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Specific Secretion of Citric Acid Induced by Al Stress in *Cassia tora* L.

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A rapid and sensitive assay method for Al-chelating activity was established to screen Al-chelating substances secreted from roots of Al-resistant species in response to Al stress. From one Al-resistant species, *Cassia tora* L., an Al-chelating substance was detected in the root exudates when they were exposed to 50 μM Al in 0.5 mM $CaCl_2$ solution at pH 4.5; the dominant component was identified as citric acid. The secretion of citric acid was very low during the first 4 h after initiation of Al treatment, but increased markedly thereafter. A 3-h pulse with 50 μM Al also induced significant secretion of citric acid after 6 h. The lag between Al addition and secretion of citric acid suggests that inducible processes are involved. A dose-response experiment showed that the amount of secreted citric acid increased with increasing external concentrations of Al. Eight-d treatment of P deficiency did not induce the secretion of citric acid. Exposure to 50 μM of either lanthanum (La^{3+}) or ytterbium (Yb^{3+}) did not induce the secretion of citric acid either. These findings indicate that the secretion of citric acid is a response specific to Al stress in *C. tora* and constitutes a mechanism of Al resistance.

Key words: Aluminum — *Cassia tora* L. — Citric acid — Secretion — Resistance.

Plant species and cultivars within species vary widely in their resistance to Al toxicity, which is a major factor limiting crop production on acid soils. Recently, much attention has been paid to understand the mechanisms of Al resistance involved in Al-resistant species. These mechanisms can be divided into two types, exclusion and internal tolerance, depending on whether the site of Al detoxification is in the apoplasm or symplasm (Taylor 1991, Kochian 1995). Many hypotheses for both exclusion and internal tolerance mechanisms have been proposed, but most of these mechanisms are still speculative, with little supporting evidence (Delhaize and Ryan 1995, Kochian 1995).

The phytotoxicity of Al varies with its chemical species. Studies have shown consistently that the chelated form of Al is less toxic to plant growth than the ionic form, Al^{3+} (e.g. Hue et al. 1986). These results led researchers to propose that secretion of Al-chelating substances may be one of the exclusion mechanisms for Al resistance (see Taylor 1991, Kochian 1995). The first direct evidence was presented by Miyasaka et al. (1991), who found that citric acid was released by roots of an Al-resistant snapbean in response to Al stress. Later, Delhaize et al. (1993) used near-isogenic wheat lines differing in Al resistance at the Al resistance locus (*Alt* 1), and found that Al-resistant genotypes excreted 5- to 10-fold more malic acid than Al-sensitive genotypes. They noted that malic acid was secreted only from the root apex (the critical site for Al toxicity) (Delhaize et al. 1993, Ryan et al. 1995). Basu et al. (1994) reported similar differences in malic acid secretion from several other Al-resistant and Al-sensitive wheat cultivars. Ryan et al. (1995) established a correlation between Al-stimulated malic acid secretion and Al resistance using a wide range of wheat genotypes differing in Al resistance. All of these findings suggest that malic acid secretion stimulated by Al is a general Al resistance mechanism in wheat. In contrast, in Al-resistant and Al-sensitive maize lines, Pellet et al. (1995) found that Al rapidly triggered excretion of citric acid from the root apex of the Al-resistant genotypes. The secretion of citric acid in response to Al was also reported in a stress-selected cell line of carrot (Ojima and Ohira 1988). However, it remains to be shown whether the mechanism of secretion of organic acids in response to Al stress is also employed by other Al-resistant species or cultivars. Substances other than organic acids which have Al-chelating activity may be secreted in response to Al stress in other plant species as an exclusion mechanism. Therefore, we examined whether the secretion of Al-chelating substances is present in Al-resistant plants and what the kinds of compounds secreted in response to Al stress are. For this purpose, a chemical assay method for Al-chelating activity was first established. Using this method, Al-chelating activity was detected in root exudates from several Al-resistant plants exposed to Al. One of them, *Cassia tora* L., was found to specifically secrete citric acid from the roots in response to Al stress. The secretion pattern is quite different from that of malic acid observed in wheat (Delhaize et al. 1993, Ryan et al. 1995). These results are reported here.

Materials and Methods

Assay method for Al-chelating activity—Solution samples (several μl) were dropped onto a filter paper of adequate size (No. 1, Advantec, Tokyo). The paper was dried and then sprayed with an $AlCl_3$ solution prepared by mixing 50 ml of 5 mM $AlCl_3$, 6 ml of 2 M HCl, 40 ml of distilled water, and 120 ml acetone. The paper was then immersed in 1/15 M phosphate buffer (pH 6.8) (Wako, Tokyo) for 3 min and washed in distilled water for 1 min. Finally, the paper was placed in pyrocatechol violet solution (37.5 mg dissolved in 100 ml of 0.2 mM acetate buffer pH 5.6) (Dojindo, Kumamoto) for 3 min. After washing in distilled water for ca.

2 min to remove excess dye, white spots on a blue background were visible if samples contained Al-chelating substances.

Solutions (3 μl) of citric acid (0.25 to 3 mM) and different organic acids were tested by the above assay method. Preliminary tests showed that malic and succinic acids could not be detected at the same concentration as citric acid. Different concentrations for citric (2.5 mM), malic (20 mM), and succinic acids (100 mM) were employed.

Plant materials—Seeds of *Cassia tora* L. were gently ground with sea sand (20–30 mesh) for ca. 10 s to facilitate germination. Treated seeds were soaked in distilled water overnight and then germinated on a net tray in the dark at 25°C. Filter paper saturated with distilled water was used to cover the seeds. After 2 d, the tray was put in a plastic container containing a nutrient solution (one-fifth strength Hoagland solution) containing 1.0 mM KNO_3, 1.0 mM $Ca(NO_3)_2$, 0.4 mM $MgSO_4$, and 0.2 mM $NH_4H_2PO_4$. Micronutrients were adjusted to full strength Hoagland solution; the solution was adjusted to pH 4.5 with 1 M HCl and renewed every 2 d. After a 5-d growth period in the nutrient solution, seedlings of similar size were selected and transplanted into the same aerated nutrient solution in a 1-liter plastic pot (15 seedlings per each pot). The solution was renewed every 2 d. After 9 d, the seedlings were subjected to different treatments as described below. Plants were grown in a growth cabinet (TGE-9H-S, TABAI ESPEC) at 25/20°C and 14-/10-h day/night cycles, 40 W m^{-2} light intensity, and 70% RH. Each experiment was conducted with three replicates and repeated at least twice independently.

Al treatment and growth—To confirm the Al resistance of *C. tora* L., the effect of Al treatment on the growth was investigated. An Al-tolerant cultivar of wheat (*Triticum aestivum* L.), Atlas 66, was used as a reference. Seedlings of both *C. tora* (7-d-old) and Atlas 66 (6-d-old) prepared as described above were subjected to the Al treatment. To avoid interaction between Al and other nutrients such as P, Al treatment was carried out by exposure to 50 μM Al in 0.5 mM $CaCl_2$ solution at pH 4.5 for one day; then the plants were returned to the nutrient solution without Al for another day. After a 10-d period of five repeated treatments, plants were harvested, separated into roots and shoots, and dried at 70°C. The fresh and dry weights of both roots and shoots were recorded.

Collection of root exudates—Before collection of root exudates, the roots were placed in 0.5 mM $CaCl_2$ solution at pH 4.5 overnight. Fifty μM Al in 0.5 mM $CaCl_2$ at pH 4.5 was used as a treatment solution unless stated otherwise. The Al solution was prepared by dissolving $AlCl_3 \cdot 6H_2O$ (Wako, Tokyo) prior to use. To examine the Al-chelating activity in root exudates, seedlings grown as described above were exposed to the Al solution for 12 h. The treatment solution was passed first through a cation exchange column (16 mm × 14 cm) filled with 5 g Amberlite IR-120B resin (H^+ form), and then through an anion exchange column (16 mm × 14 cm) filled with 2 g AG 1-×8 resin (100–200 mesh, formate form). The neutral fraction adsorbed by neither the cationic nor anionic resin was also collected. The cationic fraction was eluted with 2 M NH_4OH, and the anionic fraction was eluted with 1 M HCl. All these procedures were carried out in a cold room. Each fraction was evaporated to dryness using a rotary evaporator (40°C). The cationic fraction was dissolved in 10 ml distilled water and further passed through a column (16 mm × 14 cm) filled with 4 g Chelex 100 resin (100–200 mesh, sodium form) to remove metals. The unabsorbed fraction was evaporated to dryness. After the residue was redissolved in 1 ml distilled water, Al-chelating activity in each fraction was assayed as described above. Because Al-chelating activity was found only in the anionic fraction, this fraction was further purified by HPLC equipped with an ion-exclusion column (Shim-pack SCR-102 H, 8.0 mm × 30 cm, Shimadzu, Kyoto, Japan) and a guard column (6.0 mm × 5 cm). The eluant was Milli-Q water; the pH was adjusted to 2.1 using perchloric acid with a flow rate of 0.8 ml min^{-1} at 40°C. Detection was conducted at 210 nm. Only one dominant peak, which was not detected in the root exudates without Al treatment, was found in the root exudates treated with Al; this peak was collected and evaporated to dryness. ^{1}H-NMR spectra of the purified fraction (in D_2O) were recorded on a 500-MHz spectrometer (DMX500). Mass spectra of this purified fraction were measured on a JEOL JMX-HX110 spectrometer using FAB (fast-atom bombardment) negative ionization with dithiodiethanol matrix.

In the time course experiment, the seedlings grown as above were exposed to the Al solution and the root exudates were collected every 2 h. In the dose-response experiments, the seedlings were exposed to 0, 10, 30, or 50 μM Al in 0.5 mM $CaCl_2$ solution at pH 4.5 for 9 h. The pulse treatment was conducted by first exposing the seedlings to the Al solution for 3 h, and then to 0.5 mM $CaCl_2$ solution at pH 4.5 without Al. Root exudates were collected every 3 h. To characterize the specificity of secretion of citric acid, the response to P-deficiency was investigated. P deficiency was performed by subjecting one-week-old seedlings to a nutrient solution devoid of P. The root exudates were collected for 9 h every other day during the P-deficiency treatment period. Root exudates from the seedlings exposed to the Al solution for 9 h were also collected every other day for the comparison. At the end of the 8-d period of P-deficiency treatment, the roots were exposed to the Al solution for 9 h, and the root exudates were then collected. Responses to two other trivalent cations, lanthanum (La^{3+}) and ytterbium (Yb^{3+}), were also examined. The seedlings were exposed to 0.5 mM $CaCl_2$ solution containing 50 μM $LaCl_3$ (Nakarai, Kyoto), 50 μM $YbCl_3$ (Wako, Tokyo), or 50 μM $AlCl_3$ at pH 4.5 for 9 h. At the end of exposure, the root exudates were collected. All root exudates were passed through cation and anion exchange columns as described above. The anionic fraction was eluted using 1 M HCl, and concentrated to dryness. The residue was redissolved in 1 ml dilute perchloric acid (pH 2.1) and organic acids were determined by HPLC as described above after passing the sample through a filter (0.45 μm). Average recoveries of organic acids after cationic and anionic exchange resins were almost 100% as determined by using standard solutions.

Results

We established an assay method for Al-chelating activity in the present study based on the precipitation between $AlCl_3$ and phosphorus at a neutral pH and the subsequent reaction of this Al-P complex with pyrocatechol violet. If a sample contains an Al-chelating substance, it chelates Al^{3+}, and the precipitation of Al with phosphorus is suppressed; the extent depends on the comparative stability of each compound and its concentration. After washing, the Al chelate is removed from the filter paper, while the precipitate of Al-P is retained. With placed in a dilute solution of pyrocatechol violet, a blue complex forms with Al on the filter paper as a background, while a white spot appears corresponding to the portion where Al has been removed. Using a standard solution of citric acid, 3 μl of 1 mM citric acid was clearly detectable (Fig. 1A), indicating that the

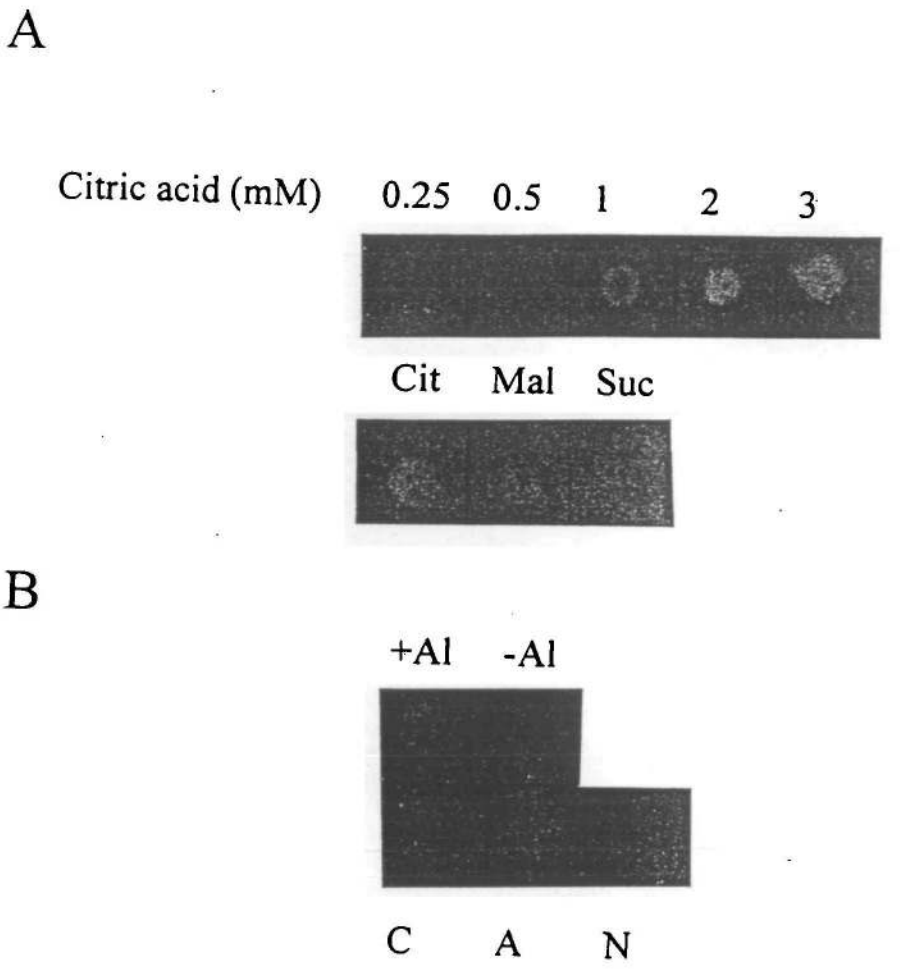

Fig. 1 Al-chelating activity assayed by a chemical method. A, 3 μl of citric acid ranging from 0.25 to 3 mM was spotted (upper panel), and 3 μl of citric acid (2.5 mM), malic acid (20 mM), and succinic acid (100 mM) were spotted (lower panel). B, Concentrated root exudates from *Cassia tora* L. treated with 50 μM Al (+Al) or without Al (−Al) (upper panel) and cationic (C), anionic (A), and neutral (N) fractions of root exudates separated by ion exchange resin (lower panel). (See Materials and Methods for details.)

minimum detection of this assay method was 3 nmol. Different organic acids had different detection limits (Fig. 1A). Sixty nmol was required for malic acid, but even 300 nmol of succinic acid was hardly detectable.

This method was applied to screen the Al-chelating substances secreted by roots of Al-resistant plants. When roots of *C. tora* L. were exposed to Al for 12 h, Al-chelating activity was detected in the root exudates, but it was not detected in the root exudates without Al treatment (Fig. 1B). Separation of the root exudates into cationic, neutral, and anionic fractions revealed that only the anionic fraction showed Al-chelating activity (Fig. 1B). Analysis of the anionic fraction by HPLC indicated one dominant peak in the root exudates from Al-treated roots but not from the roots without Al. This peak was eluted at 8.8 min, which was consistent with the retention time of citric acid. This peak was further purified by ion-exclusion column, and the structure was confirmed by ^{1}H-NMR and FAB-MASS. The ^{1}H-NMR spectrum of purified ligand displayed signals corresponding to methylene protons at 2.74 ppm (1H, doublet, J=15.8 Hz) and 2.92 ppm (1H, doublet, J=15.8 Hz). Both the chemical shifts and the coupling constants were consistent with those of citric acid. The negative-ion FAB mass spectrum of the purified fraction exhibited pseudomolecular ion peaks $[M-H]^-$ at m/z 191. These findings indicated that the dominant component secreted by the roots of *C. tora* L. in response to Al was citric acid.

C. tora L. was previously reported as an Al-resistant species (Kito and Hattori 1996). The extent of resistance of *C. tora* L. to Al toxicity was checked using hydroponic culture. The interval treatment with 50 μM Al for 10 d hardly affected the dry weight of either shoot or root (data not shown). An Al-resistant cultivar of wheat, Atlas 66, was

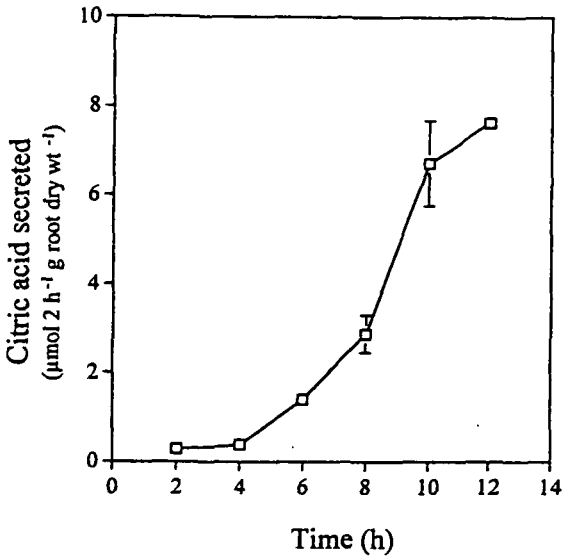

Fig. 2 Time course of citric acid secretion by *Cassia tora* L. exposed to 0.5 mM $CaCl_2$ solution (pH 4.5) containing 50 μM Al. Root exudates were collected every 2 h after initiation of Al treatment. After passing the root exudates through cationic resin (Amberlite IR-120B, H^+ form) followed by anionic resin (AG 1-×8, formic form), the anionic fraction was eluted using 1 M HCl and concentrated. Organic acids were analyzed by HPLC. Vertical bars represent ±SE (n=3).

used as a reference; the root dry weight of the wheat was decreased by about 20% by the same treatment, although the dry weight of the shoot was unaffected. This suggests that *C. tora* L. is quite resistant to Al toxicity. A difference in the amount of organic acids secreted was also observed between these two species. The rate of citric acid secretion

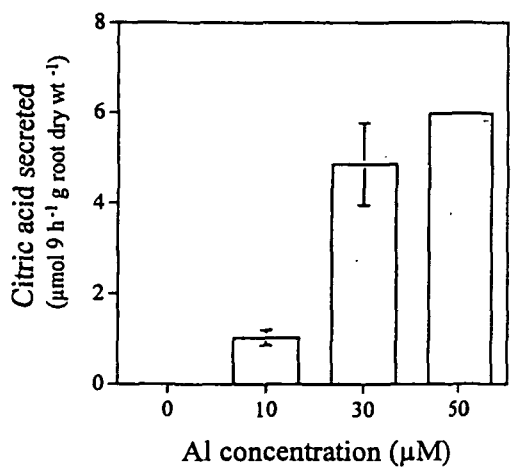

Fig. 3 Effect of external Al concentration on secretion of citric acid. Seedlings were exposed to 0.5 mM $CaCl_2$ solution (pH 4.5) containing 0, 10, 30, or 50 μM Al. During 9 h of exposure, root exudates were collected, and organic acids were analyzed as described in Fig. 2. Vertical bars represent ±SE (n=3).

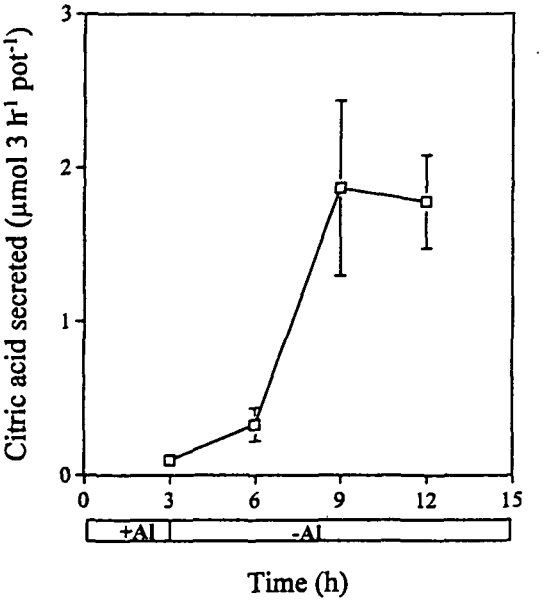

Fig. 4 Effect of a 3-h pulse of 50 μM Al on the secretion of citric acid. Seedlings were subjected to 0.5 mM $CaCl_2$ solution (pH 4.5) containing 50 μM Al for 3 h and subsequently to 0.5 mM $CaCl_2$ solution (pH 4.5) without Al. Root exudates were collected every 3 h. Organic acids were analyzed as described in Fig. 2. Vertical bars represent ±SE (n=3).

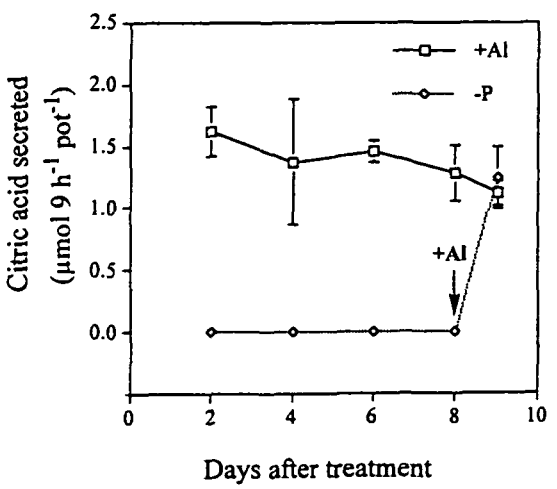

Fig. 5 Secretion of citric acid in response to P deficiency and Al. Seedlings were subjected to P deficiency for 8 d; the root exudates were collected by exposing the roots to 0.5 mM $CaCl_2$ solution for 9 h every other day during P-deficient treatment. Al treatment was conducted by exposing the seedlings to 0.5 mM $CaCl_2$ solution (pH 4.5) containing 50 μM Al for 9 h every other day; the root exudates were again collected. At the end of P-deficient treatment, the seedlings were exposed to 50 μM Al in 0.5 mM $CaCl_2$ solution (pH 4.5) for 9 h; the root exudates were yet again collected. Organic acids were analyzed as described in Fig. 2. Vertical bars represent ±SE (n=3).

was 10.1 ± 1.95 μmol 12 h^{-1} (g root dry wt)$^{-1}$ in *C. tora* L., while that of malic acid secretion was 4.36 ± 0.66 μmol 12 h^{-1} (g root dry wt)$^{-1}$ in Atlas 66.

Citric acid secretion was very low during the first 4 h after exposure to the Al solution but increased markedly thereafter (Fig. 2). The amount of citric acid secreted increased with increasing external Al concentration (Fig. 3). A 3-h pulse with 50 μM Al also induced significant secretion of citric acid after 6 h (Fig. 4), but the secretion decreased after 12 h (data not shown).

Citric acid was not detected during the 8-d period of P deficiency (Fig. 5). In contrast, Al-induced secretion of citric acid was observed every time after exposure to Al. Exposure of P deficient seedlings to Al at the end of the treatment resulted in secretion of citric acid. When the roots were exposed to lanthanum (La^{3+}) or ytterbium (Yb^{3+}) solution at 50 μM, no secretion of citric acid was detected (Fig. 6), although 50 μM Al induced secretion of citric acid at 6.47 ± 0.61 μmol (g root dry wt)$^{-1}$ over 9 h.

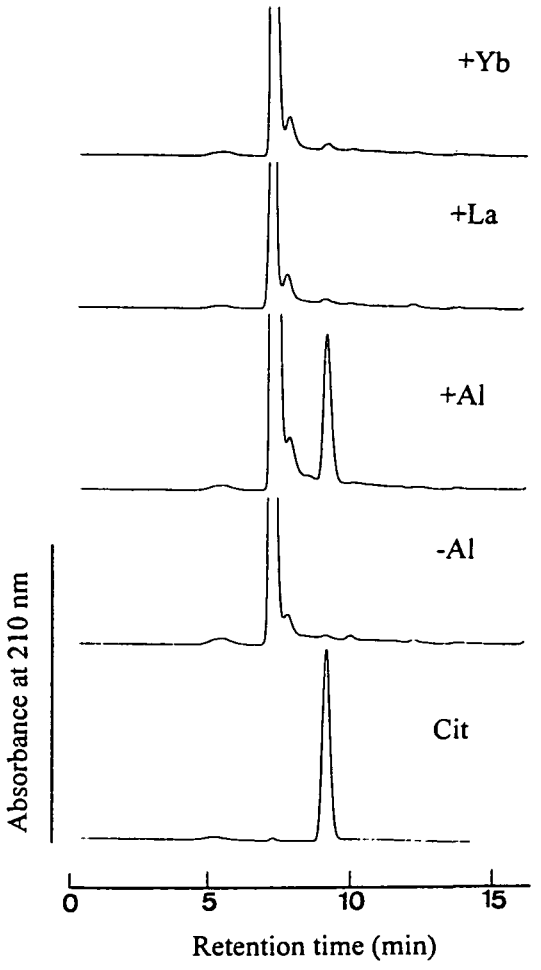

Fig. 6 HPLC profile of root exudates in the presence and absence of Al^{3+}, Yb^{3+}, La^{3+}. Seedling were exposed to 0.5 mM $CaCl_2$ solution (pH 4.5) containing 0 (−Al), 50 μM of $AlCl_3$ (+Al), $LaCl_3$ (+La), or $YbCl_3$ (+Yb). During the 9-h exposure, root exudates were collected, and organic acids were analyzed as described in Fig. 2. Cit, 2 mM citric acid. Vertical bars represent ± SE (n = 3).

Discussion

The primary visual symptom of Al toxicity is rapid inhibition of root elongation. However, the processes that lead to the inhibition of root growth remain unknown, although the root apex has been shown to be the toxicity site (Ryan et al. 1993, Kochian 1995). Many mechanisms of Al toxicity have been proposed, including Al interactions within the root cell wall, Al disruption of the plasma membrane and PM transport processes, and Al interactions with symplasmic constituents such as calmodulin (Kochian 1995). Al toxicity can be alleviated or completely prevented by Al-chelating substances such as organic acids. The alleviative extent is dependent on the substances' specific chelating ability (stability constant; log K_{eq} of Al complexes). Equimolar citric acid to Al (log K_{eq} of Al-citrate, 12.26) completely prevented the Al-induced inhibition of root elongation (Hue et al. 1986, Ma et al. 1997), while 5 to 8 times malic acid to Al (log K_{eq} of Al-malate, 6.0) was required to overcome root inhibition in Al-sensitive cultivars of wheat (Delhaize et al. 1993, Basu et al. 1994). Succinic acid (log K_{eq} of Al-succinate, 4.6) has hardly any alleviative effect (Hue et al. 1986). Citric acid reversed the inhibition of wheat root growth caused by Al (Ownby and Popham 1989). In vitro studies have indicated that citric acid reduced the Al-induced inhibition of a K^+-stimulated, Mg^{2+}-dependent, plasma membrane ATPase activity in *Pisum sativum* and *Zea mays* (Matsumoto and Yamaya 1986, Suhayda and Haug 1986). The following organic acids were able to protect calmodulin from the deleterious effects of Al in the order: citric > oxalic > malic > tartaric acids (Suhayda and Haug 1984, 1986). All these observations suggest that Al toxicity results from the binding of Al^{3+} to extracellular and intracellular components. If secretion of Al-chelating substances by roots in response to Al stress is to function as an Al-resistant mechanism, these substances must have a higher chelating ability with Al^{3+} than do cellular components and/or be secreted in sufficient amounts. To screen such substances, we first established a rapid assay method for Al-chelating activity (Fig. 1). The detection limit of this assay method for citric acid was higher than that by HPLC. This method could distinguish different Al chelating abilities of compounds (Fig. 1A) and therefore their roles in detoxifying Al.

In studying root exudates relevant to Al, both Al-resistant and Al-sensitive genotypes are usually used for comparison. As our aim is to investigate whether Al-induced secretion of Al-chelating substances is involved in the Al-resistant mechanism, only Al-resistant species were exam-

ined. Kito and Hattori (1996) found that *C. tora* L. was an Al-resistant species. Actually, its Al resistance was comparable to that of Al-resistant cultivar of wheat, Atlas 66, from the present results of 10 d interval treatment of 50 μM Al. In the root exudates of *C. tora* L. exposed to the Al solution, an Al-chelating substance was found (Fig. 1B); this substance was identified as citric acid by HPLC, ^{1}H-NMR and FAB-MASS. Although our experiments were conducted under non-sterile conditions, the amount of citric acid secreted reached 6 to 8 μmol (g root dry wt)$^{-1}$ during 9 h exposure to 50 μM Al, which was much higher than that of malic acid secreted by Atlas 66 (ca. 4 μmol (g root dry wt)$^{-1}$ 9 h^{-1}) and other Al-resistant genotypes of wheat (Basu et al. 1994). The secretion of citric acid increased with time of exposure to Al and with external Al concentration (Fig. 2, 3). This characteristic of secretion was consistent with that observed in Al-resistant genotypes of wheat (malic acid) and maize (citric acid) (Delhaize et al. 1993, Pellet et al. 1995). However, the pattern of secretion by *C. tora* L. was quite different from that by wheat. In an Al-resistant genotype of wheat, ET3, Al-stimulated secretion of malic acid from both intact roots and excised root apices was observed within 20 min after addition of Al (Delhaize et al. 1993, Ryan et al. 1995). Because there were no discernible delay between the addition of Al and the onset of malate secretion, Ryan et al. (1995) suggested that de novo protein synthesis was not required for this response. However, in *C. tora* L., the onset of citric acid secretion was obviously delayed by about 4 h after Al was added (Fig. 2). Furthermore, although malate secretion decreased rapidly after Al was removed from the external solution in wheat (Ryan et al. 1995), a significant secretion of citric acid was induced 6 h after a 3-h pulse with Al in *C. tora* L. (Fig. 4). All these observations suggest that, unlike wheat, some inducible process leading to the secretion of citric acid, such as induction of citric acid synthesis, is involved in the roots of *C. tora* L.; further research is needed to clarify this.

Miyasaka et al. (1991) presented evidence that when an Al-resistant cultivar (Dade) of snapbean (*Phaseolus vulgaris* L.) grown under aseptic conditions was exposed to Al, it secreted citric acid into the rhizosphere in a concentration that was 70 times as great as that of Dade grown without Al and 10 times as great as that of Al-sensitive cultivar (Romano) grown with or without Al. However, it was not clear whether this response was induced by Al or by P deficiency, because in their experimental conditions, P was probably precipitated as Al-phosphate in the growth solution during the 8-d culture of snapbean plants in Al. Secretion of citric acid in response to P deficiency has been reported in numerous species, including white lupine (*Lupinus albus* L.) (Gardner et al. 1983), alfalfa (*Medicago sativa* L.) (Lipton et al. 1987), and rape (*Brassica napus*) (Hoffland et al. 1989). Citric acid secreted by P-deficient white lupine can represent as much as 11% to 23% of the total plant dry weight (Gardner et al. 1983, Dinkelaker et al. 1989). To investigate the specificity of Al-induced secretion of citric acid in *C. tora* L., its response to P deficiency was compared with that to Al stress. Eight days of P deficiency did not induce the secretion of citric acid, while Al treatment did (Fig. 5). One day of P deficiency also failed to induce secretion of malic acid in wheat (Delhaize et al. 1993). Usually, there is a long lag for secretion of citric acid after P deficiency treatment. This lag was 12 d in white lupine; citric acid was first detected in the root exudates at 12 d after P deficient treatment began (Johnson et al. 1996). However, the Al-induced secretion of citric acid was observed within several hours (Fig. 2), indicating that Al-induced P deficiency was unlikely to be responsible for the secretion of citric acid in *C. tora* L.

Lanthanum shows some similarities to Al in inhibiting root growth and Ca uptake (e.g. Bennet and Breen 1992, Rengel and Elliott 1992). Root elongation was more inhibited by Yb^{3+} and La^{3+} than by Al^{3+} in both rice and pea (Ishikawa et al. 1996). The response of *C. tora* L. to Yb^{3+} and La^{3+} was investigated in terms of secretion of citric acid in the present study (Fig. 6). Neither Yb^{3+} nor La^{3+} induced secretion of citric acid. This also suggests that secretion of citric acid is a specific response to Al stress in *C. tora* L. In an Al-resistant cultivar of wheat, La^{3+} also failed to stimulate the secretion of malic acid (Delhaize et al. 1993). Recently, Ishikawa et al. (1996) presented evidence that the mechanism of Al toxicity differs from that of Yb^{3+} and La^{3+} toxicity. Al is bound to different sites in the root tips from Yb and La; this seems to be related to the ionic potential of these different metals.

In conclusion, as an Al-resistant mechanism of *Cassia tora* L., citric acid is secreted from the roots to detoxify Al. This response is specific to Al stress and some inducible processes are probably involved in the roots.

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References

Basu, U., Godbold, D. and Taylor, G.J. (1994) Aluminum resistance in *Triticum aestivum* associated with enhanced exudation of malate. *J. Plant Physiol.* 144: 747–753.

Bennet, R.J. and Breen, C.M. (1992) The use of lanthanum to delineate the aluminum signalling mechanisms functioning in the roots of *Zea mays* L. *Environ. Exp. Bot.* 32: 365–376.

Delhaize, E., Ryan, P.R. and Randall, P.J. (1993) Aluminum tolerance in wheat (*Triticum aestivum* L.). II. Aluminum-stimulated excretion of malic acid from root apices. *Plant Physiol.* 103: 695–702.

Delhaize, E. and Ryan, P.R. (1995) Aluminum toxicity and tolerance in

plants. *Plant Physiol.* 107: 315–321.

Dinkelaker, B., Romheld, V. and Marschner, H. (1989) Citric acid excretion and precipitation of calcium citrate in the rhizosphere of white lupine (*Lupinus albus* L.). *Plant Cell Environ.* 12: 285–292.

Gardner, W.K., Barber, D.A. and Parberry, D.G. (1983) The acquisition of phosphorus by *Lupinus albus* L. III. The probable mechanism by which phosphorus movement in the soil/root interface is enhanced. *Plant Soil* 70: 107–124.

Hoffland, E., Findenegg, G.R. and Nelemands, J.A. (1989) Solubilization of rock phosphate by rape. II. Local root exudation of organic acids as a response to P-starvation. *Plant Soil* 113: 161–165.

Hue, N.V., Craddock, G.R. and Adams, F. (1986) Effect of organic acids on aluminum toxicity in subsoils. *Soil Sci. Soc. Amer. J.* 50: 28–34.

Ishikawa, S., Wagatsuma, T. and Ikarashi, T. (1996) Comparative toxicity of Al^{3+}, Yb^{3+}, and La^{3+} to root-tip cells differing in tolerance to high Al^{3+} in terms of ionic potentials of dehydrated trivalent cations. *Soil Sci. Plant Nutri.* 42: 613–625.

Johnson, J.F., Vance, C.P. and Allan, D. (1996) Phosphorus deficiency in *Lupinus albus*. Altered lateral root development and enhanced expression of phosphoenolpyruvate carboxylase. *Plant Physiol.* 112: 31–41.

Kito, M. and Hattori, T. (1996) Resistance of *Cassia tora* L. to Al stress. Abstract of the annual meeting, *Japan Soc. Soil Sci. Plant Nutr.* 42: 367.

Kochian, L.V. (1995) Cellular mechanisms of aluminum toxicity and resistance in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 46: 237–260.

Lipton, D.S., Blanchar, R.W. and Blevins, D.G. (1987) Citrate, malate, and succinate concentration in exudates from P-sufficient and P-stressed *Medicago sativa* L. seedlings. *Plant Physiol.* 85: 315–317.

Ma, J.F., Hiradate, S., Nomoto, K., Iwashita, T. and Matsumoto, H. (1997) Internal detoxification mechanism of Al in *Hydrangea macrophylla*. Identification of Al form in the leaves. *Plant Physiol.* 113: 1033–1039.

Matsumoto, H. and Yamaya, T. (1986) Inhibition of potassium uptake and regulation of membrane-associated Mg^{2+}-ATPase activity of pea roots by aluminum. *Soil Sci. Plant Nutri.* 32: 179–188.

Miyasaka, S.C., Buta, J.G., Howell, R.K. and Foy, C.D. (1991) Mechanism of aluminum tolerance in snapbeans, root exudation of citric acid. *Plant Physiol.* 96: 737–743.

Ojima, K. and Ohira, K. (1988) Aluminum-tolerance and citric acid release from a stress-selected cell line of carrot. *Commun. Soil Sci. Plant Anal.* 19: 1229–1236.

Ownby, J.D. and Popham, H.R. (1989) Citrate reverses the inhibition of wheat root growth caused by aluminum. *J. Plant Physiol.* 135: 588–591.

Pellet, D.M., Grunes, D.L. and Kochian, L.V. (1995) Organic acid exudation as an aluminum-tolerance mechanism in maize (*Zea mays* L.). *Planta* 196: 788–795.

Rengel, Z. and Elliott, D.C. (1992) Mechanism of aluminum inhibition of net $^{45}Ca^{2+}$ uptake by *Amaranthus* protoplasts. *Plant Physiol.* 98: 632–638.

Ryan, P.R., DiTomaso, J.M. and Kochian, L.V. (1993) Aluminum toxicity in roots: an investigation of spatial sensitivity and the role of the root cap. *J. Exp. Bot.* 44: 437–446.

Ryan, P.R., Delhaize, E. and Randall, P.J. (1995) Malate efflux from root apices and tolerance to aluminum are highly correlated in wheat. *Aust. J. Plant Physiol.* 22: 531–536.

Suhayda, C.G. and Haug, A. (1984) Organic acids prevent aluminum-induced conformational changes in calmodulin. *Biochem. Biophys. Res. Commun.* 119: 376–381.

Suhayda, C.G. and Haug, A. (1986) Organic acids reduce aluminum toxicity in maize root membranes. *Physiol. Plant.* 68: 189–195.

Taylor, G.J. (1991) Current views of the aluminum stress response; The physiological basis of tolerance. *Curr. Top. Plant Biochem. Physiol.* 10: 57–93.

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