# Reduction of Aluminum-inhibited Root Growth of Rice Seedlings with Supplemental Calcium, Magnesium and Organic Acids

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## ABSTRACT

$AlCl_3$ dissolved in half-strength Kimura B nutrient solution was observed to be less effective in inhibiting root growth of rice seedlings than that dissolved in distilled water. Kimura B nutrient solution is composed of inorganic salts and citrate. Thus, we investigated the influence of inorganic salts and organic acids on $AlCl_3$-inhibited root growth of rice seedlings. It was observed that $CaCl_2$, $MgCl_2$, $NaH_2PO_4$, citrate, malate, tartarate, and oxalate were able to reduce $AlCl_3$-inhibited root growth of rice seedlings. Results suggest that the effect of $CaCl_2$, $MgCl_2$, and organic acids on $AlCl_3$-inhibited root growth is mediated through reducing Al level in roots of rice seedlings.

**Key words:** $AlCl_3$, $CaCl_2$, $MgCl_2$, Organic acid, Rice, Root growth.

# 氯化鈣、氯化鎂與有機酸對氯化鋁所抑制水稻幼苗根生長之影響

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## 摘要

氯化鋁溶解於半量之木村氏 B 水耕液抑制水稻根生長之能力低於氯化鋁溶解於蒸餾水中。木村氏 B 水耕液內含有無機鹽與有機酸，因此我們探討氯化鈣、氯化鎂與有機酸對氯化鋁所抑制水稻幼苗根生長之影響。試驗結果顯示，氯化鈣、氯化鎂、磷酸氫鈉與有機酸（citrate、malate、tartarate 及 oxalate）可降低氯化鋁所抑制水稻根之生長。氯化鈣、氯化鎂與有機酸之效應係經由其降低水稻根內鋁之含量所造成。

**關鍵詞：**氯化鋁、氯化鈣、氯化鎂、有機酸、水稻、根生長。

## INTRODUCTION

Aluminum (Al) does not exert any known function in plant metabolism and belongs to the non-essential metals. Under neutral soil conditions, it exists in the non-phytotoxic insoluble form, whereas acidification of soil and soil water below pH 4.5 dramatically enhances release of the phytotoxic aluminum ion (MacDonald and Martin 1988). Since acid soils occupy up to 40% of world's arable land (Kochian 1995), Al phytotoxicity may be considered as one of the major limiting factors of crop productivity in the world (Matsumoto 2000). The primary effect of Al toxicity is the inhibition of root growth; however, the mechanisms involved in this toxicity are far from clear (Matsumoto 2000).

Calcium (Ca) plays important and crucial roles in plant metabolism (Kauss 1987), development (Helper and Wayne 1985) and signal transduction (Sanders *et al.* 2002). The role of Ca in Al toxicity has been extensively examined (Foy 1988, Rengel 1992, Rengel and Zhang 2003). Al

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can decrease Ca concentration in plant and symptoms of Al toxicity are expressed as Ca deficiency (Foy 1988, Rengel 1992). Al toxicity can often be reduced by the addition of $Ca^{2+}$ to the medium (Alva and Edward 1990, Alva *et al.* 1986, Brady *et al.* 1993, Hecht-Buchholz and Schuster 1987, Kinraide and Parker, 1987, Noble and Summer 1988, Sanzonowicz *et al.*1998, Silva *et al.* 2001a). Another nutrient element apparently affected by Al to a great extent is Magnesium (Mg). Exposure to Al results in decreased Mg concentration and total Mg content in plants (Clark 1977, Grimme 1983, Kinraide and Parker 1987, Rengel 1990, Rengel and Robinson 1989a, b). $Mg^{2+}$ is also an effective ameliorant of Al toxicity (Kinraide and Parker 1987, Rhue and Grogan 1977, Silva *et al.* 2001a, b, Tan *et al.* 1992).

Al has a strong binding affinity for oxygen donor compounds such as inorganic phosphate, nucleotides, RNA, DNA, proteins, carboxylic acids, and phospholipids (Ma 2000). The binding of Al with these substances may result in structural and functional damage to the roots. Therefore, if a ligand is present that can bind Al strongly, it could reduce the activity of the free Al ions in the solution and reduce any binding to the root cells. Some organic acids such as citric, oxalic and malic acids form stable complex with Al, thereby detoxifying Al. Jones (1961) first proposed the hypothesis that Al tolerant plant species contain and exude organic acids that chelate Al and thereby reduce its toxicity. Since that time, numerous papers investigating the effect of organic acids on Al toxicity have been published (Foy *et al.* 1978, Ma 2000, Ma *et al.* 2001).

In the present study, we examined the influence of inorganic salts and organic acids on Al-inhibited root growth of rice seedlings.

## MATERIALS AND METHODS

Rice (*Oryza sativa* L., cv. Taichung Native 1) seeds were sterilized with 2.5% sodium chlorite for 15 min and washed extensively with distilled water. These seeds were germinated in a Petri dish (20 cm) on wetted filter papers at 37℃ in the dark for 24 h. Uniformly germinated seeds were selected and transferred to a Petri dish (9 cm) containing filter paper moistened with 10 ml of distilled water for 2 days. Two-day-old seedlings were then treated with distilled water, $AlCl_3$ or other test solutions. The pH of these solutions was adjusted to 4.0 by using 0.1 M HCl. Each Petri dish contained 10 germinated seeds. Each treatment was replicated four times. Incubation was carried out at 27℃ in the dark. Root or shoot length was measured at the time indicated. Harvested roots or shoots were dried at 65℃ for 48 h. Dried material was ashed at 550℃ for 20 h. Ash residue was incubated with 31% $HNO_3$ and 17.5% $H_2O_2$ at 70℃ for 12 h, and dissolved in 0.1 M HCl. Al, Ca, and Mg were then quantified using an atomic absorption spectrophotometer (Model AA-680, Shimatzu, Kyoto, Japan).

Statistical differences between measurements (n=4) on different treatments or on different times were analyzed by Duncan's multiple range test or Student's *t*-test.

## RESULTS

Figure 1 shows the influence of $AlCl_3$ on root growth and Al level in roots of rice seedlings. Increasing concentrations of $AlCl_3$ from 0.25 to 1 mM significantly decreased root growth (Fig. 1A) and increased Al level (Fig. 1B) in rice roots. The reduction of root growth is closely correlated with the increase of Al level in roots. These results are generally consistent with previous reports by other investigators (Alva and Edward 1990, Brady *et al.* 1993, Hecht-Buchholz and Schuster 1987, Kinraide and Parker 1987, Noble and Summer 1988, Sanzonowicz *et al.* 1998, Silva *et al.* 2001a, b). Contrary to the effect of $AlCl_3$ on root growth, $AlCl_3$ slightly but significantly enhanced shoot growth (Fig. 1A) and Al level (Fig. 1B) in the shoot of rice seedlings. This is an interesting finding since, to our knowledge, it has never been reported before. It appears that small amount of Al is able to enhance shoot growth of rice seedlings.

It has long been recognized that Al-inhibited root growth is observed at medium pH below 4.5 (MacDonald and Martin 1988). Here, we also observed that the inhibition of root growth in rice seedlings is more effective at pH 4.0 than in roots at pH 7.0 (Fig. 2A). Treatment of $AlCl_3$ at pH 4.0 resulted in higher Al level than that at pH 7.0 (Fig. 2B).

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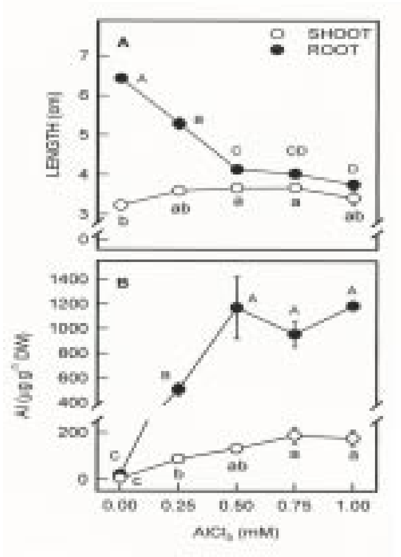


Fig. 1. Effect of $AlCl_3$ on the growth of roots and shoot and the level of Al in roots and shoot of rice seedlings. Two-day-old rice seedlings were treated with $AlCl_3$ (dissolved in distilled water, pH 4.0) for 2 days. Values with the same letter are not significantly different at $P < 0.05$, according to Duncan's multiple range test.

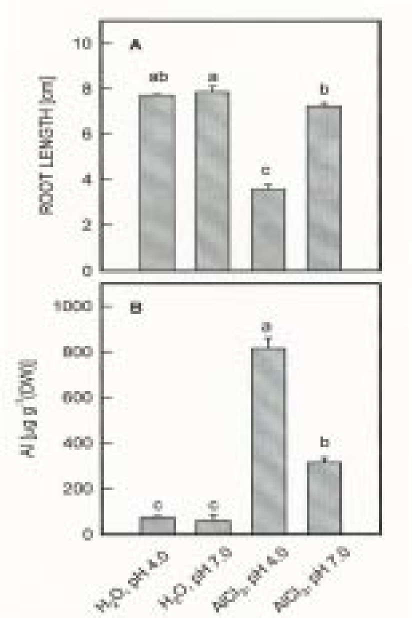


Fig. 2. Effect of $AlCl_3$ and pH on root growth and Al level in roots of rice seedlings. Two-day-old rice seedlings were treated with 0.5 mM $AlCl_3$ at pH 4.0 and 7.0 for 2 days. Values with the same letter are not significantly different at $P < 0.05$, according to Duncan's multiple range test.

When root growth of rice seedlings treated with $AlCl_3$ in distilled water was compared with that in half-strength Kimura B solution, it was observed that the former was more effective in root growth inhibition and had higher Al level in roots than the latter (Fig. 3A and Fig.4). However, the medium pH of the treatment that $AlCl_3$ dissolved in water was higher than that of $AlCl_3$ dissolved in Kimura B solution (Fig. 3B). Thus, medium pH cannot be used to explain the less root growth inhibition of rice seedlings treated with $AlCl_3$ in Kimura B solution compared to that treated with $AlCl_3$ in distilled water.

Kimura B solution is composed of inorganic salts, such as $K^+$, $Na^+$, $Ca^{2+}$, $Mg^{2+}$, $Mn^{2+}$, phosphorus, $Fe^{2+}$, and $H_3BO_3$ (Chu and Lee 1989). These inorganic salts may interfere with the uptake of Al by rice roots. If this is indeed the case, then inorganic salts are expected to reduce Al-inhibited growth. Figures 5B, 5F, and 5G show that $CaCl_2$, $MgCl_2$ and phosphorus were effective in reducing root growth inhibition caused by $AlCl_3$ dissolved in $H_2O$. However, KCl, NaCl,

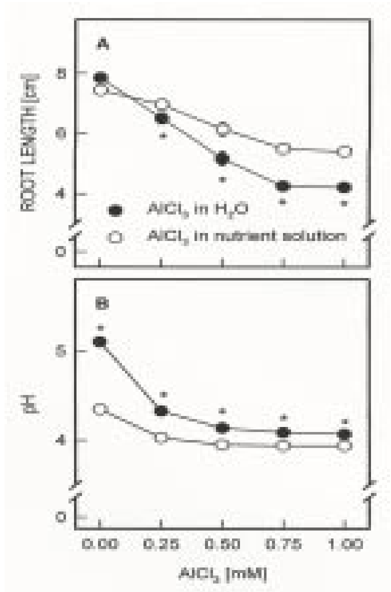


Fig. 3. Effect of $AlCl_3$ on root growth of rice seedlings and medium pH. Two-day-old seedlings were treated with $AlCl_3$ dissolved in distilled water (pH 4.0) or dissolved in nutrient solutions (pH 4.0) for 2 days. Nutrient solution represents half-strength Kimura B solution. Asterisks indicate values that are significantly different at $P < 0.05$ level by Student's $t$-test when compare to $AlCl_3$ in $H_2O$.

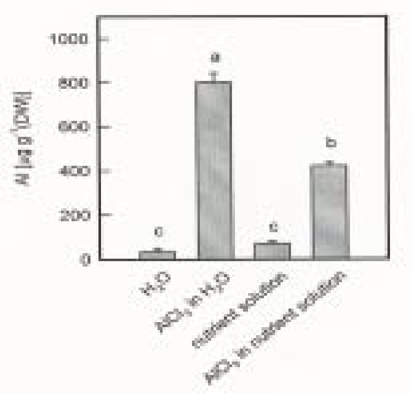


Fig. 4. Effect of $AlCl_3$ on Al level in roots of rice seedlings. Two-day-old seedlings were treated with $AlCl_3$ dissolved in distilled water (0.5 mM, pH 4.0) or $AlCl_3$ dissolved in nutrient solution (0.5 mM, pH 4.0) for 2 days. Nutrient solution represents half-strength Kimura B solution. Values with the same letter are not significantly different at $P < 0.05$, according to Duncan's multiple range test.

$MnCl_2$, $FeSO_4$, and $H_3BO_3$ had no effect on Al-inhibited root growth of rice seedlings (Figs. 5A, 5C, 5D, 5E, and 5H).

Figure 6A shows that the increase in Al level in roots caused by $AlCl_3$ was reduced by $CaCl_2$ and $MgCl_2$. Ca level was observed to be higher in roots treated with $CaCl_2$ together with $AlCl_3$ compared with roots treated with $AlCl_3$ alone (Fig. 6B). Similarly, Mg level was higher in roots treated with $MgCl_2$ together with $AlCl_3$ when compared with $AlCl_3$ alone (Fig. 6C). Compared with water control, $AlCl_3$ treatment did not reduce Ca or Mg level in roots of rice seedlings (Figs. 6B and 6C).

Since Kimura B solution is also composed of citrate, thus we examined the effects of several organic acids (such as acetate, succinate, malate, oxalate, citrate, and tartarate) on growth inhibition of roots caused by $AlCl_3$ (Fig. 7). When roots were treated with $AlCl_3$ and these organic acids, it was observed that malate, oxalate, citrate, and tartarate were effective in reducing Al-inhibited root growth (Figs. 7C, 7D, 7E, and 7F). However, acetate and succinate had no effect on Al-inhibited root growth (Figs. 7A and 7B). Figure 8 shows that citrate, malate, tartarate, and oxalate were able to reduce the increase in Al level in roots caused by $AlCl_3$.

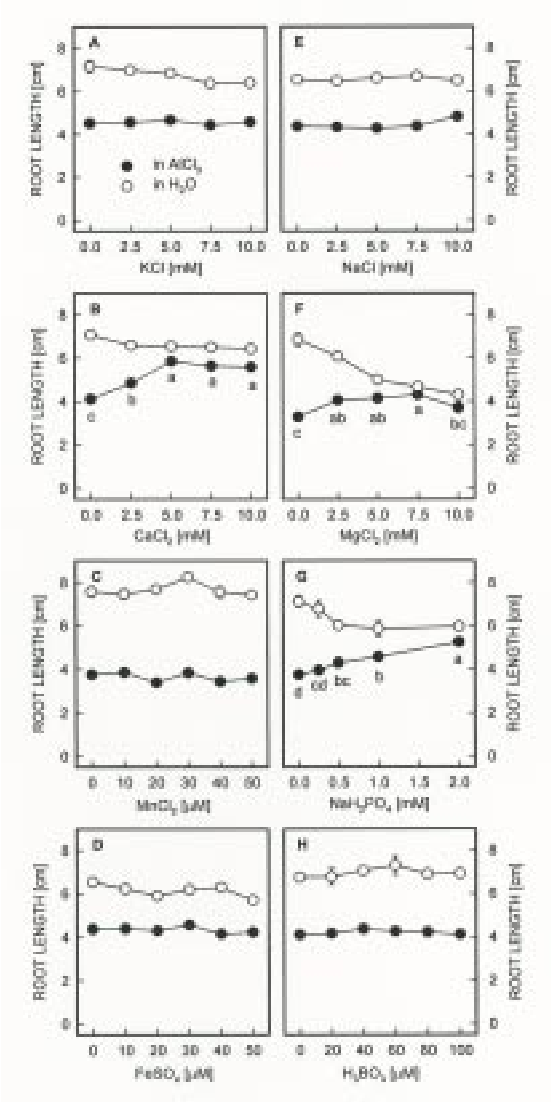


Fig. 5. Effect of KCl, NaCl, $CaCl_2$, $MgCl_2$, $NaH_2PO_4$, $FeSO_4$, and $H_3BO_3$ on Al-inhibited root growth of rice seedlings. Two-day-old seedlings were treated with various salts in the presence or absence of $AlCl_3$ (0.5 mM, pH 4.0) for 2 days. Values with the same letter are not significantly different at $P < 0.05$, according to Duncan's multiple range test.

## DISCUSSION

In this study, we found that supplement of $CaCl_2$ and $MgCl_2$ significantly ameliorate growth inhibition of rice roots caused by $AlCl_3$ (Figs. 5B and 5F). These results are consistent with the general contention that $Ca^{2+}$ and $Mg^{2+}$ are able to reduce the toxic effects of Al (Foy 1988, Rengle 1992, Rengle and Zhang 2003). $AlCl_3$ treatment results in an increase in Al level in roots of rice seedlings (Figs. 1B, 2B, and 6A). This increase in Al level could be reduced by the presence of $CaCl_2$ and $MgCl_2$ in the medium solution (Fig. 6A). Thus, the alleviative effect of $Ca^{2+}$ or $Mg^{2+}$ is mostly likely attributable to $Ca^{2+}$- or $Mg^{2+}$-reduced Al uptake of rice roots.

It has been shown that the symptoms of Al toxicity resemble those of $Ca^{2+}$ or $Mg^{2+}$ deficiency (Foy 1988). This does not seem to be the case in the roots of rice seedlings, because $AlCl_3$ treatment had no effect on Ca and Mg level (Figs. 6B and 6C).

Increasing the phosphorus supply has been reported to exert certain roles in eliminating Al toxicity (Foy 1988, Tan and Keltjens 1990a, 1990b). We also observed that addition of $NaH_2PO_4$ was able to reduce Al-inhibited root growth of rice seedlings (Fig. 5G). Lenoble *et al.* (1996a, b) demonstrated that supplemental B prevented Al inhibition of root growth of alfalfa. However, we were unable to show the alleviative effect of $H_3BO_3$ on Al-inhibited growth of rice seedlings (Fig. 5H). It appears that Al toxicity in rice roots may not induce B deficiency. Although interactions of Al with Fe, Mn, and K has previously been reported (Foy 1988), we found no effect of $FeSO_4$, $MnCl_2$, and KCl on Al-inhibited root growth of rice seedlings (Figs. 5A, 5C, and 5D).

Organic acids are known to detoxify Al toxicity (Ma 2000, Ma *et al.* 2001). Based on pure solution experiments, Hue *et al.* (1986) classified citrate, oxalate, and tartarate as strong Al detoxifiers, malate and malonate as moderate Al detoxifiers, and acetate and succinate were weak Al detoxifiers. Here, among six organic acids tested, we show that malate, citrate, and tartarate were the most effective, and oxalate was moderate effective in reducing Al-inhibited root growth of rice seedlings (Figs. 7C, 7D, 7E, and 7F). However, acetate and succinate were observed to be ineffective in detoxify Al toxicity in rice seedlings (Figs. 7A and 7B). Citrate, malate, oxalate, and tartarate treatments significantly reduced the increase of Al level in roots of rice seedlings (Fig. 8). These results are consistent with the general contention that organic acids detoxify Al in root medium solution by chelating Al (Ma 2000, Ma *et al.* 2001).

In conclusion, the presence of calcium, magnesium, phosphorus, and citric acid in Kimura B seems to be the reasons that $AlCl_3$ dissolved in Kimura B solution is less effective in inhibiting root growth of rice seedlings than $AlCl_3$ dissolved in distilled water.

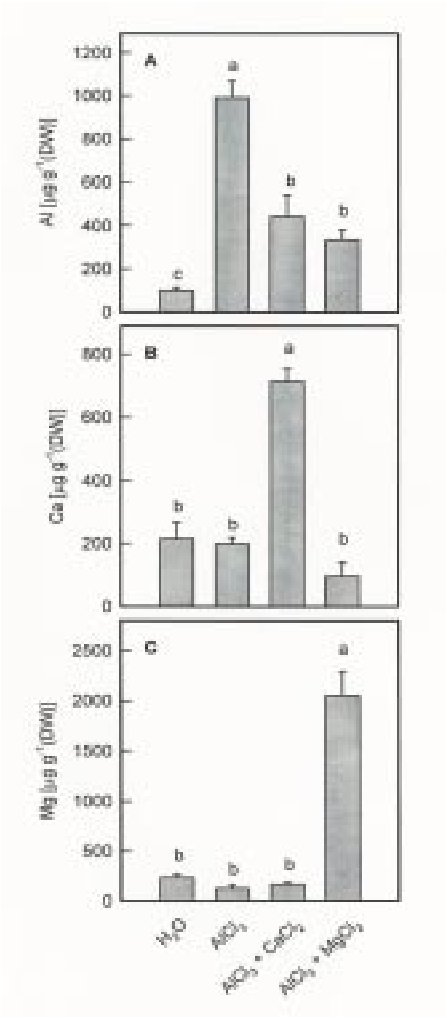


Fig. 6. Effect of $AlCl_3$, $CaCl_2$, and $MgCl_2$ on the levels of Al, Ca, and Mg in roots of rice seedlings. Two-day-old seedlings were treated with distilled water (pH 4.0), 0.5 mM $AlCl_3$ (pH 4.0), 0.5 mM $AlCl_3$ + 5mM $CaCl_2$(pH 4.0) or 0.5 mM . $AlCl_3$ + 5mM $MaCl_2$ (pH 4.0) for 2 days. Values with the same letter are not significantly different at $P < 0.05$, according to Duncan's multiple range test.

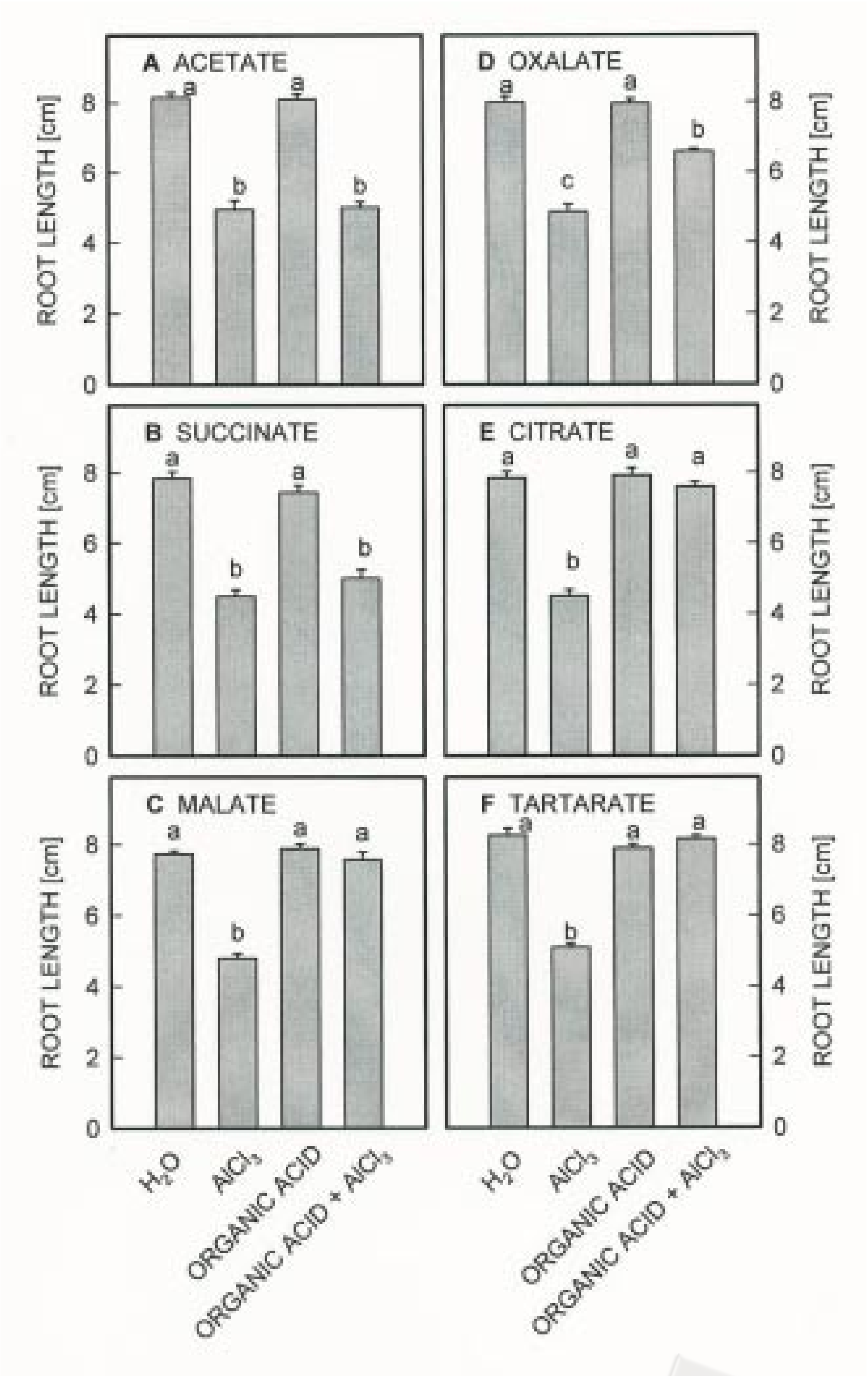


Fig. 7. Effect of organic acids on $AlCl_3$-inhibited root growth of rice seedlings. Two-day-old seedlings were treated with various organic acids (0.5 mM, pH 4.0) in the presence or absence of $AlCl_3$ (0.5 mM, pH 4.0) for 2 days. Values with the same letter are not significantly different at $P < 0.05$, according to Duncan's multiple range test.

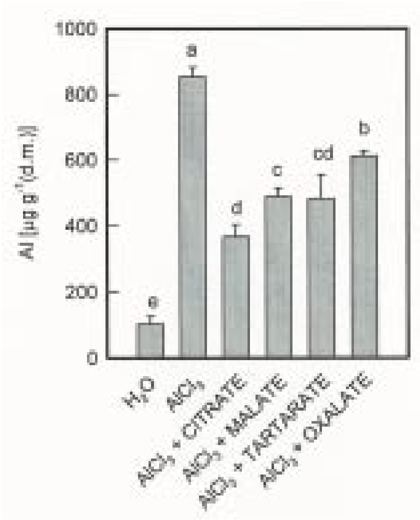


Fig. 8. Effect of $AlCl_3$ and organic acids on Al level in roots of rice seedlings. Two-day-old seedlings were treated with distilled water (pH 4.0), 0.5 mM $AlCl_3$ (pH 4.0), 0.5 mM $AlCl_3$ and 0.5 mM organic acids (pH 4.0) for 2 days. Values with the same letter are not significantly different at $P < 0.05$, according to Duncan's multiple range test.

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