Aluminum-Activated Citrate and Malate Transporters from the MATE and ALMT Families Function Independently to Confer Arabidopsis Aluminum Tolerance

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ABSTRACT

Aluminum (Al) activated root malate and citrate exudation play an important role in Al tolerance in many plant species. *AtALMT1*, an Al-activated malate transporter, is a major contributor to Arabidopsis Al tolerance. Here, we demonstrate that *AtMATE* encodes an Arabidopsis Al-activated citrate transporter. *AtMATE* is expressed primarily in roots and is induced by Al. A loss-of-function *AtMATE* mutant line lacks Al-activated root citrate exudation. An *AtALMT1 AtMATE* double mutant lacks both Al-activated root malate and citrate exudation and exhibits greater Al sensitivity than the single *AtALMT1* mutant. Therefore, *AtMATE* makes a significant although smaller contribution to Arabidopsis Al tolerance.

AtMATE shares the highest sequence identity with *SbMATE*

In Arabidopsis, the MATE family contains at least 56 members, which can be further classified into several clusters based on sequence similarity (Li et al., 2002; Rogers and Guerinot, 2002). *AtMATE* is the Arabidopsis MATE family member with the highest sequence similarity to *SbMATE*, the recently cloned sorghum Al tolerance gene (Magalhaes et al., 2007) (Figure 1). The *AtFRD3* clade contains 4 members, including *AtFRD3* (*At3g08040*), *AtMATE* (*At1g51340*), *At2g38330* and *At4g38380*.

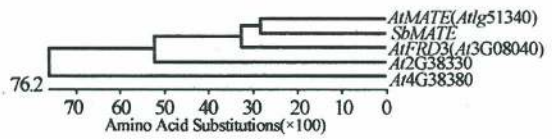

Figure 1 *AtMATE* shares the highest sequence similarity with *SbMATE* in Arabidopsis

AtMATE expression in roots is induced by Al treatment

By conducting semi-quantitative reverse-transcriptase (RT)-PCR analysis, we demonstrated that within the *AtFRD3* clade, *AtMATE* is the only member whose gene expression is induced by Al and is localized primarily to the root, which is the site where Al tolerance must occur (Figure 2).

AtMATE is responsible for Al-activated root citrate exudation

In Arabidopsis, Al induces a high level of malate and lower level of root citrate exudation (*WT*, Figure 3a, b). The *AtALMT1* knock-out line (*AtALMT1-KO*) and the *AtMATE-KO* line lack Al-activated root malate and citrate exudation, respectively. The *AtALMT1 AtMATE* double knock-out (*double-KO*) line lacks both Al-activated root malate and root citrate exudation.

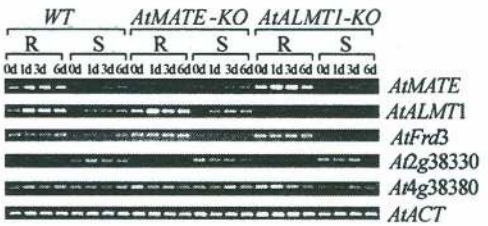

Figure 2 Spatial and temporal gene expression patterns for *AtMATE* and other members of the *AtFRD3* clade in the Arabidopsis MATE family, as well as the major Al tolerance gene, *AtALMT1*. The 6-day-old Arabidopsis seedlings, wild type (*WT*), *AtMATE* knock-out (*AtMATE-KO*) and *AtALMT1* knock-out (*AtALMT1-KO*) lines, were treated with Al^{3+} for 0 day (0d, the control), 1 day (1d), 3 days (3d), and 6 days (6d). Total RNA was extracted from roots (R) and shoots (S).

The *AtMATE AtALMT1* double mutant is highly hypersensitive to Al stress

Although *AtALMT1* is clearly the major determinant of Al tolerance in Arabidopsis as knocking out this gene caused

an approximately 60% reduction in Al tolerance with respect to the wild type at 1.5 μM Al^{3+} activity (Hoekenga et al., 2006), *AtMATE* also contribute a moderate (approx. 30%) component to the overall Arabidopsis Al tolerance. The double *AtMATE AtALMT1* T-DNA knock-out (*Double-KO*) line displayed more Al sensitivity compared to the *AtALMT1* single mutant (Figure 4).

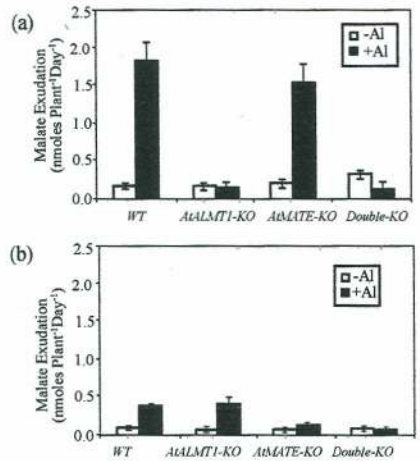

Figure 3 Root malate (a) and citrate (b) exudation under +/- Al conditions for the wild-type (*WT*) control, the *AtMATE* knock-out (*AtMATE-KO*) line, the *AtALMT1* knock-out (*AtALMT1-KO*) line, and the *AtMATE*, *AtALMT1* double knock-out (*Double-KO*) line

DISCUSSION

It appears that *AtALMT1*-mediated Al-activated malate exudation and *AtMATE*-mediated Al-activated citrate exudation evolved and function independently in conferring the full range of Arabidopsis Al tolerance.

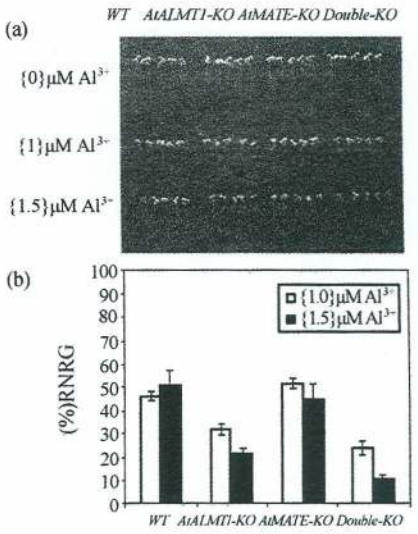

Figure 4 The *AtMATE*, *AtALMT1* double mutant is hypersensitive to Al stress. (a) Aluminum (Al) tolerance (root growth in nutrition solutions containing {0} μM, {1.0} μM, or {1.5} μM Al^{3+}) for the wild-type (*WT*) control, the *AtALMT1-KO*, the *AtMATE-KO* and the *double-KO*. (b) Aluminum tolerance as measured by percentage of relative net root growth (% RNRG = root growth in Al / control root growth × 100) in wild type and the three knock-out lines

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