Role of organic acids in aluminum accumulation and plant growth in *Melastoma malabathricum*

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Received August 1, 2001; accepted January 5, 2002; published online July 2, 2002

Summary  *Melastoma malabathricum* L. (melastoma) is an Al-accumulating woody plant that grows in tropical Southeast Asia in acid soils with high aluminum (Al) concentrations and low nutrient concentrations. Because oxalate serves as a ligand for Al accumulation in melastoma leaves and citrate is the ligand associated with Al translocation from roots to shoots, we investigated the role of organic acids in the adaptation of melastoma to growth on these soils. Phosphorus starvation increased oxalate concentration in the rhizosphere, enabling melastoma to solubilize insoluble aluminum phosphate in the rhizosphere. Increased availability of P and Al in the rhizosphere enhanced growth. In the xylem sap, the concentration of citrate increased with increasing Al concentration. In contrast, the concentrations of malate, succinate and α-ketoglutarate in the xylem sap decreased with increasing Al concentration, suggesting that tricarboxylic acid cycle enzymes were affected by Al treatment.

Keywords: acid soil, aluminum-accumulator species, aluminum translocation, citrate, oxalate, phosphorus.

Introduction

Generally, a high soil aluminum (Al) concentration restricts plant growth in acid soils. An Al ion can easily form a complex with intracellular substances at cytosolic pH, and thereby inhibit metabolism (Matsumoto 2000). However, Al-accumulator species are found in acid soils, particularly in the tropics, and these species have developed tolerance to high tissue Al concentrations, enabling them to survive and grow in acid soils. Some species that have adapted to acid soils accumulate more than 10,000 mg Al kg\(^{-1}\) in leaves in the form of monomeric Al and Al-oxalate complexes (Watanabe et al. 1998a). High tissue Al concentrations in melastoma are the result of a high capacity for Al retention in root symplasts, rather than a high Al uptake rate into the symplasts (Watanabe et al. 2001). In addition, the presence of Al in water culture increases oxalate concentrations in root apoplasts of melastoma plants (Watanabe et al. 2001). Organic acid exudation from roots is generally considered to be an important mechanism for avoiding Al toxicity (Ma 2000), as a result of the formation of Al chelates with organic acids (e.g., Bartlett and Riego 1972, Hue et al. 1986, Luo et al. 1999). However, melastoma hyper-accumulates Al, and the rate of Al accumulation in the root symplastic fractions does not decline after the onset of oxalate exudation (Watanabe et al. 2001). These findings suggest that oxalate exudation in melastoma does not prevent Al accumulation.

We recently investigated the form of Al translocated from roots to shoots in melastoma (Watanabe and Osaki 2001). An \(^{27}\)Al NMR analysis indicated that, in plants grown in a nutrient solution with 0.2 mM Al for 3 weeks, the translocation form of Al is an Al-citrate complex (Al:citrate = 1:1). In addition, we found that the xylem sap of melastoma plants grown in the absence of Al contains high concentrations of malate, whereas high concentrations of citrate are found in the presence of Al.

Because the mechanisms of Al hyper-accumulation in melastoma remain unclear, we investigated the role of oxalate exudation from roots as well as the form of Al transported from roots to shoots.

Materials and methods

**Experiment 1: Effects of oxalate application on Al uptake**

To investigate whether oxalate exudation from roots prevents Al uptake by melastoma, we examined the effect of 0.5 mM oxalate application on Al uptake by melastoma plants. Cuttings were prepared from adult melastoma plants and grown in an Al-free nutrient solution for 4 months to obtain plants of...
uniform size. All experiments were carried out in a greenhouse at Hokkaido University under natural conditions (13–15 h photoperiod and a day/night temperature of 20–28/18–22 °C). Cuttings were rooted in tap water and then transferred to 56-l containers containing standard nutrient solution at pH 4.0: 2.14 mM N (NH₄NO₃), 0.77 mM K (K₂SO₄·KCl = 1:1), 2.0 mM Ca (CaCl₂·2H₂O), 0.82 mM Mg (MgSO₄·7H₂O), 35.8 µM Fe (FeSO₄·7H₂O), 9.1 µM Mn (MnSO₄·H₂O), 46.3 µM B (H₃BO₃), 3.1 µM Zn (ZnSO₄·7H₂O), 0.16 µM Cu (CuSO₄·5H₂O) and 0.05 µM Mo ((NH₄)₆Mo₇O₂₄·4H₂O) with P = 0.1 mM as NaH₂PO₄. After 2 months, well-rooted uniform plants (shoot height = 15 cm, main root length = 12–15 cm) were selected for study. Three plants were transferred to each 1.5-l pot containing treatment solution that was constantly aerated. The treatment solutions, which consisted of the standard nutrient solution except that no P was added, also contained 0.5 mM AlCl₃ with or without 0.5 mM oxalic acid. The treatment solutions were renewed daily. At the end of the 1-week treatment period, plants were sampled for analysis.

Analysis Roots were washed with deionized water, and plants separated into roots, stems and leaves. Each set of organs was dried at 80 °C for 72 h and weighed. For Al analysis, 100 g of the dried samples was digested in H₂SO₄/H₂O₂ and Al concentrations were determined by inductively coupled plasma emission spectrometry (ICPES) (ICP-7000, Shimadzu, Kyoto, Japan).

Experiment 2: Determination of ability to utilize aluminum phosphate

In Experiment 2, the relationship between concentrations of organic acids in the rhizosphere and the ability to utilize aluminum phosphate was examined. Cuttings were rooted in tap water and transferred to a 56-l container filled with a perlite:vermiculite mixture (1:1), precultured for 3 weeks in a greenhouse at Hokkaido University. The plants were fertilized every 3 days with an adequate amount of the standard nutrient solution containing 0.1 mM P. After the 3-week pre-culture, three melastoma plants were transferred to each 1.5-l pot filled with treatment solution, consisting of standard nutrient solution except that no P was added, also contained 0.5 mM AlCl₃ with or without 0.5 mM oxalic acid. The treatment solutions were renewed daily. At the end of the 1-week treatment period, plants were sampled for analysis.

Analysis The dried plant samples were digested as described in Experiment 1 and Al concentrations determined by ICPES. The P concentrations were determined by the vanado-molybdate yellow method, and organic acid concentrations were determined by capillary electrophoresis (Quanta-4000CE, Waters, Milford, MA; Watanabe et al. 1998a).

Experiment 3: Changes in organic acid and mineral concentrations in the xylem sap with time

Watanabe and Osaki (2001) found that Al is complexed with citrate for translocation, whereas Al is complexed with oxalate for accumulation in leaves (Watanabe et al. 1998a). In Experiment 3, changes in Al and organic acid concentrations (total and chelated) with time were examined. The melastoma plants were prepared and precultured as described in Experiment 1. After preculture, the plants were transferred to 40-l containers filled with treatment solution, consisting of standard nutrient solution (P-free) plus Al or P or both. The treatments were: +Al (0.09 mM AlCl₃), ++Al (0.2 mM AlCl₃), +P (0.1 mM NaH₂PO₄) and +Al+P (0.2 mM AlCl₃ and 0.1 mM NaH₂PO₄) (soluble Al and P = 0.09 and 0.05 mM, respectively). The pH of each solution was adjusted to 4.0 every day. The Al and P concentrations in the treatment solutions, which were filtered through membrane filters (pore size = 0.45 µm), were determined daily, and adequate amounts of AlCl₃ and NaH₂PO₄ were added to maintain the target soluble Al and P concentrations in the treatment solutions.

Sampling was performed on Days 1, 3 and 10 after the start of the treatments. Xylem sap was collected according to the modified method of Krämer et al. (1996). Briefly, each shoot was carefully cut with a razor. The cut end was washed with deionized water, wiped with tissue and then covered with cotton and a plastic bag and kept in the dark for 12 h at about 20 °C. The volume of xylem sap collected was estimated by the change in the weight of the cotton. The xylem sap was then extracted from the cotton by pressing in a syringe. Fresh leaves and roots were homogenized in cold 0.01 M HCl (1 g leaves per 10 ml HCl or 1 g roots per 5 ml of HCl) to determine organic acid concentrations in the plant. To determine tissue mineral concentrations, xylem sap and extract were filtered through a membrane filter (pore size = 0.45 µm) before analysis. Plants were sampled as described in Experiment 1.
Analysis  The dry plant samples were digested as described in Experiment 1 and the Al concentrations in the xylem sap and plant tissue were determined by ICPES. Organic acid concentrations were determined by capillary electrophoresis. Phosphate concentrations in xylem sap and in plant tissue were determined by the vanado-molybdate yellow method.

Organic acid concentrations in the xylem sap were measured after either two dilutions with deionized water or two dilutions with 0.02 M HCl to compare the concentrations of free organic acid with those of chelated organic acid. The organic acid–cation complex was broken down by acidification, and the total organic acid concentrations determined by capillary electrophoresis.

Statistics  Results in Experiment 1 were analyzed with Student’s t-test. Results in Experiments 2 and 3 were evaluated by analysis of variance, and Fisher’s LSD when significant ($P < 0.05$) treatment effects were found.

Results  Experiment 1: Effects of oxalate application on Al uptake  Oxalate application had no significant effect on Al concentrations in leaves (Figure 1). In contrast, Al concentrations in roots were significantly increased in response to oxalate application (Figure 1).

Experiment 2: Determination of the ability to utilize aluminum phosphate  Growth of melastoma plants in the Al-P treatment was significantly superior to that in the +P and –P treatments (Figure 2). Among treatments, P concentrations in the rhizosphere solution were highest in the +P treatment (Table 1). In the Al-P treatment, high P concentrations were detected in the rhizosphere solution, whereas P concentrations in the plant-free-medium solution were almost negligible (Table 1). Shoot P concentrations were significantly higher in the Al-P treatment than in the –P treatment, and shoot P contents were similar in the Al-P and +P treatments (Tables 1 and 2).

The Al concentrations in the plant-free-medium solution were very low for all of the treatments (Table 1). However, Al concentrations were significantly increased in the rhizosphere solution in the Al-P treatment. Among treatments, Al concentrations in both shoots and the rhizosphere solution were highest in the Al-P treatment, and significantly higher in the –P treatment than in the +P treatment (Tables 1 and 2). The Al concentrations measured in roots were inaccurate because of contamination with vermiculite (data not shown). Oxalate, malate and citrate were detected in the rhizosphere solutions. Oxalate concentrations were significantly higher in the Al-P treatment than in the +P treatment, whereas malate and citrate concentrations did not differ among treatments (Table 2). Root oxalate concentrations were significantly higher in the Al-P and –P treatments than in the +P treatment.

Experiment 3: Changes in organic acid and mineral concentrations in the xylem sap with time  Aluminum in the xylem sap was detected on the first day of all treatments with Al, and Al concentrations increased with time (Figure 3). On Day 10, Al concentrations in the xylem sap were significantly lower in the +Al+P treatment than in the +Al and ++Al treatments. There was almost no significant difference in Al concentration in the xylem sap between the +Al treatment (0.09 mM Al) and the ++Al treatment (0.2 mM Al). Phosphorus concentrations in xylem sap were significantly higher in the +Al+P treatment than in the other treatments, even on the first day of the treatments (Figure 3).

Changes in citrate concentrations in the xylem sap showed
the same trend as changes in Al concentrations (Figure 4). Concentrations of succinate, α-ketoglutarate and malate decreased up to the third day of the treatment, and remained at low values in the presence of Al, whereas they were unchanged in the absence of Al. The pH was around 4.8 and did not differ among treatments (Figure 4). Total citrate concentrations in the xylem sap were closely correlated with Al concentrations (Figure 5A). However, the ratio of Al to total citrate increased with treatment time (Figure 5A). The ratio of free citrate (including loosely bound citrate) to total citrate was above 0.8 during the period of +P treatment (in the absence of Al) (Figure 5B). In contrast, the ratio was significantly lower in the treatments with Al application than in the +P treatment, and decreased with time. Results of two-way ANOVA showed that there was a significant interaction between treatment and time for all of the elements determined in the xylem sap ($P > 0.05$ or 0.01).

The Al concentrations in leaves did not differ significantly between treatments in the presence of Al, although root Al concentrations were significantly higher in the +Al+P treatment (Table 3). Despite lower soluble P concentrations in the +Al+P treatment solution (see Materials and methods), leaf P concentrations were higher in the +Al+P treatment than in the +P treatment (Table 3). Leaf oxalate concentrations increased 2–4 times in response to 10 days of exposure to Al compared with values in control leaves (+P treatment), whereas root oxalate concentrations were unaffected by exposure to Al (Table 4). The presence of Al increased citrate concentrations more than 10 and 20 times in leaves and roots, respectively, compared with the controls (Table 4). Malate concentrations in leaves and roots were low, but leaf malate concentrations were significantly increased by the absence of P (+Al and ++Al treatments) (Table 4).

### Discussion

Watanabe et al. (2001) reported that Al application increases

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Table 1. Concentrations of oxalate, malate, citrate, phosphorus (P) and aluminum (Al) (µM) in rhizosphere extracts at Day 21. Values are the means of three replicates ± SE. Different letters indicate statistically significant treatment effects ($P < 0.05$). Values in parenthesis are µM g–1.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oxalate (µM)</th>
<th>Malate (µM)</th>
<th>Citrate (µM)</th>
<th>P (µM)</th>
<th>Al (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+Plant Superphosphate</td>
<td>28 ± 8 a</td>
<td>5 ± 1 a</td>
<td>98 ± 22 a</td>
<td>365 ± 57 a</td>
<td>9 ± 2 a</td>
</tr>
<tr>
<td>Aluminum phosphate</td>
<td>(62 ± 18 A)</td>
<td>(10 ± 3 A)</td>
<td>(224 ± 62 A)</td>
<td>(801 ± 106 A)</td>
<td>(20 ± 2 A)</td>
</tr>
<tr>
<td>–P</td>
<td>136 ± 40 b</td>
<td>11 ± 4 a</td>
<td>131 ± 30 a</td>
<td>130 ± 17 b</td>
<td>102 ± 16 b</td>
</tr>
<tr>
<td>(222 ± 38 B)</td>
<td>(17 ± 6 A)</td>
<td>(216 ± 37 A)</td>
<td>(220 ± 24 B)</td>
<td>(171 ± 18 B)</td>
<td></td>
</tr>
<tr>
<td>80 ± 21 ab</td>
<td>8 ± 2 a</td>
<td>131 ± 23 a</td>
<td>28 ± 10 c</td>
<td>21 ± 5 a</td>
<td></td>
</tr>
<tr>
<td>(174 ± 50 B)</td>
<td>(18 ± 5 A)</td>
<td>(282 ± 52 A)</td>
<td>(57 ± 17 C)</td>
<td>(44 ± 10 A)</td>
<td></td>
</tr>
</tbody>
</table>

1 Extraction was conducted with 5 g of the medium that was cultured without plant.
2 nd = Not detected.

Table 2. Concentrations of Al and P, and P contents in shoots of melastoma at Day 21. For comparison, the values at Day 0 are also presented. Values are the means of three replicates ± SE. Different letters indicate statistically significant treatment effects ($P < 0.05$). Abbreviations: +P = superphosphate; Al-P = aluminum phosphate; and –P = no phosphate.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Al (mg kg–1)</th>
<th>P (mg g–1)</th>
<th>P (mg shoot–1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+P</td>
<td>253 ± 29 a</td>
<td>2.48 ± 0.06 a</td>
<td>2.94 ± 0.13 a</td>
</tr>
<tr>
<td>Al-P</td>
<td>1737 ± 39 b</td>
<td>2.08 ± 0.04 b</td>
<td>2.97 ± 0.22 a</td>
</tr>
<tr>
<td>–P</td>
<td>646 ± 59 c</td>
<td>1.45 ± 0.05 c</td>
<td>1.37 ± 0.05 b</td>
</tr>
<tr>
<td>Day 0</td>
<td>234 ± 25 a</td>
<td>2.39 ± 0.13 a</td>
<td>–</td>
</tr>
</tbody>
</table>

Figure 3. Changes in concentrations of Al and P in the xylem sap and changes in volume of the xylem sap with time (Experiment 3). Vertical bars at each sampling date indicate LSD at $P < 0.05$ when ANOVA indicated significant treatment effects ($P < 0.05$).
Root exudation of organic acid is generally considered a mechanism to exclude Al (Ma 2000). However, we found that the increased rate of Al accumulation in the symplastic fraction of melastoma roots did not decline after the onset of oxalate exudation (Watanabe et al. 2001), suggesting that oxalate exudation does not prevent Al uptake by melastoma roots. The results presented in Figure 1 confirm the suggestion that oxalate does not inhibit Al uptake by melastoma.

In a second experiment to determine the role of oxalate exudation in melastoma, we found higher concentrations of oxalate in the rhizosphere solution of melastoma plants in the Al-P and –P treatments than in the +P treatment (Table 1). Melastoma plants in the –P treatment took up more Al from the medium than plants in the +P treatment (Table 2), indicating that high concentrations of oxalate in the rhizosphere help to solubilize insoluble Al compounds (mainly vermiculite). However, we could not determine whether the large amount of oxalate in the rhizosphere solution came directly from the roots or was derived from the degradation products of other exuded organic compounds (e.g., citrate). Increased concentrations of oxalate in the rhizosphere in response to P starvation may have solubilized insoluble aluminum phosphate in the rhizosphere of melastoma plants in the Al-P treatment (Table 1). We found that melastoma plants in the Al-P treatment took up similar amounts of P to plants in the +P treatment (Table 2) despite the lower amounts of available P in the medium (Table 1). Because available P is limited in many acid soils where melastoma grows (Osaki et al. 1998a, 1998b, Onthong et al. 1999), the ability to solubilize insoluble aluminum phosphate in the rhizosphere is extremely important. It has been demonstrated that roots of some dicotyledonous plants exude large amounts of organic acid into the rhizosphere in response to P deficiency (Jones 1998).

In Experiment 3, P concentrations were significantly higher in leaves and xylem sap of plants in the +Al+P treatment than in the +P treatment (Table 3 and Figure 3), indicating that Al stimulated P uptake by melastoma. High concentrations of Al in the rhizosphere solution may also have stimulated P uptake by melastoma in the Al-P treatment in Experiment 2. However, maximum growth in the Al-P treatment in Experiment 2 could not be explained by enhanced P uptake alone (Figure 2 and Table 2), suggesting that Al itself has a beneficial effect on growth. A beneficial effect of Al on growth has also been reported for Miconia albicans (Sw.) Triana (Melastomataceae). When M. albicans plants growing in calcareous soil developed chlorotic leaves, the symptom was eliminated when a portion of the root system was exposed to 10 mg l–1 Al solution (without nutrients) (Haridasan 1988). The mechanism underlying this effect of Al is unknown.

We found that Al concentrations in xylem sap were lower in the +Al+P treatment than in the +Al and ++Al treatments (Figure 3). This finding may reflect dilution of the xylem sap by an increased flow rate in response to the +Al+P treatment rather than precipitation of Al as aluminum phosphate in the roots, because shoot Al concentrations did not significantly differ between Al treatments (Table 3). There was no significant difference in Al concentration in shoot and xylem sap between the +Al treatment (0.09 mM Al) and the ++Al treatment (0.2 mM Al) (Figure 3 and Table 3). This finding suggests that 0.09 mM Al in the external solution results in maximum Al uptake, which does not depend on transpiration.

Total concentration of citrate, a primary ligand of Al in the xylem sap, increased with time after the start of Al exposure, and the pattern of increase was almost identical to that for Al concentration (Figures 3 and 4). Although the ratio of Al to total citrate in the Al treatment was nearly 1:1 on the first day of the treatment, it increased with time (Figure 5A). This increase was inversely related to changes in the ratio of free citrate (including loosely bound citrate) to total citrate with time (Figure 5B). These results indicate that Al formed a complex with citrate in the xylem sap, and that the rate of biosynthesis of citrate was slightly less than the rate of uptake of Al. In the absence of Al (+P treatment), trace amounts of citrate were present in the xylem sap, and the primary organic acid was malate (Figure 4). Oxalate, which is a ligand of Al in the leaves of melastoma, was not detected in the xylem sap regardless of the
treatment. Although malate was the primary organic acid in the xylem sap on the first day of the Al treatment, malate concentrations decreased markedly on the third day of the Al treatment (Figure 4). In addition, the concentrations of α-ketoglutarate and succinate also decreased in response to Al exposure (Figure 4). The decrease in concentrations of malate, α-ketoglutarate and succinate in the xylem sap corresponded to an increase in citrate and Al concentrations. Because these organic acids are intermediates in the tricarboxylic acid (TCA) cycle, we speculate that the activities of several TCA cycle enzymes are affected by Al signals.

The increase in citrate synthase (CS) activity in roots is associated with root exudation of citrate. De la Fuente et al. (1997) introduced a Pseudomonas aeruginosa CS gene into tobacco and papaya, causing both CS activity and citrate exudation to increase and Al tolerance to be enhanced in the transgenic plants. Li et al. (2000) demonstrated that one explanation for high Al tolerance in rye (Secale cereale L.) is related to citrate and malate exudation from roots, which is associated with an Al-induced increase in CS activity. The Al-induced increase in citrate concentration in the xylem sap of melastoma may also be related to increased CS activity. Root citrate concentrations increased more than 20 times over the 10-day exposure to Al compared with the control (+P treatment) (Table 4). Buckwheat (Fagopyrum esculentum Moench) also accumulates Al in shoots (< 1500 mg kg⁻¹) (Ma et al. 1997b, Osaki et al. 1997). In buckwheat, Al in leaves occurs as the Al-oxalate complex (Ma et al. 1997b), whereas Al is translocated as an Al-citrate complex (Ma and Hiradate 2000), which is similar to that in melastoma. In the case of buckwheat, however, the citrate concentrations in the xylem sap are constitutively high, and Al application does not affect the concentrations of citrate and malate in the xylem sap (Ma and Hiradate 2000). The different responses of organic acid me-

Table 3. Concentrations of Al and P in each organ of melastoma grown in treatment solution for 10 days. For comparison, the concentrations at Day 0 are also presented. Values are the means of three replicates ± SE. Different letters indicate statistically significant treatment effects (P < 0.05). Treatments: +Al = 0.09 mM Al; ++Al = 0.2 mM Al; +P = 0.1 mM P; and +Al+P = 0.09 mM Al and 0.05 mM P (soluble form).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Al (mg kg⁻¹)</th>
<th>P (mg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf</td>
<td>Root</td>
</tr>
<tr>
<td>+Al</td>
<td>6819 ± 276 a</td>
<td>9341 ± 326 a</td>
</tr>
<tr>
<td>++Al</td>
<td>6133 ± 619 a</td>
<td>9187 ± 562 a</td>
</tr>
<tr>
<td>+P</td>
<td>224 ± 17 b</td>
<td>600 ± 37 b</td>
</tr>
<tr>
<td>+Al+P</td>
<td>6691 ± 1063 a</td>
<td>14,648 ± 455 c</td>
</tr>
<tr>
<td>Day 0</td>
<td>269 ± 24 b</td>
<td>680 ± 20 b</td>
</tr>
</tbody>
</table>
Metabolism to Al application may be related to differences in Al accumulation capacity of shoots between melastoma (Table 3, Watanabe et al. 1997) and buckwheat (< 1500 mg kg\(^{-1}\)).

In conclusion, the role of organic acids in melastoma can be summarized as shown in Figure 6. Oxalate has two roles: (1) it solubilizes insoluble P (aluminum phosphate) in the rhizosphere, and (2) it serves as a ligand for Al accumulation in the leaves (Watanabe et al. 1998\(^a\)). It is unknown whether the large amounts of oxalate in the rhizosphere come directly from the roots or are degradation products from other exuded organic compounds. Because the cation exchange sites in melastoma roots show a high capacity for, and a high affinity to, Al (Watanabe et al. 2001), we hypothesize that the cell-wall sites on the root surface absorb Al from Al-oxalate complexes, leaving large amounts of oxalate in the rhizosphere. Melastoma decreases rhizosphere pH when absorbing NH\(_4\), which is the primary inorganic nitrogen source in many acid soils (Watanabe et al. 1998\(^b\)). Aluminum that is adsorbed to the root cation-exchange sites, and the Al present as Al-oxalate on the root surface (Figure 1) may be solubilized by this acidification. The Al ion absorbed by roots forms a complex with citrate for translocation in the xylem. In leaves, Al changes the ligand from citrate to oxalate or becomes free.

References


Table 4. Concentrations of organic acids in each organ of melastoma grown in treatment solution for 10 days. For comparison, the concentrations at Day 0 are also presented. Values are the means of three replicates ± SE. Different letters indicate statistically significant treatment effects (\(P<0.05\)). Treatments: +Al = 0.09 mM Al; ++Al = 0.2 mM Al; +P = 0.1 mM P; and +Al+P = 0.09 mM Al and 0.05 mM P (soluble form).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oxalate (µmol g(^{-1}))</th>
<th>Malate (µmol g(^{-1}))</th>
<th>Citrate (µmol g(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf</td>
<td>Root</td>
<td>Leaf</td>
</tr>
<tr>
<td>+Al</td>
<td>152 ± 32 a</td>
<td>173 ± 22 a</td>
<td>1.74 ± 0.25 a</td>
</tr>
<tr>
<td>++Al</td>
<td>104 ± 35 a</td>
<td>169 ± 19 a</td>
<td>2.21 ± 0.41 a</td>
</tr>
<tr>
<td>+P</td>
<td>40 ± 2 b</td>
<td>132 ± 19 b</td>
<td>0.60 ± 0.39 b</td>
</tr>
<tr>
<td>+Al+P</td>
<td>115 ± 22 a</td>
<td>192 ± 11 a</td>
<td>0.78 ± 0.22 b</td>
</tr>
<tr>
<td>Day 0</td>
<td>35 ± 8 b</td>
<td>121 ± 18 b</td>
<td>0.21 ± 0.26 b</td>
</tr>
</tbody>
</table>

Figure 6. Diagrammatic representation of the role of organic acids in the growth of melastoma. (a) Oxalate solubilizes insoluble aluminum phosphate. (b) Aluminum (including Al in Al-oxalate complex) is adsorbed to the cation exchange sites of the roots (Watanabe et al. 2001). Root-induced acidification in the rhizosphere (Watanabe et al. 1998\(^b\)) releases Al from the cation exchange sites and from Al-oxalate precipitation. The released Al is absorbed by the roots. (c) Absorbed Al forms a complex with citrate and (d) is transported to the shoots in the xylem. (e) In the leaves, Al exchanges its citrate ligand for an oxalate ligand or becomes free.