Role of exudation of organic acids and phosphate in aluminum tolerance of four tropical woody species

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Summary Responses of Melaleuca leucadendra (L.) L., Melaleuca cajuputi Powell, Acacia auriculiformis A. Cunn. ex Benth. and Eucalyptus camaldulensis Dehn. to aluminum (Al) toxicity at low pH are poorly understood. We investigated effects of low pH and exudation of ligands by roots on Al tolerance of these species. Seedlings were grown hydroponically in nutrient solutions at pH 4.2 or 3.5 containing AlCl₃, at concentrations ranging from 0 to 4 mM Al. The presence of 4 mM Al at pH 3.5 depressed growth in all species. Growth depression was greatest in E. camaldulensis, least in A. auriculiformis. In the low Al treatment (0.5 mM Al), roots of M. cajuputi tended to have the highest Al concentration among species, whereas in the 4 mM Al treatment, the highest Al concentration was found in roots of E. camaldulensis. Aluminum application enhanced root exudation of citrate in all species, with the enhancement in M. cajuputi, M. leucadendra and A. auriculiformis being similar and much greater than in E. camaldulensis. Exudation of oxalate and phenolic compounds was greater in E. camaldulensis than in the other species. The presence of Al enhanced phosphate exudation in all species, particularly in A. auriculiformis. Acacia auriculiformis was tolerant to low pH, probably because the presence of an unknown substance increased the pH. Application of 0.38 mM Al alleviated the toxicity of the pH 3.5 treatment in E. camaldulensis and M. cajuputi, whereas low pH alleviated Al toxicity in A. auriculiformis. We conclude that exudation of ligands such as citrate and phosphate only partly accounts for interspecific differences in Al tolerance among the tropical woody plants studied, whereas the reciprocal alleviation of Al toxicity and low pH differed considerably among the species.

Keywords: Acacia auriculiformis, Eucalyptus camaldulensis, low pH tolerance, Melaleuca cajuputi, Melaleuca leucadendra.

Introduction

Aluminum is toxic to plants growing in acidic soil and is a major cause of stress in trees in forest ecosystems (Cronan and Grigal 1995). The first symptom of Al phytotoxicity is inhibited root elongation with subsequent impairment of nutrient and water uptake (Delhaize and Ryan 1995; Kochian 1995). This process is thought to occur as a result of Al³⁺ ions binding extracellular and intracellular substances, such as inorganic phosphate, nucleotides and carboxylic acids (Foy et al. 1978, Kochian 1995), thereby injuring the root apex (Ryan et al. 1993, Kinraide 1997), particularly the distal part of the transition zone (Sivaguru and Horst 1998). Both internal and external mechanisms have been shown to reduce the phytotoxic effects of free Al³⁺ ions (Taylor 1991, Kochian 1995, Ma 2000). Internal mechanisms detoxify Al³⁺ ions in the symplasm by chelating Al with organic acids (Ma et al. 1997a, 1997b, 1998) or other organic ligands (Nagata et al. 1992). External (or exclusion) mechanisms detoxify Al³⁺ ions in the apoplasm, on root surfaces, or in the rhizosphere, thereby protecting sensitive symplastic sites from free Al³⁺ ions. External mechanisms include immobilization of Al at the cell wall (Heim et al. 1999), formation of a plant-induced pH barrier in the rhizosphere (Foy et al. 1978, Degenhardt et al. 1998), release of an Al-binding mucilage (Horst et al. 1982, Li et al. 2000a), exudation of organic ligands (Li et al. 2000a, Ma 2000, Ma et al. 2000, Zhang et al. 2001), exudation of phosphate (Pettersson and Strid 1989, Lindberg 1990, Pellet et al. 1995, 1996), and exudation of phenolic compounds (Heim et al. 1999, Kidd et al. 2001, Ofei-Manu et al. 2001a). Wenzl et al. (2001) suggested that active Al extrusion from the symplasm contributed to the high Al tolerance of signal grass.

In addition to Al toxicity, plants growing on highly acidic soil are subject to proton toxicity. The binding of H⁺ ions and consequent reduction of cell-surface negativity may lead to inhibition of nutrient absorption and translocation by roots (Kinraide et al. 1992, Malkanthi et al. 1995a, 1995b). A high proton concentration induces membrane leakage (Fawzy et al. 1954, Horst et al. 1992), inhibits H⁺ ion extrusion from roots (Bennet et al. 1987, Keltjens 1988), and reduces the viability of root cells (Yokota and Ojima 1995). An inverse relationship
between H⁺ ion toxicity and Al toxicity has been observed, with Al³⁺ ions alleviating H⁺ ion toxicity (Noble et al. 1988, Kinraide and Parker 1990, Kinraide et al. 1992, Huang and Bachelard 1993, Osaki et al. 1997, Ofei-Manu et al. 2001a) and H⁺ ions alleviating Al³⁺ ion toxicity (Kinraide and Parker 1990, Kinraide et al. 1992, Godbold et al. 1995).

We compared Al tolerance at low pH of four tropical woody species, *Melaleuca cajuputi* Powell, *Melaleuca leucadendra* (L.) L., *Acacia auriculiformis* A. Cunn. ex Benth. and *Eucalyptus camaldulensis* Dehnh. We also examined the exudation of ligands, including organic acid, phosphate, and phenolic compounds, by roots of these species in response to Al exposure.

**Materials and methods**

**Plant culture, treatment and sample collection**

Seeds of *A. auriculiformis* from Melville Island, *E. camaldulensis* from the Laura River, Australia, *M. leucadendra* from Kuru, Papua New Guinea, and *M. cajuputi* from An Giang Province, Vietnam, were germinated in trays containing sand washed with deionized water. The trays were soaked in nutrient solution containing (µM): 1190 NO₃⁻, 113 NH₄⁺, 50 H₂PO₄⁻, 625 K⁺, 125 Mg²⁺, 500 Ca²⁺, 134 SO₄²⁻, 9 Fe²⁺, 0.25 Mn²⁺, 0.0125 Cu²⁺, 0.05 Zn²⁺, 0.005 Na⁺, 0.00025 Co²⁺, 0.005 MoO₄²⁻, and 1.25 B(OH)₃; the pH was adjusted to 4.2 with HCl. Thirty days (*A. auriculiformis* and *E. camaldulensis*) and 50 days (*M. cajuputi* and *M. leucadendra*) after germination, 243 seedlings of each species were floated on aerated nutrient solution in 2-l plastic pots. Each pot contained nine seedlings. All plants in each pot were harvested, separated into leaves, stems and roots, washed with deionized water and blotted with tissue paper. The fresh mass of each plant part and the length of taproots were measured. Samples were stored at −80 °C until analyzed.

**Experiment 1** For each species, 54 uniform plants at 34 (*A. auriculiformis* and *E. camaldulensis*) and 54 days after sowing (DAS) (*M. cajuputi* and *M. leucadendra*) were exposed to six treatments in which the treatment solution described above was adjusted as follows: (1) control: unmodified nutrient solution; (2) low pH: nutrient solution was adjusted to pH 3.5; (3) low Al: nutrient solution contained 0.5 mM AlCl₃ at pH 4.2, with the pH adjusted with KOH before addition of AlCl₃ (Larsen et al. 1996) (the concentration of [Al³⁺] = 160 µM, calculated by GEOCHEM 2.0 (Wenzl et al. 2001)); (4) low Al + low pH: nutrient solution contained 0.38 mM AlCl₃ at pH 3.5, [Al³⁺] = 160 µM; (5) high Al + low pH: nutrient solution contained 4 mM AlCl₃ at pH 3.5, [Al³⁺] = 950 µM; and (6) low P: the concentration of H₂PO₄⁻ was reduced to 2.5 µM. Solutions were renewed every 2 days. Each treatment was performed in triplicate. Thirty days after the start of each treatment (DAT), all plants in each pot were harvested, separated into leaves, stems and roots, washed with deionized water and blotted with tissue paper. The fresh mass of each plant part and the length of taproots were measured. Samples were stored at −80 °C until analyzed.

**Experiment 2** For each species, 27 uniform plants at 50 DAS (*A. auriculiformis* and *E. camaldulensis*) and 70 DAS (*M. cajuputi* and *M. leucadendra*) were kept in 0.2 mM CaCl₂ at pH 4.2 or 3.5 overnight, and then exposed to 0.2 mM CaCl₂ solution containing 0 (pH 4.2), 0.26 (pH 4.2) and 1.35 mM AlCl₃ (pH 3.5). The pH was adjusted with HCl. The concentration of [Al³⁺] ions was 0, 160 and 950 µM in the 0, 0.26 and 1.35 mM AlCl₃ treatments, respectively. Three replications were made. An aliquot of culture solution was taken 24 h after the start of treatment and stored at 4 °C until analyzed.

**Plant sample preparation**

Frozen samples were thawed and centrifuged at 11,000 g for 30 min to obtain cell sap, and the pellets dried at 80 °C in a forced convection oven for 3 days. Samples were ground to fine powder with a vibrating sample mill (Model TI-100, Heiko, Iwaki, Fukushima, Japan) and digested with nitric sulfate.

**Measurement of organic acids and phenolic compounds**

Organic acids in the treatment solution and cell sap were quantified by HPLC as described by (Ma et al. 1997a, 1997c) with some modifications. One liter of treatment solution or 0.5 ml of root cell sap (RCS) was first passed through a cation exchange column filled with 10 g (5 g for RCS) of Amberlite IR-120B resin (H⁺ form), and then through an anion exchange column filled with 10 g (2 g for RCS) of AG 1-X 8 resin (100–200 mesh, formate form). Anionic fractions were eluted with 60 ml (12 ml for RCS) of 1 N HCl. Organic acids were detected at 210 nm.

Cell sap was diluted 1:1 (v/v) with 50% methanol. Treatment solution was filtered, concentrated and solubilized in 50% methanol. The content of total phenolic compounds was determined by the Folin-Denis method (Nakabayashi 1967, Ofei-Manu et al. 2001a). The results were expressed as caffeic acid equivalents.

**Measurement of Al and P**

Aluminum and P in cell sap and the digested samples were measured with an inductively coupled plasma atomic emission spectrometer (ICAP-575 II; Nippon Jarrell-Ash, Kyoto, Japan). The P concentration in the treatment solution was measured by the molybdenum blue method.

**Statistical analysis and calculation**

The experiments were arranged in a split-plot design (Steel and Torrie 1987). Analysis of variance (ANOVA) and standard error of difference were calculated using DATAPLUS and GENSTAT software (Williams and Matheson 1994). Significant differences between treatment means were tested by a least significant difference test, with values of P < 0.05 considered significant. Some data sets were transformed to logarithmic scale before analysis to equalize variances.

Relative values (RV) were calculated as RV = 100TV/CV, where TV is the treatment value and CV is the control value. The 0 mM Al pH 4.2 treatment in Experiment 1 and the 0 mM [Al³⁺] pH 4.2 treatment in Experiment 2 served as the controls unless otherwise indicated.
Results

Growth response

Low pH had no effect on whole-plant biomass of *A. auriculiformis*, but significantly decreased whole-plant biomass of the other species (Figures 1 and 2). The presence of 0.5 mM Al at pH 4.2 had no effect on whole-plant and root biomass of *M. cajuputi*, but caused significant reductions in both parameters in the other species. Whole-plant biomass of all species was significantly decreased by the presence of 4 mM Al, with reductions of 54, 69, 87 and 96% relative to the controls for *A. auriculiformis*, *M. cajuputi*, *M. leucadendra* and *E. camaldulensis*, respectively. Application of 160 µM {Al^3+} increased whole-plant biomass in *M. cajuputi* and *E. camaldulensis* (Figure 2) at pH 3.5, but not at pH 4.2, and the pH 3.5 treatment alleviated the effect of 160 µM {Al^3+} in *A. auriculiformis* (Figure 2).

Taproot lengths of *M. cajuputi* and *M. leucadendra* were slightly reduced by Al application and low pH (Figure 1), whereas the reduction was marked in *E. camaldulensis* and intermediate in *A. auriculiformis*.

Whole-root exudates

Organic acids In all species, roots released organic acids, mainly oxalate and citrate (Figure 3). The release of organic acids was only slight when Al was absent from the culture solution, but increased with increasing Al concentration. When Al concentration was increased from 160 to 950 µM {Al^3+}, the increase in citrate release was larger than the increase in oxalic acid release. At 950 µM {Al^3+}, the rate of oxalate release was highest in *E. camaldulensis* (0.3 µmol (g root FW)⁻¹ day⁻¹), intermediate in *M. cajuputi* and *M. leucadendra* (0.2 µmol (g root FW)⁻¹ day⁻¹), and lowest in *A. auriculiformis* (0.12 µmol (g root FW)⁻¹ day⁻¹), whereas the rates of citrate release were similar among *A. auriculiformis*, *M. cajuputi* and *M. leucadendra* (0.25 µmol (g root FW)⁻¹ day⁻¹), but higher than in *E. camaldulensis*.

Phosphate Aluminum application stimulated the release of phosphate in all species (Figure 3), and the release rate tended to increase with increasing Al concentration. At 950 µM {Al^3+}, the P release rate was highest in *A. auriculiformis* (0.7 µmol (g root FW)⁻¹ day⁻¹), followed by *M. cajuputi* and *M. leucadendra*, and least in *E. camaldulensis*. At 160 µM {Al^3+}, the rate of P release was lower than that of citrate and oxalate, but the reverse was found at 950 µM {Al^3+} except in *E. camaldulensis*.

Phenolic compounds The rate of phenolic compound (PC) release was much lower than the rates of organic acid and phosphate release (Figure 3). All species released PC regardless of the concentration of Al in the culture solution. The highest PC release rate, 81 nmol (g root FW)⁻¹ day⁻¹, was recorded in *E. camaldulensis* in the 950 µM {Al^3+} treatment, and the lowest rate was found in *M. cajuputi*.

Change of culture solution pH by root plant

The pH values of the nutrient solutions in Experiment 1 were measured from 20 to 26 DAT. In the 0 mM Al + pH 3.5 treatment, nutrient solution pH was much higher for *A. auriculiformis* than for the other species 24 and 48 h after renewing (Table 1). Although this pH alleviation was reduced when Al was present, the largest increase in pH was again recorded for
A. auriculiformis. For example, in the 4 mM Al + pH 3.5 treatment, nutrient solution pH was increased from 3.5 to 3.8 by A. auriculiformis 48 h after solution renewal, whereas nutrient solution pH was almost unchanged by the other species.

Concentrations of Al in shoots and roots

Aluminum concentration in roots was much higher than in shoots, regardless of the Al treatment and plant species (Figure 4). In all roots except those of E. camaldulensis, the Al concentration was higher in the 0.5 mM Al + pH 4.2 treatment than in the Al treatments at pH 3.5. The Al concentration in roots of M. cajuputi tended to be higher than in the other species in the low Al treatments (0.5 mM Al + pH 4.2 and 0.38 mM Al + pH 3.5), but for the high Al treatment (4 mM + pH 3.5), the highest Al concentration occurred in the roots of E. camaldulensis. In shoots, Al concentration was only slightly different between the 0.5 mM Al + pH 4.2 and 0.38 mM Al + pH 3.5 treatments. However, in all species, the Al concentration of shoots was higher in the 4 mM Al + pH 3.5 treatment than in the other treatments. Among species, M. leucadendra had the highest shoot Al concentration in all treatments. In the 0.5 mM Al + pH 4.2 treatment, the Al concentration in roots was positively correlated with relative whole-plant fresh mass (Figure 5). This relationship was also found in the 0.38 mM Al + pH 3.5 treatment, where the Al concentration was about twice as high in M. cajuputi and A. auriculiformis as in E. camaldulensis or M. leucadendra. This relationship became negative in the 4 mM Al + pH 3.5 treatment, where the highest Al concentration was recorded in E. camaldulensis (2–3 times higher than in the other species) followed by M. leucadendra, M. cajuputi and A. auriculiformis.
Concentrations of organic acid, phosphate, and phenolic compounds in root cell sap

In the absence of Al, the concentrations of oxalate and malate in root cell sap were higher in *E. camaldulensis* (Table 2), and the concentration of citrate was higher in *A. auriculiformis* than in other species. Aluminum treatment increased the concentration of citrate and decreased the concentration of oxalate in all species; malate concentration was also decreased in *A. auriculiformis* and *M. leucadendra*. Although low pH without Al application reduced citrate concentration in all species and oxalate concentration in *E. camaldulensis* and *M. leucadendra*, low pH with Al treatment reduced the organic acid concentration only in *E. camaldulensis*.

Without Al treatment, the P concentration in root cell sap was twice as high in *E. camaldulensis* as in the other species (Table 2). Aluminum application at pH 4.2 sharply reduced the concentration of P in root cell sap of all species. The reduction was as large as that in the P-deficiency treatment and more pronounced in *A. auriculiformis* than in the other species. Aluminum applied at pH 3.5 did not reduce the P concentration except in *A. auriculiformis*, and it significantly increased the P concentration in the root cell sap of *E. camaldulensis*.

The concentration of phenolic compounds in root cell sap was highest in *E. camaldulensis* and lowest in *A. auriculiformis* (Table 2). At pH 3.5, Al treatments increased the phenolic concentration in *A. auriculiformis* and decreased it in *M. cajuputi* and *M. leucadendra*. The pH 3.5 and P-deficiency treatments significantly increased the concentration of phenolic compounds in the root cell sap of all species.

**Discussion**

*Al* tolerance in tropical woody plants

We found interspecific differences in Al tolerance among four tropical woody species. Based on whole-plant biomass production, tolerance to Al toxicity at pH 3.5 was in the order *A. auriculiformis* > *M. cajuputi* > *M. leucadendra* > *E. camaldulensis* (Figure 1). Because a low pH (e.g., 3.5) is necessary to obtain a high concentration of Al in solution (e.g., 4 mM), the sensitivity of plants to high Al concentrations could be associated with proton toxicity or Al toxicity, or both. The inhibitory effects of low pH and high Al concentration on whole-plant biomass production were independent of each other (Figure 1). *Acacia auriculiformis* was more tolerant to low pH, but not Al, than the other species, suggesting that it is more tolerant to Al toxicity at low pH mainly because of its greater pH tolerance. The reverse was observed for *M. cajuputi* (Figure 1), which was tolerant to Al at low pH owing to tolerance to Al itself rather than low pH. Because *E. camaldulensis* was more susceptible to low pH as well as Al toxicity than the other species, it was less tolerant to Al at low pH than the other species.

Signal grass (*Brachiaria decumbens* Stapf) is reported to be more tolerant to Al than many other crop species (Wenzl et al. 2001). For instance, 49.5 µM [Al] reduced root elongation by 50%, and exposure to 115 µM [Al] for 13 days reduced root fresh mass by 24% relative to the control value. We found that a 30-day exposure to 160 µM [Al] had no significant effect on growth of *M. cajuputi* seedlings, as measured by whole-plant fresh mass, root fresh mass and taproot length (Figures 1 and 2), indicating that *M. cajuputi* is more Al-tolerant than many Al-tolerant crops and woody species (Wenzl et al. 2001).

Why is the Al tolerance of *M. cajuputi* and *A. auriculiformis* higher than that of other species?

All of the study species released organic acid and inorganic phosphate in response to Al application, and the rates of release increased with increasing Al concentration in the nutrient solution (Figure 3). *Eucalyptus camaldulensis* released phenolic compounds at a higher rate than the other species, al-
though its Al tolerance was lower (Figures 1 and 2). This is consistent with studies showing that exudation of phenolic compounds is not a major Al tolerance mechanism in *Camellia sinensis* L., *Picea abies* (L.) Karst. or *Gleditsia triacanthos* L. (Heim et al. 1999, Ofei-Manu et al. 2001a, 2001b). Similarly, *E. camaldulensis* had a higher oxalate release rate than the other species. These results suggest that release of phenolic compounds and oxalate can be excluded as factors controlling Al tolerance in these tropical woody species. The rate of citrate release was lower in *E. camaldulensis* than in *A. auriculiformis*, *M. cajuputi* and *M. leucadendra* (Figure 3). But among the last three species, the rate was not significantly different, though their Al tolerance differed. Furthermore, the rate of organic acid release from our study species was low compared with other crop species in which the release of organic acid is associated with Al tolerance, such as *Cassia tora* L. (Ma et al. 1997b), wheat cv. Atlas 66 and rye cv. King (Li et al. 2000), taro (*Colocasia esculenta* (L.) Schott) (Ma and Miyasaka 1998) and buckwheat (*Fagopyrum esculentum* Moensch) (Zheng et al. 1998). Our results agree with previous findings showing no association between the Al-induced release of organic acid and Al tolerance in signal grass and ruzi grass (*Brachiaria ruziziensis* Germ. & Evrard), which are considered highly Al-tolerant species (Wenzl et al. 2001). We conclude that interspecific differences in Al tolerance among our

Table 1. Changes in pH of the nutrient solutions induced by plant roots 24 and 48 h after renewal of the solution. Values are means of three replicates, each measured three times between 20 and 26 days after treatment in Experiment 1.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (h)</th>
<th>A. auriculiformis</th>
<th>E. camaldulensis</th>
<th>M. cajuputi</th>
<th>M. leucadendra</th>
<th>lsd(0.5)</th>
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<tbody>
<tr>
<td>0 mM Al + pH 4.2</td>
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<td>4.58</td>
<td>4.59</td>
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</table>

Figure 4. Aluminum concentrations in shoots and roots of the study species. Seedlings were grown for 30 days in nutrient solution containing 160 µM {Al³⁺} + pH 4.2 (filled columns), 160 µM {Al³⁺} + pH 3.5 (cross hatched columns) and 950 µM {Al³⁺} + pH 3.5 (striped columns). Values are means of three replicates. The numbers and letters above the columns present the comparison (by lsd(0.05)) between treatments within a species and between species within a treatment, respectively; columns with the same number or letter are not significantly different.

Figure 5. Relationship between Al concentration in roots and relative value of whole-plant fresh mass (FW). Seedlings were grown for 30 days in nutrient solution containing 160 µM {Al³⁺} + pH 4.2 (○), 160 µM {Al³⁺} + pH 3.5 (△) and 950 µM {Al³⁺} + pH 3.5 (□). Values are means of three replicates.
four tropical woody species cannot be accounted for by differences in organic acid release alone.

Aluminum significantly increased the release of inorganic phosphate by roots of our study species, indicating that phosphate release could contribute to Al tolerance in these plants (cf. Pellet et al. 1996). A higher rate of phosphate release was observed in *A. auriculiformis* than in the other species at 4 mM Al (Figure 3), suggesting that the higher Al tolerance of *A. auriculiformis* compared with the other tropical woody species we examined is partly associated with phosphate release. The alleviation of Al toxicity by phosphate release may be associated with (1) the formation of an Al–Pi complex (Pellet et al. 1996) either in the apoplasm, on the root surface or in the rhizosphere (Taylor 1991, Luttge and Clarkson 1992); and (2) an increase in the rhizosphere pH leading to a decrease in Al$^{3+}$ activity owing to the high affinity of phosphate for H$^+$ as well as Al$^{3+}$ (Pellet et al. 1996).

Low pH tolerance was higher in *A. auriculiformis* than in the other species (Figures 1 and 2) and may be associated with the greater capacity of *A. auriculiformis* to amend low pH. In the absence of Al in the nutrient solution at pH 3.5, *A. auriculiformis* increased the pH from 3.5 to about 4.9 and 6.6 at 24 and 48 h after renewal, respectively (Table 1), whereas *M. cajuputi* increased the pH to about 3.7 and 5.1 at 24 and 48 h, respectively. The release of phosphate can increase rhizosphere pH; however, the pH 3.5 treatment without Al application did not induce the release of phosphate (data not shown), indicating that the ability of *A. auriculiformis* to amend low pH is not associated with phosphate release. Therefore, low pH and Al need to be examined independently to determine the mechanism underlying Al tolerance of *A. auriculiformis* at low pH.

Exudation of ligands for Al, such as citrate and phosphate, is only partly involved in tolerance of our study species to Al at low pH. Adaptation to high-Al toxic-acid-sulfate soil by *M. cajuputi* could involve Al tolerance mechanisms other than the release of Al ligands, because organic acids, phosphate and phenolic compounds released from roots of *M. cajuputi* are inadequate to detoxify Al. Wenzle et al. (2001) suggested that the amount of organic acids secreted by the root apices of Al-tolerant wheat and maize genotypes might be adequate to detoxify Al only at concentrations well below those inhibiting root growth of signal grass ([{Al$^{3+}$}] < 115 µM).

Although shoot Al concentrations of *E. camaldulensis* were similar or less than those of *A. auriculiformis* and *M. cajuputi*,

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Table 2. Effects of excess Al, low pH and P deficiency on the concentrations (mM) of organic acids, phenolic compounds and P in root cell sap. Seedlings were grown in nutrient solution containing six treatments, (1) 0 mM Al + pH 4.2, (2) 0 mM Al + pH 3.5, (3) 0.5 mM Al + pH 4.2, (4) 0.38 Al + pH 3.5, (5) 4 mM Al + pH 3.5 and (6) 0 mM Al + pH 4.2 + low P (2.5 µM). Samples were taken 30 days after treatment. Values are means of three replicates. The concentration of phenolic compounds is expressed as caffeic acid equivalents; nd = not detectable; a dash means the value was not measured. Within a treatment, values followed by the same letter are not significantly different at $P = 0.05$.

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<th>1</th>
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<th>6</th>
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<td></td>
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4.2 to 3.5 and that the reduction was significantly decreased by A. auriculiformis only in the presence of Al toxicity by low pH may occur only in plant species. Alleviation of H⁺ toxicity by Al³⁺ has been observed in turnip roots (Kinraide and Parker 1990), soybeans (Noble et al. 1988), wheat (Kinraide et al. 1992), Eucalyptus manifera (Mudie and Pinus radiata D. Don (Huang and Bachelard 1993), and several other woody plants (Osaki et al. 1997, Ofei-Manu et al. 2001). Our results suggest a negative relationship between low pH tolerance and degree of alleviation of H⁺ toxicity by Al³⁺ (Figure 6) in all the study species except M. leucadendra. Growth of M. leucadendra was greatly reduced at pH 3.5; however, application of 160 µM Al³⁺ had no alleviating effect on H⁺ toxicity. This may have been because the Al concentration was too high; only Al concentrations that are below a certain threshold concentration for a particular pH alleviate H⁺ toxicity. Although 160 µM Al³⁺ decreased plant growth at pH 4.2 in all species except M. cajuputi, pH 3.5 alleviated Al toxicity only in A. auriculiformis (Figure 2), suggesting that the alleviation of Al toxicity by low pH may occur only in plant species tolerant to low pH.

Concentration of organic acids in roots

Except in A. auriculiformis, where the concentration of citrate was much higher than that of oxalate, Al application increased the concentration of citrate and decreased the concentration of oxalate in root cell sap, resulting in a sharp increase in the citrate:oxalate ratio (Table 2). Watanabe and Osaki (2001) reported a decrease in malate concentration and an increase in citrate concentration in xylem sap of Melastoma seedlings in the presence of Al. The increase in citrate synthesis could serve to detoxify Al³⁺, because citrate can detoxify 2–3 times more Al (Ma et al. 1997a, Ma et al. 1998, Zheng et al. 1998, Ma 2000). These results suggest that an increase in citrate synthesis could be an advantageous response to Al stress in some plant species.

In conclusion, the higher tolerance to Al toxicity at low pH of A. auriculiformis and M. cajuputi compared with E. manifera and M. leucadendra was associated with a higher tolerance to low pH in A. auriculiformis and a higher tolerance to Al in M. cajuputi. The release of organic acids and inorganic phosphate could not explain the interspecific differences in Al tolerance among the examined species. At low pH, the lower the tolerance to low pH of the plant, the larger the growth stimulation by low Al concentration in the culture solution. Alleviation of Al toxicity by low pH may occur only in plant species that are tolerant to low pH, such as A. auriculiformis.

References


