

Mini Review

Role of Organic Acids in Detoxification of Aluminum in Higher Plants

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Phytotoxicity of aluminum ion (Al^{3+}) is a serious problem limiting crop production on acid soils. Organic acids with Al-chelating ability play an important role in the detoxification of Al both externally and internally. Al is detoxified externally by the secretion of organic acids such as citric, oxalic, and /or malic acids from the roots. The secretion of organic acids is highly specific to Al and the site of secretion is localized to the root apex. The kind of organic acids secreted as well as secretion pattern differ among plant species. There are two patterns of Al-induced secretion of organic acids: In pattern I, there is no discernible delay between the addition of Al and the onset of the release of organic acids. Activation of the anion channel seems to be involved in this pattern; In pattern II, there is a marked lag phase between the addition of Al and the onset of organic acid release. The action of genes related to the metabolism and secretion of organic acids seems to be involved in this pattern. Internal detoxification of Al in Al-accumulating plants is achieved by the formation of Al-organic acid complex. For instance, the complex of Al-citrate (1 : 1) in hydrangea and Al-oxalate (1 : 3) in buckwheat has been identified.

Key words: Al tolerance — Al toxicity — Chelation — External detoxification — Internal detoxification — Organic acids.

Aluminum ion (Al^{3+}) is toxic to plants at micromolar concentrations. The chemistry of Al in solution is complicated because Al hydrolyses in a pH-dependent manner to form various complexes with hydroxyl groups. The toxicity of these soluble Al species varies considerably with the trivalent Al^{3+} ion likely to cause greatest stress to plants. Therefore the focus of this review will be on studies conducted in acidic conditions (<pH 5.0) where the Al^{3+} cation predominates speciation. (Al^{3+} will be represented as Al for the remainder of the text). Phytotoxicity of Al is characterized by rapid inhibition of root elongation and subsequent decrease in the uptake of nutrients and water (for a review, see Kochian 1995). Al toxicity has been recognized as a major factor limiting crop productivity on acid soil, which comprises about 40% of the arable land in the world (Foy et al. 1978). The concentration of Al in acid

soil solutions ranges from 10 to 100 μM .

However, some plant species and cultivars show tolerance to Al toxicity. The mechanism of Al tolerance has been categorized into external or exclusion and internal detoxification mechanisms (Taylor 1991, Kochian 1995). The main difference between these two types is in the site of Al detoxification: symplasm (internal) or apoplasm (external). The proposed mechanisms for external detoxification include immobilization of Al at the cell wall, selective permeability of the plasma membrane, a plant-induced pH barrier in the rhizosphere, exudation of chelate ligands, exudation of phosphate and Al efflux (Taylor 1991, Kochian 1995). By contrast, the internal detoxification mechanisms include chelation in the cytosol by organic acids, proteins, or other organic ligands, compartmentation in the vacuole, evolution of Al-tolerant enzymes and elevated enzyme activity. Most of these mechanisms remain to be examined in the future. However, recently accumulating evidence shows that organic acids play an important role in both the internal and external Al detoxification. In this paper, the role of organic acids in Al tolerance of higher plants is reviewed.

How do organic acids detoxify Al?

The primary event in Al toxicity is rapid inhibition (within 1 h) of root elongation (e.g. Ownby and Popham 1989, Ryan et al. 1992) and the targeting site of Al toxicity is the root apex (Ryan et al. 1993). Although root elongation consists of cell division and cell elongation, the contribution of cell division to the rapid elongation would be small. Thus, initial Al-induced inhibition of root elongation is likely to be caused by the inhibition of cell elongation. However, little is known about how Al causes rapid inhibition of cell elongation. Many different mechanisms of Al toxicity have been proposed (for reviews, see Delhaize and Ryan 1995, Kochian 1995). Al may interact with the root cell wall, disrupt the plasma membrane and inhibit transport processes on the plasma membrane (Fig. 1). It may inhibit enzyme activity and DNA replication, disrupt signal transduction pathways and inhibit the formation of microtubules. Al may also interact with Ca homeostasis within the root cell and other symplasmic constituents such as calmodulin. Thus, Al seems to inhibit root elongation by targeting multiple sites of the root cells simultaneously, not by targeting only one site. This speculation is supported

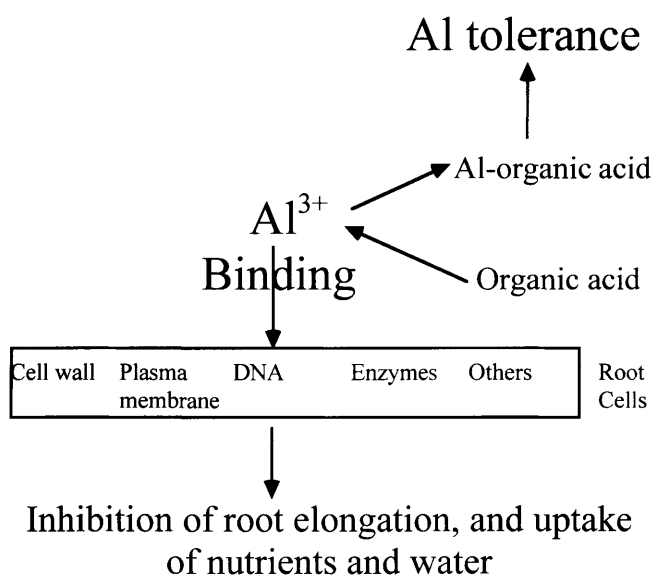


Fig. 1 Schematic representation of Al toxicity and the role of Al-chelating substance such as some organic acids in detoxifying Al.

by the fact that Al induces quantitative and qualitative changes of numerous proteins in the root tips of wheat exposed to Al for a short period (Delhaize et al. 1991, Ownby and Hruschka 1991). A number of genes are also induced in wheat roots by Al exposure (Snowden and Gardner 1993, Richards et al. 1994). Mechanisms involved in Al toxicity may vary with Al concentrations. In the roots exposed to a low concentration of Al, only the apoplasm of the roots such as the cell wall may be influenced by Al. However, in the roots exposed to a high concentration of Al, the plasma membrane, DNA, and enzymes may be also affected. It should be noted that the Al concentration in an acid soil solution rarely exceeds $140 \mu\text{M}$ (Haug 1984). Some previous results derived from laboratory experiments on the plants grown in the presence of Al at millimolar concentrations may not be applied to the plants under field conditions. It seems necessary to re-examine the toxicity of Al at low concentrations and more attention should be paid to the action of Al in the apoplasm (Horst 1995).

Although the mechanisms responsible for Al-induced inhibition of root elongation are complicated, all these inhibitory effects result from the binding of Al with extracellular and intracellular substances (Fig. 1). Al has a strong binding affinity for the oxygen donor compounds such as inorganic phosphate, nucleotides, RNA, DNA, proteins, carboxylic acids, phospholipids, polygalacturonic acids, heteropolysaccharides, lipopolysaccharides, flavonoids, and anthocyanins (Martin 1988). 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 (Fig. 1). Some organic acids such as citric, oxalic, malic, tartaric, salicylic, and malonic acids form stable complex with Al, thereby detoxifying Al.

The Al-detoxifying capacity of organic acids depends on the stability constants of the Al-organic acid complexes (Hue et al. 1986). For example, equimolar citric acid can detoxify Al (Ma et al. 1997a, Fig. 2A), but 3 times more oxalic acid (Ma et al. 1998) and 6–8 times more malic acid than Al (Delhaize et al. 1993b, Ryan et al. 1995b) are required to detoxify Al. The different Al-detoxifying capacity of organic acids results from their structural configurations (relative positions on the main carbon chain of OH and COOH groups) (Hue et al. 1986). The most effective detoxifying acids have either two pairs of OH/COOH attached to two adjacent carbons (citric and tartaric) or two COOHs directly connected (oxalic) (Fig. 3), forming stable 5- or 6-bond ring structures with Al.

External detoxification of Al by organic acids

Organic acids have been known to alleviate Al toxicity in vitro for many years (e.g. Bartlett and Riego 1972), but the secretion of organic acids from the roots as an Al tolerance mechanism was first suggested by Kitagawa et al. (1986). They found that the secretion of malic acid from the roots was stimulated by Al in an Al-tolerant cultivar of wheat, Atlas 66, and more malic acid was secreted from Atlas 66 than from an Al-sensitive cultivar of wheat, Brevor. However, convincing data on the relationship between Al tolerance and organic acid secretion was presented by Delhaize and his co-workers (Delhaize et al. 1993a, b), who used a pair of near-isogenic wheat lines differing in Al tolerance at a single dominant locus (*Alt1*) (Fig. 2B). Since then, intensive studies on Al-induced secretion of organic acids have been carried out in a number of Al-tolerant species and cultivars. Here, the characteristics of the Al-induced organic acid secretion are discussed.

Kinds and amounts of organic acids secreted—Although many organic acids are present in the roots, only some specific organic acids are secreted into the rhizosphere in response to Al. The kinds of organic acids secreted from the roots under Al stress differ among plant species and the secretion of malic, oxalic, and citric acid has been reported in different plant species. Malic acid is secreted from the roots of Al-tolerant cultivars of wheat in response to Al (Kitagawa et al. 1986, Delhaize et al. 1993b, Basu et al. 1994). Delhaize et al. (1993b) found that Al-tolerant genotypes (ET3) excreted 5- to 10-fold more malic acid than Al-sensitive genotypes (ES3). Basu et al. (1994) also reported that exposure to $100 \mu\text{M}$ Al increased the exudation of malic acid from the roots of Al-tolerant cultivars by 100–120%, while in the Al-sensitive cultivars, it reduced the exudation of malic acid.

Citric acid secretion in response to Al was found

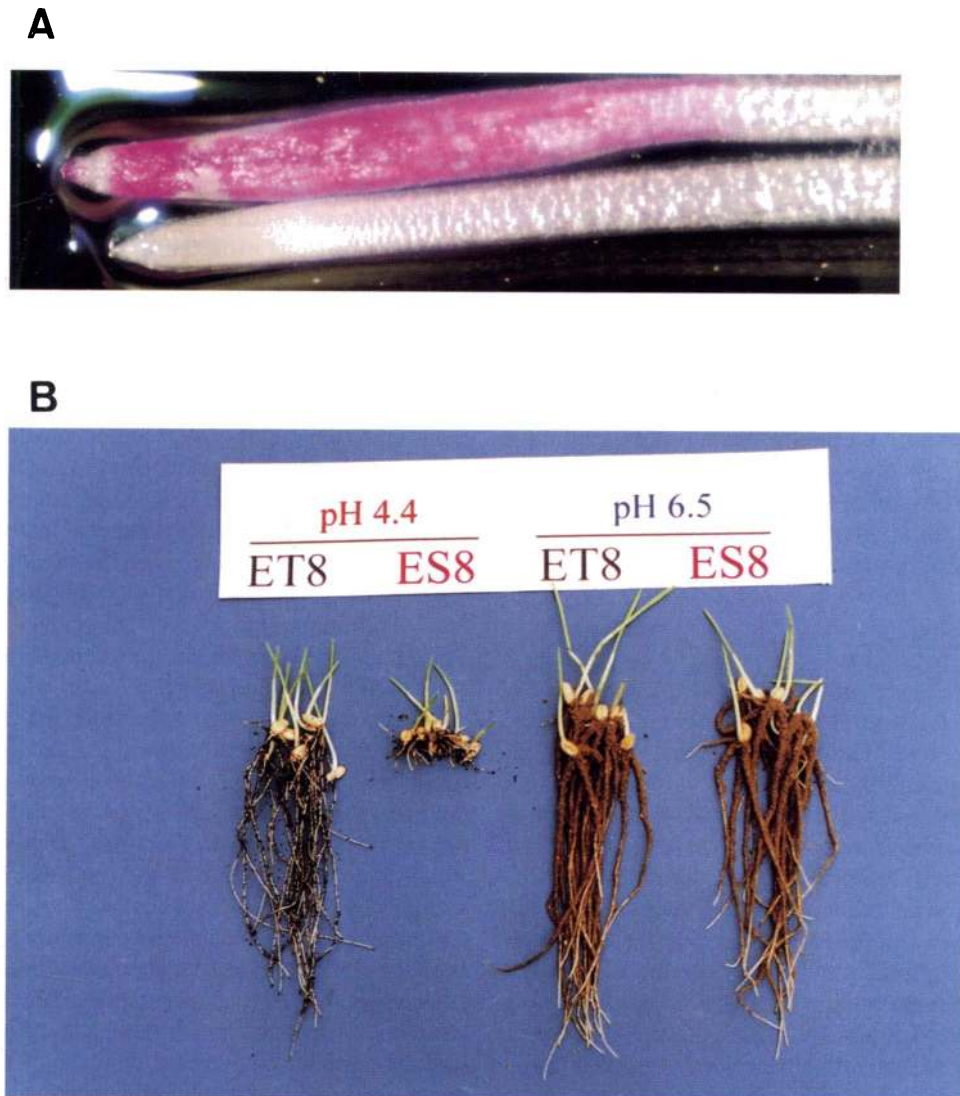


Fig. 2 (A) Effect of citric acid on the detoxification of Al. Wheat (Al-sensitive cultivar, Scout 66) roots were exposed to 0.5 mM CaCl_2 (pH 4.5) solution containing either 20 μM AlCl_3 (upper) or 20 μM Al-citrate (lower) (1 : 1) for 20 h. The roots were then stained with 0.1% Eriochrome Cyanine R for 10 min. Pink color shows Al accumulation. In the presence of citric acid, Al binding to the root cells was prevented. (B) Root growth of a pair of near-isogenic wheat lines differing in Al tolerance at a single dominant locus (*Al1*). ET8 (Al-tolerant) and ES8 (Al-sensitive) were grown in a non-allophanic Andosol (pH 4.4, Al toxic) or slightly acid soil (pH 6.5, no Al toxicity) for 6 d. Their difference in Al tolerance results from secretion of malic acid from the roots of ET8 (Delhaize et al. 1993b). The seeds were kindly provided by Drs. Delhaize and Ryan at CSIRO.

in Al-tolerant cultivars of snapbean (*Phaseolus vulgaris*) (Miyasaka et al. 1991) and maize (Pellet et al. 1995), *Cassia tora* L. (Ma et al. 1997b) and *Paraserianthes falcataria* L. Neilson (Osawa et al. 1997). Miyasaka et al. (1991) showed that an Al-tolerant cultivar of snapbean secreted 10-fold more citric acid than an Al-sensitive cultivar. Pellet et al. (1995) reported that an Al-tolerant maize line secreted 10-fold more citric acid than a sensitive line. *Cassia tora* L., an Al-tolerant species (Ma et al. 1997b, c) and *Paraserianthes*

falcataria L. Neilson, an Al-tolerant tree species (Osawa et al. 1997) also secrete citric acid in response to Al treatment.

Recently, oxalic acid has been reported to be secreted from the roots of buckwheat (*Fagopyrum esculentum* Moench, cv. Jianxi) and taro. Buckwheat shows high Al tolerance (Ma et al. 1997d, Zheng et al. 1998b), and the exposure of the roots to Al elicited the secretion of oxalic acid (Ma et al. 1997d). Taro is naturally tolerant to excess Al, and excreted oxalic acid from the roots in response to

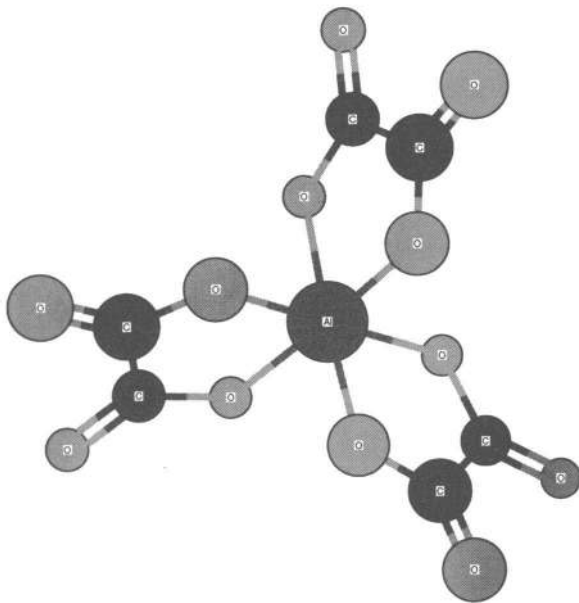


Fig. 3 Structural model of 1 : 3 Al-oxalate complex. Al is coordinated with carboxylic groups from three molecules of oxalic acid, forming a stable complex which is non-toxic to plants.

Al (Ma and Miyasaka 1998).

In some plant species, two organic acids are secreted from the roots in response to Al. Rapeseed, oats, and radish secreted both malic and citric acids (Zheng et al. 1998a). Al-tolerant mutants of *Arabidopsis thaliana* that mapped together on chromosome 1 released greater amounts of citric and malic acids compared with wild type (Larsen et al. 1998). Both citric and malic acids were also found to be secreted from the roots of an Al-tolerant triticale line (Ma et al. 2000). The secretion of organic acids in this line has been linked to the short arm of rye chromosome 3R.

In either case, the secretion of organic acids in Al-tolerant species and cultivars is stimulated by Al and the amount of organic acids secreted increases with increasing external Al concentrations (e.g. Delhaize et al. 1993b, Ma et al. 1997b, d).

The amount of organic acids secreted in response to Al varies with the plant species, but it is difficult to directly compare the published data which were obtained under different experimental conditions. This is because the amount of organic acids secreted varies with the Al activity, exposure time to Al, plant age, growth conditions and others. Ryan et al. (1995b) investigated 36 wheat cultivars differing in Al tolerance under the same condition and found that the amount of malic acid secreted was highly correlated with Al tolerance. Zheng et al. (1998a) examined the relationship between Al tolerance and the secreted amount of Al-induced organic acids in 8 plant cultivars

belonging to 5 species over relatively long period and suggested that the continuous secretion of organic acids at a high level is related to high Al tolerance.

Site of organic acid secretion—The secretion site of organic acids in the roots has been investigated in wheat, maize, and buckwheat. Approximately 35-fold more malic acid was released from the root apex (3–5 mm) than from the mature portion of the root in an Al-tolerant cultivar of wheat (Delhaize et al. 1993b). The secretion of malic acid was stimulated by Al, at the root apex but not from the mature root tissue. The Al-induced citric acid release was localized to the root apex of the Al-tolerant cultivar of maize (Pellet et al. 1995); a comparable mature root region of either the Al-tolerant or sensitive variety did not release citric acid in the absence or presence of Al. Using a non-destructive method, oxalic acid was found to be secreted in the region 0 to 10 mm from the root tip of buckwheat (Zheng et al. 1998b). The root apex is also the targeting site of Al toxicity (Ryan et al. 1993). Therefore, the secretion of organic acids from the same site can protect the root apex from Al-induced injury.

Specificity of organic acid secretion—Organic acids are secreted in response to P deficiency in some plant species such as white lupin (Gardner et al. 1983), alfalfa (Lipton et al. 1987), and rape (Hoffland et al. 1989). Because Al is easily precipitated by P, organic acid secretion might be caused indirectly by Al-induced P deficiency. In the work with snapbean mentioned above (Miyasaka et al. 1991), it was not clear whether the secretion of citric acid was induced by Al or by P deficiency because in their experimental conditions, P was probably precipitated as insoluble Al-phosphate in the culture solution during the 8-d culture. However, later studies with a short exposure clearly showed that the secretion of organic acids is a specific response to Al. One day of P deficiency failed to induce secretion of malic acid in wheat (Delhaize et al. 1993b). In *Cassia tora* L., 8 d of P deficiency did not induce the secretion of citric acid (Ma et al. 1997b), but a short exposure to Al did. P deficiency also did not result in the secretion of oxalic acid in the buckwheat (Zheng et al. 1998b). Usually, the induction of organic acid secretion by P deficiency takes a long time (more than 10 d) (Johnson et al. 1996), but Al-induced secretion of organic acid occurs within several hours (e.g., Ryan et al. 1995a, Ma et al. 1997b). The mechanism involved in the secretion of organic acids by P deficiency seems to differ from that by Al.

Other polyvalent cations failed to induce the secretion of organic acids. La shows some similarities to Al in inhibiting root growth and Ca uptake (Bennet and Breen 1992, Rengel and Elliott 1992). It inhibited the root elongation of both rice and peas more strongly than Al (Ishikawa et al. 1996). However, exposure to La could not induce the secretion of malic acid in wheat (Delhaize et al. 1993b), citric acid in *Cassia tora* L. (Ma et al. 1997b), and

oxalic acid in buckwheat (Zheng et al. 1998b). Iron, ytterbium, gallium, indium and the tridecamer Al_{13} failed to stimulate the secretion of organic acids (Ryan et al. 1995a, Ma et al. 1997b).

Secretion patterns and possible mechanisms—The Al-induced secretion of organic acids can be classified into two patterns depending on plant species. In pattern I, there is no discernible delay between the addition of Al and the onset of release of organic acids. For example, in an Al-tolerant genotype of wheat, ET3, Al-stimulated secretion of malate from both intact roots and excised root apices was observed within 20 min after the exposure to Al (Delhaize et al. 1993b, Ryan et al. 1995a). In buckwheat, the secretion of oxalic acid occurred within 30 min after the exposure to Al (Ma et al. 1997d) (Fig. 4). In pattern II there is a marked lag phase between the addition of Al and the onset of organic acid release. In *Cassia tora* L., the secretion of citrate in response to Al was increased after 4 h (Ma et al. 1997b) (Fig. 4). In an Al-resistant cultivar of maize, a considerable lag phase before the maximal citrate efflux is observed (Pellet et al. 1995, Jorge and Arruda 1997). Recently, Al-induced secretion of malic and citric acids was found to be significantly increased after 6 and 12 h, respectively, in a triticale line (Ma et al. 2000).

Different mechanisms seem to be involved in the two secretion patterns. Organic acids have been suggested to be secreted through an anion-channel located on the plasma membrane (Ryan et al. 1995a). The rapid secretion of organic acids upon Al exposure in Pattern I suggests that gene induction is not involved. Activation of the anion channel by Al is a possible mechanism involved in rapid release (Delhaize and Ryan 1995). Three possibilities have been proposed by Delhaize and Ryan (1995). (1). Al interacts directly with a channel protein, causing a change in the conformation and increasing its mean open time or con-

ductance. (2). Al interacts with a specific receptor on the membrane surface or with the membrane itself, which, through a series of secondary messengers in the cytoplasm, changes channel activity. (3). Al enters the cytoplasm and alters channel activity either directly by binding with the channel or indirectly through a signal transduction pathway. In fact, an anion channel in the plasmalemma of protoplasts isolated from wheat roots was found to be activated by Al (Ryan et al. 1997). The channel could not be activated by La and was observed in the protoplasts isolated from the root apex but not in the protoplasts isolated from mature root tissue. These findings are consistent with the site and specificity of organic acid secretion discussed above. The secretion of organic acids was also found to be inhibited by some anion-channel inhibitors although the inhibitory effects differ among plant species. For example, niflumic acid significantly inhibited the Al-induced secretion of malic acid in wheat (Ryan et al. 1995a), but it did not inhibit the secretion of oxalic acid in buckwheat (Zheng et al. 1998b). It seems that the characteristics of anion channels differ with the kind of organic acids. Furthermore, in Pattern I, the activities of phosphoenolpyruvate carboxylase and NAD-malate dehydrogenase did not differ between Al-sensitive and Al-tolerant cultivars of wheat and between the plants treated and not treated with Al (Ryan et al. 1995a). The internal malic acid content was not changed by the exposure to Al during a short time (Delhaize et al. 1993b). All of these facts suggest that the *in vivo* synthesis of organic acids is not altered by Al in the wheat plants.

In contrast, gene induction may be involved in the Pattern-II secretion. The gene(s) may be related to metabolism (biosynthesis and decomposition) of organic acids, anion channel on plasma membrane and/or tonoplast, or transport of organic acids from mitochondria. It is interesting that only the secretion of citric acid has been

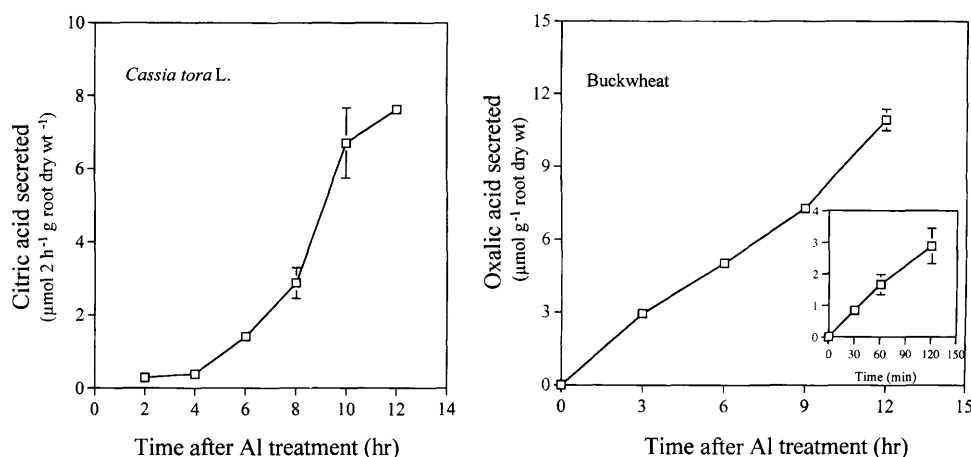


Fig. 4 Different secretion pattern of organic acids in response to Al. Roots of *Cassia tora* L. (Ma et al. 1997b) and the buckwheat (cv. Jianxi) (Ma et al. 1997d) were exposed to 0.5 mM CaCl_2 solution (pH 4.5) containing 50 μM AlCl_3 .

found in this pattern. Our preliminary results showed that NADP-specific isocitrate dehydrogenase (NADP-ICDH), an enzyme catalyzing a reaction from isocitrate to 2-oxoglutarate in the cytosol, was inhibited by Al in *Cassia tora* L. (Chiba 1999). This may result in increased accumulation of citric acid. In an Al-tolerant tree species, *Paraserianthes falcataria* L. Neilson, internal citric acid concentration, citrate synthase (CS) activity in mitochondria and the amount of mRNA of CS were increased by the exposure to Al (Osawa 1998). However, further work on the effect of Al on the enzyme activity related to metabolism of organic acids as well as gene induction is needed.

Significance of organic acid secretion—Against the idea that the secretion of organic acids is a tolerance mechanism of Al toxicity, the argument is whether the amount of organic acids secreted is sufficient to detoxify Al. The primary site of Al toxicity is in the root apex as mentioned above (Ryan et al. 1993), therefore, it is a prerequisite to protect the root apex from Al injury. The secretion of organic acids is localized to the root apex (Delhaize et al. 1993b, Pellet et al. 1995, Zheng et al. 1998b), but it is difficult to estimate the concentration of organic acids around the root apex because only the organic acids secreted into the bulk solution could be measured. Taking the diffusion coefficient of citric acid, mucilage production rate into account in an Al-tolerant maize, Pellet et al. (1995) estimated that citric acid in the unstirred layer of the solution adjacent to the root apex would be approximately 260 μM , which is much higher than the Al concentration. Recently, more convincing data on the role of organic acids in detoxifying Al has been reported. de la Fuente et al. (1997) introduced a *Pseudomonas aureginosa* citrate synthase gene into tobacco and papaya. As a result, the transgenic plants showed enhanced Al tolerance which was associated with an increase in citric acid secretion. Al-induced secretion of oxalic acid in buckwheat was found to be inhibited by an anion channel inhibitor, phenylglyoxal (Zheng et al. 1998b). In the presence of phenylglyoxal, Al tolerance of the buckwheat was significantly decreased. These results strongly suggest that the secretion of organic acids plays an important role in the external detoxification of Al.

Internal detoxification of Al with organic acids

When the root elongation is inhibited by Al at micromolar concentrations, most Al is localized in the epidermis and outer cortex cells of the roots (e.g. Ishikawa et al. 1996). Moreover, Al binds mainly to the component of the cell wall (Zhang and Taylor 1990, 1991) although recent research indicated that Al can enter the symplasm of root cells fairly quickly (Lazof et al. 1994, Vitorello and Haug 1996). Further penetration of Al to the stele seems to be prevented, resulting in a high Al content in the roots and low content in the shoot in most plant species. However, it

is also well known that some plant species accumulate Al at a high concentration in the top without showing Al toxicity. Old leaves of tea can accumulate Al up to 30,000 mg kg^{-1} on a dry-weight basis (Matsumoto et al. 1976). Hydrangea plants accumulated high Al ($>3,000 \text{ mg kg}^{-1}$) in both leaves and sepals during a several-month growth period (Ma et al. 1997a). After 10 d of intermittent treatment with 50 μM Al, the Al concentration of the buckwheat leaves reached about 450 mg Al kg^{-1} on a dry-weight basis, in contrast to other species such as wheat, oat, radish and rape, which contained less than 50 mg Al kg^{-1} after the same treatment (Ma et al. 1997d). These facts suggest that Al is transported across the plasma membrane into the symplasm in Al-accumulating plant species. Symplasmic solutions usually have a pH above 7.0. Although the concentration of free Al is decreased to less than 10^{-10} M at pH 7.0 due to formation of insoluble $\text{Al}(\text{OH})_3$, such low concentrations are still potentially phytotoxic because of the strong affinity of Al for oxygen donor compounds as discussed above. For example, Al binds almost 10^7 time more strongly to ATP than does Mg; therefore, less than nanomolar amounts of Al can compete with Mg for the P sites (Martin 1988). These facts suggest that Al-accumulating plants must possess effective mechanisms to detoxify Al internally. However, until recently, there has been little direct evidence for an internal detoxification mechanism of Al. Ma et al. (1997a) found that about 80% of total Al was present in a soluble form in the hydrangea leaves and the Al concentration in the cell sap was as high as 13.7 mM. Using ^{27}Al -nuclear magnetic resonance analysis, the form in the hydrangea has been identified as a complex of Al-citrate (1 : 1) (Ma et al. 1997a). The standard stability constant of Al-citrate complex was reported to be 8.1 (Martin 1988). However, the conditional stability constant becomes 11.7 and 12.4 at pH 7.0 and 7.4, respectively, which are significantly higher than that for the Al-ATP complex (10.9). This strong chelation capacity could effectively reduce the activity of Al in the cytosol at a pH above 7.0 and prevent the formation of the complex between Al and cellular components such as ATP, DNA, and hence, decrease Al phytotoxic effects. For instance, it was demonstrated that the application of citric acid can partially restore the Al-induced loss of structure in calmodulin once an Al-calmodulin complex had been formed, or, if added prior to Al addition, citric acid protects the regulatory protein from undergoing a loss of α -helix content (Suhayda and Haug 1984, 1986).

Al form in the buckwheat was also investigated (Ma et al. 1997d, 1998). It was demonstrated that Al in both the roots and leaves of the buckwheat is present in the form of 1 : 3 Al-oxalate complex (Fig. 3). Furthermore, about 90% of Al accumulated in the leaves was in the cell sap. Oxalic acid can form three species of complexes with Al at an Al to oxalic acid molar ratio of 1 : 1, 1 : 2, and 1 : 3, but 1 : 3

Al-oxalate complex is the most stable, with a stability constant of 12.4 (Nordstrom and May 1996). This stability constant is much higher than that of Al-ATP, meaning that the formation of a 1 : 3 Al-oxalate complex can also prevent binding of Al to cellular components, thereby detoxifying Al (Ma et al. 1998).

In conclusion, the internal detoxification of Al in the Al-accumulating plants is achieved by complexation with organic acids. The organic acids used for the complexation may not be induced by Al because there is no big difference in the concentration of oxalic acids in the cell sap between buckwheat leaves treated with and without Al (Ma et al. 1998).

Concluding remarks

Multiple mechanisms of Al tolerance in higher plants have been suggested (Pellet et al. 1995) and the secretion of organic acids with Al-chelating capacity from the root tips has been considered as an important one. However, little is known about the mechanisms leading to the secretion of organic acids. Alteration in the metabolism of organic acids and activation of anion channel have been suggested to be involved in the Al-induced secretion of organic acids depending on the secretion patterns, but the responsible mechanisms need to be examined, and the genes controlling these processes remain to be cloned in the future. The mechanisms which regulate the Al-induced organic acid secretion also need to be elucidated. Concerning the internal detoxification mechanisms in Al-accumulating plants, it is still unknown how Al crosses the plasma membrane of the roots and is translocated into the upper parts of the plants. The localization of the Al-organic acid complex, that is, whether the complex is present in the cytosol or in vacuoles, also needs to be addressed in future studies. The introduction of a bacterial citrate synthase gene into plants and the resulting increase in Al tolerance suggest the possibility of increasing Al tolerance of crops by genetic manipulation.

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