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. \textit{AtAlMT1}, an Al-activated malate transporter, is a major contributor to Arabidopsis Al tolerance. Here, we demonstrate that \textit{AtMATE} encodes an Arabidopsis Al-activated citrate transporter. \textit{AtMATE} is expressed primarily in roots and is induced by Al. A loss-of-function \textit{AtMATE} mutant line lacks Al-activated root citrate exudation. An \textit{AtAlMT1 AtMATE} double mutant lacks both Al-activated root malate and citrate exudation and exhibits greater Al sensitivity than the single \textit{AtAlMT1} mutant. Therefore, \textit{AtMATE} makes a significant although smaller contribution to Arabidopsis Al tolerance.

\textit{AtMATE} shares the highest sequence identity with \textit{SbMATE}

In Arabidopsis, the MATE family contains at least 56 members, which can be further classified into several clusters based on sequence similarity \cite{Li2002, Rogers2002}. \textit{AtMATE} is the Arabidopsis MATE family member with the highest sequence similarity to \textit{SbMATE}, the recently cloned sorghum Al tolerance gene \cite{Magalhaes2007} (Figure 1). The \textit{AtFRD3} clade contains 4 members, including \textit{AtFRD3} (At3g08040), \textit{AtMATE} (At1g51340), At2g38330 and At4g38380.

\textit{AtMATE} expression in roots is induced by Al treatment

By conducting semi-quantitative reverse-transcriptase (RT) -PCR analysis, we demonstrated that within the \textit{AtFRD3} clade, \textit{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).

\textit{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 \textit{AtAlMT1} knock-out line (\textit{AtAlMT1-KO}) and the \textit{AtMATE-KO} line lack Al-activated root malate and citrate exudation, respectively. The \textit{AtAlMT1 AtMATE} double knock-out (\textit{double-KO}) line lacks both Al-activated root malate and root citrate exudation.

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Figure 1 \textit{AtMATE} shares the highest sequence similarity with \textit{SbMATE} in Arabidopsis

Figure 2 Spatial and temporal gene expression patterns for \textit{AtMATE} and other members of the \textit{AtFRD3} clade in the Arabidopsis MATE family, as well as the major Al tolerance gene, \textit{AtAlMT1}. The 6-day-old Arabidopsis seedlings, wild type (WT), \textit{AtMATE} knock-out (\textit{AtMATE-KO}) and \textit{AtAlMT1} knock-out (\textit{AtAlMT1-KO}) lines, were treated with Al\textsuperscript{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 \textit{AtMATE AtAlMT1} double mutant is highly hypersensitive to Al stress

Although \textit{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 Al³⁺ 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).

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.