Negative regulation of aluminum-responsive citrate efflux from roots of *Cassia tora* by an anion channel antagonist

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**ABSTRACT.** In a search for the regulatory basis of citrate efflux, three anion channel antagonists, anthracene-9-carboxylic acid (A9C), niflumic acid (NIA), and phenylglyoxal (PG) were examined for their effects on the aluminum-responsive citrate release. Treatment with 8 µM A9C for 9 h resulted in a 60% decrease in Al-responsive citrate release in *Cassia tora*. However, no inhibitory effects of NIA and PG on the citrate efflux were found. Because Al-induced citrate efflux was linked to the plant tolerance to Al toxicity, the root growth and Al accumulation were measured at the same time. Treatment of the seedlings with 20 µM Al alone inhibited root elongation, but simultaneous incubation with A9C resulted in an additional inhibition of root growth and Al accumulation. By contrast, NIA and PG exerted no effects on root growth. Three antagonists examined in the present study had no effect on the activities of citrate synthase (CS, EC 4.1.3.7) and aconitase (Aco, EC 4.2.1.3), or on the citrate accumulation in the Al-treated root tips, suggesting that the inhibition of Al-responsive citrate efflux by the anion channel antagonists was not involved in citrate metabolism. Further, we examined the interaction between A9C and salicylic acid (SA), which has been found to promote the Al-responsive citrate efflux and thereby Al tolerance in *C. tora*. Treatment with A9C at 8 µM exerted a negative effect on the SA promotion of Al-responsive citrate efflux. We also found that the cancellation of SA effect by A9C on citrate efflux caused an additional inhibition of root growth in the presence of Al. Taken together, we speculate that a putative A9C-sensitive anion channel may be responsible for the mediation of Al-activated citrate efflux in the roots of *Cassia tora*.

**Keywords:** Aluminum; Anion channel; Antagonist; Citrate exudation.

**INTRODUCTION**

Acid soils make up about 40% of the arable land of the world (Foy et al., 1978). In China, they cover 21% (Wang and Lin, 1993). The solubility of aluminum in neutral and alkaline soils is low. However, in acidic soils aluminum becomes soluble, and the concentration of free Al³⁺ increases considerably, resulting in rapid root growth inhibition, the most easily recognized symptom of aluminum toxicity (Kochian, 1995; Tesfaye et al., 2001). So far, a variety of mechanisms for Al toxicity to plants have been proposed (Barceló and Poschenrieder, 2002; Kochian et al., 2004). Meanwhile, mechanisms for Al tolerance in a wide range of plant species have also been identified (Matsumoto, 2000). Among them, the exudation of organic acids from roots and chelation of Al with organic acids outside cells is the most often advanced (Barceló and Poschenrieder, 2002). Citrate, malate, and oxalate are organic acids commonly released in plants upon Al exposure (Tesfaye et al., 2001; Yang et al., 2001; Anoop et al., 2003), and these can chelate Al³⁺, thus preventing it from entering the root cells. Although a number of tolerant plant species exhibiting the exudation of organic acids upon Al exposure have been identified, the regulatory mechanism relating to physiological processes remains to be elucidated.

Increasing evidence has shown that exudation of organic acids might occur through an Al-activated (Ryan et al., 1997; Kollmeier et al., 2001) or phosphorus deficiency-induced (Zhang et al., 2004) anion channel localized in the plasma membrane of root apexes. Several lines of study have indicated that the anion channels are closely associated with citrate or malate efflux (Ryan et al., 1997; Kollmeier et al., 2001; Piñeros and Kochian, 2001; Zhang et al., 2001). Since the relationship between Al-activated opening of anion channels and permeable organic acids has been established, the anion transport systems are considered likely candidates mediating the Al-responsive efflux of organic acids (Piñeros et al., 2002).
However, these channels may be different in type and nature (Ryan et al., 1997; Kollmeier et al., 2001; Piñeros et al., 2001; Zhang et al., 2001; Zhang et al., 2004). For example, in wheat the Al-activated channels required extracellular Al\(^{3+}\) to maintain channel activity (Ryan et al., 1997) while in maize root cortical cells, the opening of Al-activated channels did not require a continuous Al exposure for their active state (Kollmeier et al., 2001). The suggestion has been made that the efflux of organic acids may be mediated by different transporters (Piñeros et al., 2001). Meanwhile, some researchers have employed pharmacological or physiological antagonists to investigate the properties of anion transporters in plants (Yamamoto et al., 1997; Mithöfer et al., 2001). By using antagonists of anion channels, Ryan et al. (1995) were able to demonstrate that the Al-responsive efflux of malate from tolerant wheat plant was blocked by niflumic acid. However, in another Al tolerant plant species, buckwheat, no inhibition of oxalate release by niflumic acid was observed (Zheng et al., 1998).

*Cassia tora* possesses an Al tolerance mechanism responsible for Al exclusion by citrate release in roots (Ishikawa et al., 2000). However, the regulatory process of Al-responsive citrate efflux is still unknown. Recently, we have identified several key enzymes involved in the pathway of citrate synthesis (Yang et al., 2004) and an intermediate component that is involved in the modulation of citrate release (Yang et al., 2003). In this report, we further investigated the physiological effects of three different anion channel antagonists, anthracene-9-carboxylic acid (A9C), niflumic acid (NIA) and phenylglyoxal (PG) on the Al-responsive citrate efflux. In addition, we have identified the responses of citrate synthase and aconitase activities and root citrate accumulation, which are closely linked to citrate metabolism. The outcome of the present study may help us in understanding the underlying mechanisms for the regulation of Al-induced citrate efflux and their relation to Al tolerance of the plant species.

**MATERIALS AND METHODS**

**Plant material and treatment**

Seeds of *Cassia tora* L. were selected and soaked in distilled water overnight and distributed on a net tray. When seeds were germinated, seedlings were transferred to an aerated solution containing 0.5 mM CaCl\(_2\) (pH 4.5) and grown at 22°C for 3 days, with a light intensity of 100 µmol m\(^{-2}\) s\(^{-1}\) and 14 h photoperiod. The solution was changed daily. After that, the seedlings were transferred to plastic containers containing 0.5 mM CaCl\(_2\) (pH 4.5) solution with 20 µM Al and/or different concentrations of anion-channel antagonists, anthracene-9-carboxylic acid (A9C), niflumic acid (NIA), and phenylglyoxal (PG). The concentrations of antagonists were 0, 2, 4, 8 and 12 µM, and the SA concentration was 0.5 µM, where the Al-responsive citrate efflux is optimally stimulated (Yang et al., 2003). Plants were harvested, and 30 root tips (for one treatment) of 0.5 cm were collected. Harvested root tips were immediately frozen in liquid nitrogen and stored at -80°C for further analysis. Both antagonists and salicylic acid were prepared in the 0.5 mM CaCl\(_2\) solution.

**Collection of root exudates and citrate measurement**

Following the treatments, seedlings were removed from the treatment solutions. The remaining solutions containing root exudates were collected, frozen, and lyophilized (Yang et al., 2003). The dried exudates were dissolved in 5 mL distilled water and passed through a cationic exchange resin column (16 mm × 25 cm) containing 4 g of Dowex 5W × 8 (100-200 mesh, H\(^+\) form). The eluates were freeze-dried again, and the residue was finally dissolved in 1 mL distilled water for citrate measurement.

Citrate concentrations in exudates and root tips were measured according to the modified method described by Yang et al. (2004). Root tips were ground in 1 mL solution of ice-cold 0.6 N perchloric acid, and the homogenate was centrifuged at 15000 g at 4°C for 5 min. The supernatant was collected and neutralized with 80 µL of K\(_2\)CO\(_3\) (69%, w/v). The neutralized solution was centrifuged at 15000 g at 4°C for 5 min and then used to determine citrate content. Usually, 300 µL of sample solution was incubated with 120 µL of buffer (1 M Tris-HCl, pH 7.8), 15 µL of 10 mM NADH, 2 µL of a lactate dehydrogenase (1.25 units)/malate dehydrogenase (6 units) mixture, and 650 µL distilled water. After a stable reading, 10 µL citrate lyase (0.5 unit) was added, and the decline in A\(_{340}\) due to oxidation of NADH was monitored.

**Enzyme assay**

Root tips were homogenized in iced-cold 50 mM HEPES-NaOH buffer (pH 7.5) containing 5 mM MgCl\(_2\), 5 mM EDTA, 10% (v/v) glycerol, and 0.1% (v/v) Triton X-100. The activity of citrate synthase was spectrophotometrically assayed by recording a decrease in acetyl CoA at 412 nm for 4 min. The reaction mixture contained 100 mM Tris-HCl buffer (pH 8.0), 5 mM MgCl\(_2\), 0.5 mM 5,5-dithio-bis-2-nitrobenzoic acid, 0.2 mM acetyl CoA, and 1 mM oxalacetic acid (Yang et al., 2003). Aconitase activity was spectrophotometrically measured at 240 nm by following the formation of cis-aconitate from isocitrate in the solution containing 75 mM Tris-HCl (pH 7.6), 30 mM citric acid (Kennedy et al., 1983). The total protein content in enzyme extracts was determined by the method of Bradford using bovine serum albumin as a standard (Bradford, 1976).

**Determination of Al content in root tips**

The collected root tips were placed in a 1.5 mL Eppendorf tube, and to it 1 mL of 2 N HCl was added. Al in root apexes was extracted and measured by a graphite furnace atomic absorption spectrophotometer (180-80 Hitachi, Tokyo).
**Statistical analysis**

In citrate efflux experiments, 70 seedlings were used for a treatment. For measurement of root elongation, a minimum of 15 seedlings was sampled for a treatment. Each result shown in tables and figures was the mean of at least three replicated treatments. The significance of differences between treatments was statistically evaluated by standard deviation and Student’s *t*-test methods.

**RESULTS**

**Effect of anion-channel antagonists on Al-responsive citrate efflux, Al content and root growth**

Citrate in the root-bathing solution without Al was undetectable based on the method used in the present study (Table 1). Also, application of A9C, NIA or PG in the absence of Al had no effect on citrate efflux (Table 1). Treatment of seedlings with 20 µM Al for 9 h induced a high level of citrate release from roots. However, application of 8 µM A9C for 9 h resulted in a 60% smaller release. No inhibitory effects of either NIA or PG were observed on the citrate efflux from Al-treated roots.

Since efflux of organic acids confers Al tolerance in plants, a decrease in citrate efflux after treatment with A9C might cause decline in Al exclusion from root tips. As shown in Table 1, Al content in root tips treated simultaneously with 20 µM Al and 8 µM A9C increased by 1.45% over 20 µM Al alone. Nevertheless, neither NIF nor PG had any effect on the accumulation of Al.

Because inhibition of root growth is the primary response to Al toxicity, the root elongation was measured. Although treatment with 20 µM Al alone was able to inhibit root growth, simultaneous treatment with A9C caused an additional inhibition of root growth, with a 33% decrease as compared to the control (Table 1). By contrast, neither NIF nor PG had any significant effects on root growth.

The significant impact of A9C on both citrate release and root growth was displayed in a dose-dependent manner in the presence of Al (Figure 1). For instance, A9C (12 µM) decreased Al-induced citrate efflux by 67%. Likewise, A9C-induced inhibition of citrate efflux was observed in a time-course study, in which the inhibition caused a 33% decrease as compared to the control (Table 1). By contrast, neither NIF nor PG had any significant effects on root growth.

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![Figure 1](image-url)  
Figure 1. Effect of A9C on the citrate efflux and root elongation of *C. tora* in the presence of Al. Seedlings were exposed to the 0.5 mM CaCl$_2$ (pH 4.5) solutions containing various concentrations of A9C with 20 µM Al for 9 h. Vertical bars represent standard deviation of the mean (*n*=3). Asterisks indicate that mean values are significantly different between the A9C treated and control seedlings (0 µM A9C) (*p* < 0.05).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Citrate efflux (nmol plant$^{-1}$ 9 h$^{-1}$)</th>
<th>Al content (nmol tip$^{-1}$)</th>
<th>Root elongation (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-Al</td>
<td>–</td>
<td>0.11±0.01</td>
<td>0.81±0.06</td>
</tr>
<tr>
<td>-Al+A9C</td>
<td>–</td>
<td>0.12±0.02</td>
<td>0.79±0.06</td>
</tr>
<tr>
<td>-Al+NIF</td>
<td>–</td>
<td>0.09±0.01</td>
<td>0.83±0.07</td>
</tr>
<tr>
<td>-Al+PG</td>
<td>–</td>
<td>0.10±0.01</td>
<td>0.73±0.07</td>
</tr>
<tr>
<td>+Al</td>
<td>1.47±0.15</td>
<td>1.46±0.20</td>
<td>0.42±0.04</td>
</tr>
<tr>
<td>+Al+A9C</td>
<td>0.59±0.07</td>
<td>2.11±0.18</td>
<td>0.28±0.03</td>
</tr>
<tr>
<td>+Al+NIF</td>
<td>1.52±0.06</td>
<td>1.37±0.09</td>
<td>0.44±0.03</td>
</tr>
<tr>
<td>+Al+PG</td>
<td>1.51±0.09</td>
<td>1.41±0.14</td>
<td>0.39±0.04</td>
</tr>
</tbody>
</table>

“–” Undetectable.

Table 1. Effects of anion channel antagonists, A9C, NIA and PG on the root citrate efflux, Al content and the root elongation of *Cassia tora*. Seedlings were exposed to 0.5 mM CaCl$_2$ (pH 4.5) solutions containing 8 µM antagonists with 0 or 20 µM Al for 9 h. Values are the means ± SD (*n*=3).
in the Al-treated roots exposed to 8 µM A9C for 9 h was threefold greater than the controls (20 µM Al alone) (Figure 2). In a parallel experiment, the correlation between the Al-responsive citrate efflux and inhibition of root growth in the presence of A9C was observed (Figures 1 and 2).

A 3-h pulse of Al treatment experiment was performed to check the possibility that A9C interferes with Al\(^{3+}\) action in the treatment solution, thus resulting in a decreased citrate release. The result showed that the Al-activated citrate efflux was blocked by the late presence of 8 µM A9C (Table 2), indicating that the interference of A9C with Al\(^{3+}\) action in the solution was of minimal importance in the experiment.

**Effect of anion-channel antagonists on the citrate content and enzyme activities in root tips**

Treatment with Al at 20 µM stimulated the citrate accumulation and citrate synthase activity, but caused decreases in the aconitase activity in root tips as compared with controls (Table 3). However, application of A9C, NIF and PG had no marked effect on the Al-responsive citrate content or the activities of enzymes. In another experiment, citrate content and activities of the enzymes in the root tips were measured as a function of A9C concentrations in the growth medium (Figure 4). The citrate content (Figure 3) and citrate synthase or aconitase activities (Figure 4) underwent no marked changes even though the concentration of A9C was raised up to 12 µM in the treatment medium.

**Effect of A9C on SA-promoted Al-responsive citrate efflux and root growth**

In our previous study, salicylic acid was found to promote the Al-responsive citrate efflux in *C. tora* (Yang et al., 2003). This finding prompted us to investigate whether A9C affected the SA-promotion of citrate exudation. At first, seedlings were pretreated with 8 µM A9C for 3 h.

![Figure 2. Effect of A9C on the pattern of Al-induced citrate efflux in *C. tora* over time. Seedlings were exposed to 0.5 mM CaCl\(_2\) (pH 4.5) solutions containing 20 µM Al (filled circles) alone or 20 µM Al + 8 µM A9C (open circles). Root exudates were collected every 3 h after the initiation of treatments. Vertical bars represent standard deviation of the mean (n=3). Asterisks indicate that the mean values are significantly different between the A9C treated and control seedlings (0 µM A9C) (p < 0.05).](image-url)

![Table 2. Effect of A9C on the citrate efflux from roots of *Cassia tora* after a 3-h pulse of Al treatment. Seedlings were first exposed to 0.5 mM CaCl\(_2\) (pH 4.5) solution containing 20 µM Al for 3 h, and then rinsed with 0.5 mM CaCl\(_2\) (pH 4.5) solution. Afterwards, the seedlings were transferred to the Al-free solution (0.5 mM CaCl\(_2\), pH 4.5) or the solution containing only 8 µM A9C and incubated for 7 h. Values are the means ± SD (n=3).](table-url)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Citrate efflux (nmol plant(^{-1}) 3 h(^{-1}))</th>
<th>Citrate synthase activity (units mg(^{-1}) protein)</th>
<th>Aconitase activity (units mg(^{-1}) protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-Al</td>
<td>0.97±0.09</td>
<td>0.12±0.01</td>
<td>0.11±0.01</td>
</tr>
<tr>
<td>-Al+A9C</td>
<td>1.02±0.07</td>
<td>0.11±0.01</td>
<td>0.10±0.02</td>
</tr>
<tr>
<td>-Al+NIF</td>
<td>1.13±0.09</td>
<td>0.12±0.01</td>
<td>0.11±0.01</td>
</tr>
<tr>
<td>-Al+PG</td>
<td>0.99±0.06</td>
<td>0.11±0.02</td>
<td>0.12±0.02</td>
</tr>
<tr>
<td>+Al</td>
<td>1.58±0.12</td>
<td>0.16±0.01</td>
<td>0.06±0.01</td>
</tr>
<tr>
<td>+Al+A9C</td>
<td>1.48±0.16</td>
<td>0.17±0.02</td>
<td>0.05±0.01</td>
</tr>
<tr>
<td>+Al+NIF</td>
<td>1.46±0.09</td>
<td>0.15±0.01</td>
<td>0.06±0.01</td>
</tr>
<tr>
<td>+Al+PG</td>
<td>1.65±0.17</td>
<td>0.16±0.02</td>
<td>0.06±0.01</td>
</tr>
</tbody>
</table>

![Table 3. Effects of anion channel antagonists, A9C, NIF and PG on the citrate content and citrate synthase and aconitase activities in the root tips of *Cassia tora*. Seedlings were exposed to 0.5 mM CaCl\(_2\) (pH 4.5) solutions containing 8 µM antagonists with 0 or 20 µM Al for 9 h. Values are the means ± SD (n=3).](table-url)
They were then treated with 20 µM Al alone and 20 µM Al plus 5 µM SA. We found that A9C-pretreated seedlings excluded sufficient amounts of citrate after exposure to 20 µM Al for 9 h (Figure 5). However, the citrate exudation from either Al-treated or Al+SA-treated seedlings was significantly suppressed by the pretreatment with A9C.

To confirm that A9C independently acted against the effect of SA, a time-course experiment was performed. We first allowed the two sets of seedlings to receive the same treatment with both 20 µM Al and 5 µM SA for 9 h. Then, one set of the seedlings was subjected to the treatment with 8 µM A9C only for 6 h (Figure 6). The citrate efflux was analyzed. Control seedlings exhibited a progressive increase in citrate efflux after activation of Al and SA while seedlings treated with A9C for 6 h showed an immediate inhibition of citrate efflux. These results indicate a strong effect of A9C on the SA action in Al-responsive citrate efflux.

Exudation of organic acids confers Al resistance in many Al-tolerance plant species (Barceló and Poschenrieder, 2002). An increase in citrate efflux due to SA application was observed in C. tora (Yang et al., 2003). As a result, an increase in root elongation was found. After demonstrating a negative effect of A9C on the SA-regulated citrate efflux, it could be expected that

**Figure 3.** Effect of A9C on the citrate content in root tips of *Cassia tora* in the presence of Al. Seedlings were exposed to 0.5 mM CaCl2 (pH 4.5) solutions containing various concentrations of A9C and 20 µM Al for 9 h. Values are the means ± SD (n=3).

![Graph](image1)

**Figure 4.** Effects of A9C on the activities of citrate synthase (a) and aconitase (b) in the root tips of *Cassia tora* in the presence of Al. Seedlings were exposed to 0.5 mM CaCl2 (pH 4.5) solutions containing various concentrations of A9C and 20 µM Al for 9 h. Values are the means ± SD (n=3).

![Graph](image2)

**Figure 5.** A9C inhibition of Al-induced citrate efflux response to salicylic acid. Seedlings were first exposed to 0.5 mM CaCl2 (pH 4.5) solution containing 8 µM A9C for 3 h, and then transferred to the solution (0.5 mM CaCl2, pH 4.5) containing 20 µM Al alone and 20 µM Al + 5 µM SA for 9 h. Values are the means ± SD (n=3). Asterisks indicate that the mean values are significantly different between seedlings treated with 20 µM Al in the presence and absence of 5 µM SA (p < 0.05).

![Graph](image3)
application of A9C removed the effect of SA-stimulated root elongation. It was found that the root growth was strongly inhibited by the pretreatment with A9C even though the SA was present in the growth medium (Figure 7).

DISCUSSION

In acidic soils, Al-induced exudation of organic acids from plants protects plant roots from Al$^{3+}$ toxicity (Kochian, 1995). In the present study we confirmed such a response by testing the inhibitory effect of an anion channel antagonist, namely anthracene-9-carboxylate, which is frequently used for animal electrophysiology (Chao and Mochizuki, 1992; Tamai et al., 2000; Kaya et al., 2002; Parai and Tabrizchi, 2002). We found a strong negative effect of A9C on the Al-responsive citrate efflux from roots of Cassia tora. Such antagonists are useful because they may provide a basic approach to understanding the nature of anion transporters in plants. Application of anion channel antagonists in identifying different ionic transporters in plant cells has been reported (Yamamoto et al., 1997; Mithöfer et al., 2001). For example, by using several antagonists, ion-transport systems such as H$^+$-ATPase and carriers and anion channels associated with plasma or tonoplast membrane were characterized (Ryan et al., 1995; Yamashita et al., 1996). However, their function and specificity in higher plants remains to be elucidated.

Exudation of organic acids is considered to occur through Al-activated anion channels located on the plasma membrane of root tip cells in some plant species such as wheat (Ryan et al., 1997), maize (Kollmeier et al., 2001; Piñeros et al., 2001) and white lupin (Zhang et al., 2004). The response of these plants to Al differs greatly among species, and even genotypes within a species vary. These characteristics may bring about great changes in the types or the amount of released organic acids (Ryan et al., 1997). A good understanding of regulatory mechanisms for organic acid efflux by an anion channel requires more evidence from a variety of plant species. We examined the effect of anion channel antagonists on Al-responsive citrate efflux from roots of C. tora. A9C has been shown to be effective against citrate efflux (Table 1, Figure 2). These results suggest that Al-responsive citrate efflux may be associated with the activation of a putative anion channel responsible for the citrate release.

Because exudation of organic acids confers Al tolerance in C. tora (Yang et al., 2003) and other Al-tolerant plant species (Miyasaka et al., 1991; Zheng et al., 1998; Yang et al., 2001), the inhibition of citrate efflux by A9C would affect the Al exclusion from roots and thus cause an additional inhibition of root growth. Our results showed that the simultaneous treatment with 8 µM A9C for 9 h led to a more than 30% decrease in root elongation over 20 µM Al treatment alone (Table 1). Also, treatment with A9C caused relatively higher accumulation of Al in root tips (Table 1). It was noted that A9C alone was not able to exert toxicity on the roots, evidenced by the data on roots treated with 8 µM A9C showing no significant difference from the controls (Table 1). To exclude the possibility that A9C added to the root-bathing medium might trick Al$^{3+}$, thus reducing its activity or toxicity to roots, a 3-h pulse experiment was performed. Our data indicate that A9C

![Figure 6. A9C inhibition of Al-responsive citrate efflux response to salicylic acid over time. Seedlings were first incubated in 0.5 mM CaCl$_2$ (pH 4.5) solution containing 20 µM Al + 5 µM SA for 9 h, and then transferred to the 0.5 mM CaCl$_2$ (pH 4.5) solution containing 0 µM (filled circles) and 8 µM A9C (open circles) for the continuous exudation of citrate for 6 h. Root exudates were collected every 3 h after the initiation of treatments. Values are the means ± SD (n=3). Asterisks indicate that the mean values are significantly different between the two treatments.](image1)

![Figure 7. Effect of A9C on SA promotion of root growth in the presence of Al. Seedlings were first exposed to 0.5 mM CaCl$_2$ (pH 4.5) solution containing 8 µM A9C for 3 h before being transferred to the solution (0.5 mM CaCl$_2$, pH 4.5) containing 0, 20 µM Al and/or 5 µM SA for 9 h. Values are the means ± SD (n=3). Asterisks indicate that the mean values of seedlings treated with 20 µM Al in the presence of 5 µM SA differ significantly from those treated in its absence (p < 0.05).](image2)
was able to independently affect Al-responsive citrate efflux (Table 3).

Since Al-responsive efflux of citrate in *Cassia tora* (Ishikawa et al., 2000; Yang et al., 2004) and several other plant species (Miyasaka et al., 1991; Piñeros et al., 2002) is associated with the activation of citrate metabolism, it becomes important to determine the activities of enzymes closely linked to citrate synthesis and degradation. Our results demonstrate that neither citrate synthase nor aconitase activities were affected by the tested anion channel antagonists in this study. We also measured the citrate content and did not find a marked change in the level of citrate in the root tips. Therefore, we conclude that the action of these antagonists was not involved in the mediation of the citrate metabolism in the presence of aluminum.

Earlier study with *C. tora* showed that exogenous application of SA at 5 μM promoted the Al-responsive citrate efflux, and this effect was correlated with decreases in Al-induced inhibition of root growth as well as in Al accumulation in root tips (Yang et al., 2003). These data could allow us to analyze whether the inhibitor A9C could block the effect of SA on the Al-responsive citrate efflux. Nearly 70 percent of citrate efflux was inhibited by the addition of 8 μM A9C to the medium in the presence of Al and SA as compared to the control (Al and SA only) (Figure 5). However, it caused no difference in citrate efflux between the +Al+ A9C- and +Al+ A9C+SA-treated plants. This result suggests that A9C can abolish the release of citrate from both Al-induction and SA-promotion in root tips. To confirm the A9C action, a similar experiment for root elongation was performed. The root elongation in the presence of Al and SA decreased by about 50 percent due to addition of 8 μM A9C when compared with control (Figure 7). Although the process by which A9C acted on SA effects is not clear in the present study, A9C might possibly block the opening of a putative citrate permeable anion channel stimulated by SA. More detailed studies are required to characterize the anion channels of the plasma membrane of *C. tora*.

**LITERATURE CITED**


Ryan, P.R., M. Skerrett, G.P. Findlay, E. Delhaize, and S.D. Tyerman. 1997. Aluminum activates an anion channel in...


本文研究了三種陰離子通道抑制劑 anthracene-9-carboxylic acid (A9C), niflumic acid (NIA) 和 phenylglyoxal (PG) 對鉄應答的檸檬酸分泌調節的影響。8 µM A9C 處理 9 小時導致決明植株檸檬酸分泌量減少 60%。但 NIA 和 PG 對檸檬酸分泌沒有任何影響。因為鉄誘導檸檬酸分泌與植株對鉄毒害的耐性有相關性，因此，同時測定了根的生長和鉄的積累。試驗觀察到，與單獨 20 µM Al 處理比較，同時用 A9C 處理則使根的伸長受到更大的抑制，而 NIA 和 PG 對根生長沒有影響。三種抑制劑對檸檬酸合成酶（EC 4.1.3.7）和順烏頭酸酶（EC 4.2.1.3）活性及根尖中檸檬酸的含量沒有影響，表明陰離子通道抑制劑對檸檬酸分解的分泌和檸檬酸的代謝沒有關係。由於水楊酸（salicylic acid, SA）能促進鉄誘導檸檬酸分泌和植株的耐鉄性，本研究測定了 A9C 對 SA 的作用效應。結果表明，A9C 對 SA 的作用具有負效應。上述結果使我們推測，在決明植株中可能存在一個對 A9C 敏感的陰離子通道，負責鉄啟動的檸檬酸的分泌。

**關鍵詞：**鉄；檸檬酸分泌；陰離子通道；抑制劑。