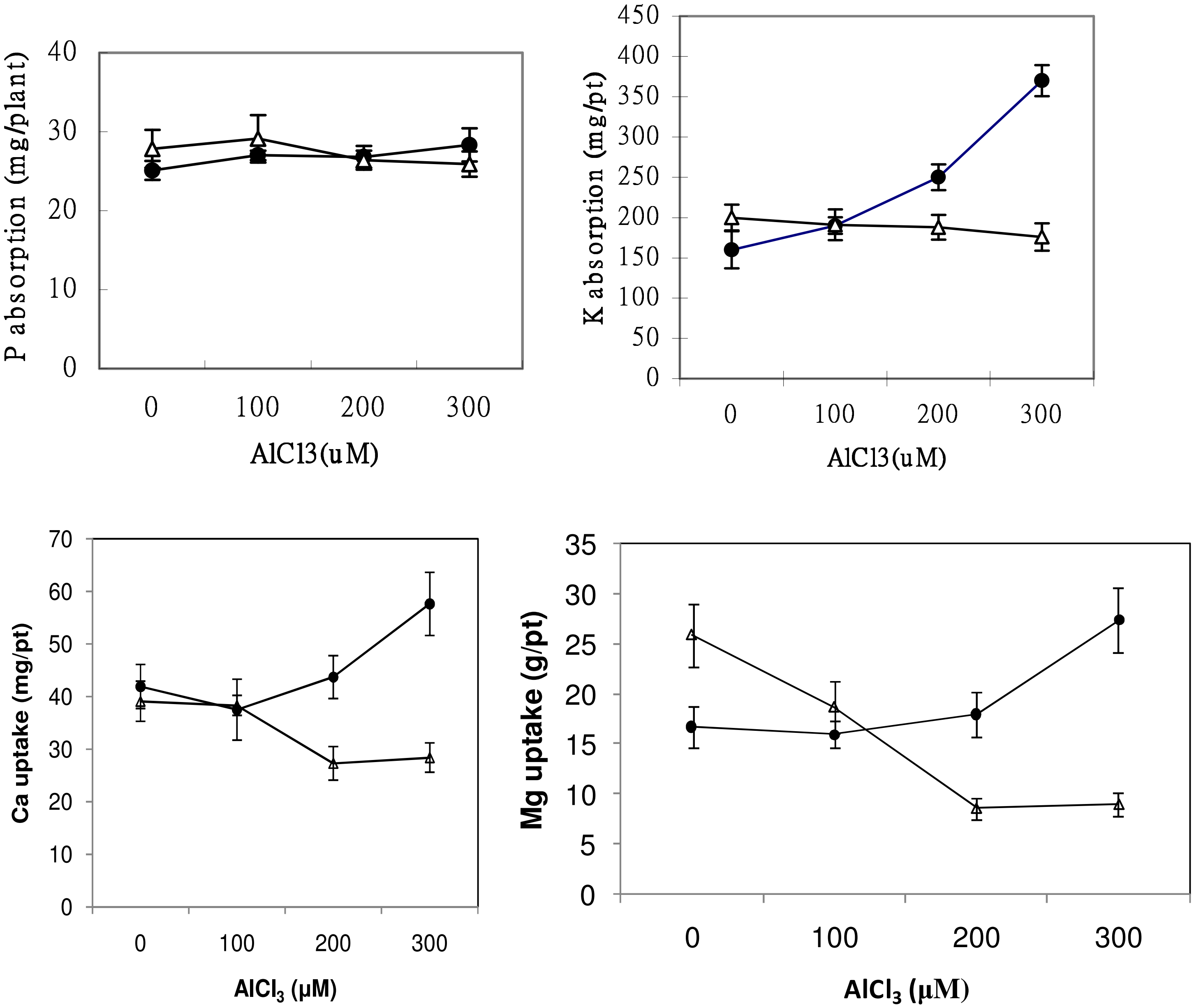

Figure 4. The absorption of macronutrients by Cayenne and Tainung No.17 pineapple in different $AlCl_3$ concentration.

reported work (Moral et al.,1994). Absorptions of Fe, Mn and Cu were not affected in Cayenne with different concentrations of Al; however, they were affected in Tainung No.17 with theatments of 300 μM $AlCl_3$.

The present study thus provides data and comparisons among the nutrient trends in the Al-resistant and Al-sensitive pineapples grown on different Al concentrations.

Conclusion

In this experiment, the root elongation of Tainung No.17 was affected negatively by Al and root biomass decreased significantly at 300 μM $AlCl_3$. Aluminum accumulation in roots of Tainung No.17 was higher than Cayenne up to the treatment of 200μM $AlCl_3$. The nutrients absorption of Cayenne was not affected. The absorption of nutrients of Tainung No.17 mainly affected by Al was K, Ca and Mg at the treatment of 200 μM $AlCl_3$ and Fe, Mn, Cu at the treatment of 300 μM $AlCl_3$. The absorption of P and Zn were not affected in Tainung No.17. An increase of Ca, Mg and K absorption under Al stress would be a possible mechanism for reducing the toxic effects of Al in Al-resistant pineapple.

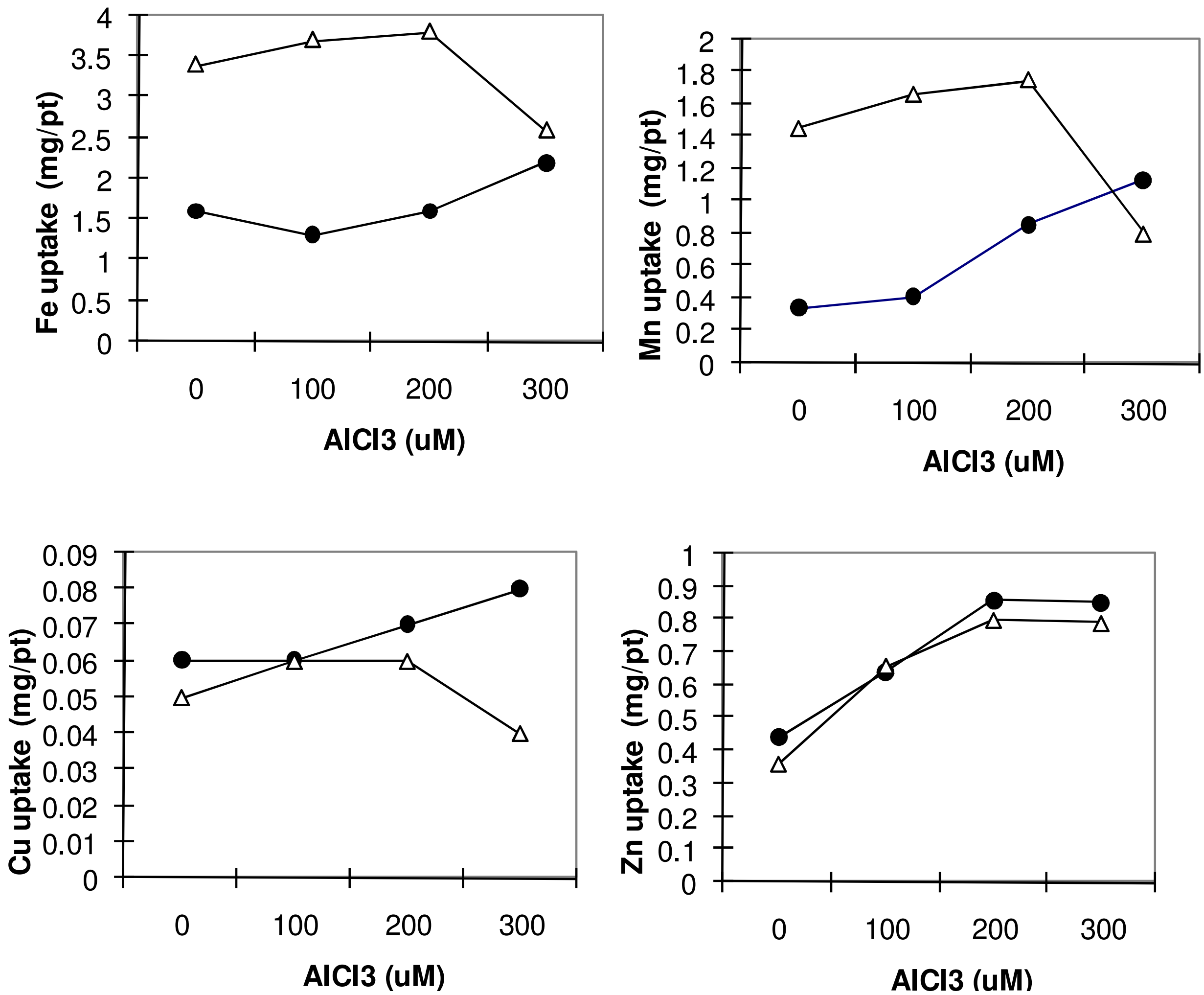

Figure 5. The absorption of micronutrients by Cayenne and Tainung No.17 pineapple in different $AlCl_3$ concentration.

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