Overexpression of AtALMT1 in the Arabidopsis thaliana ecotype Columbia results in enhanced Al-activated malate excretion and beneficial bacterium recruitment

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AtALMT1 (Arabidopsis thaliana Alumin activated Malate Transporter 1) encodes an Arabidopsis thaliana malate transporter that has a pleiotropic role in Arabidopsis stress tolerance. Malate released through AtALMT1 protects the root tip from Al rhizotoxicity, and recruits beneficial rhizobacteria that induce plant immunity. To examine whether the overexpression of AtALMT1 can improve these traits, the gene, driven by the cauliflower mosaic virus 35S promoter, was introduced into the Arabidopsis ecotype Columbia. Overexpression of the gene enhanced both Al-activated malate excretion and the recruitment of beneficial bacteria Bacillus subtilis strain FB17. These findings suggest that overexpression of AtALMT1 can be used as an approach to enhance a plant’s ability to release malate into the rhizosphere, which can enhance plant tolerance to some environmental stress factors.

The excretion of organic acids (OAs) from the roots plays various roles in stress tolerance in many plant species.1-4 OAs, mainly malate and citrate, form stable chelating compounds of various metals, and these are generally less toxic than soluble ions. This chemical property enables the detoxification of rhizotoxic aluminum (Al) ions in the rhizosphere (a major Al-exclusion mechanism in Al-tolerance),3 the solubilizing of iron from hardly soluble forms at an alkaline pH (a component of the strategy I iron acquisition),1 and enhancement of the Pi availability of inorganic Pi-salts in soils.2 In addition, OAs released from the roots are involved in the process of plant immune responses; this is because they recruit beneficial rhizobacteria that trigger induced systemic resistance (ISR).4 Taken together, the capacity of OA excretion from the roots is an important trait for the improvement of stress tolerance in plant breeding programs.

A series of molecular physiological studies have identified that malate excretion through AtALMT1 (Arabidopsis thaliana Aluminum activated Malate Transporter 1) plays critical roles in both Al tolerance and in the recruitment of beneficial bacterium.

Reverse genetics of AtALMT1 revealed that knocking out the gene fully suppressed Al tolerance and the recruiting of the Bacillus subtilis strain FB17 (a beneficial bacterium carrying malate chemotaxis that can trigger ISR), thus identifying the essentiality of AtALMT1 in these Arabidopsis defensive responses. Malate excretion of AtALMT1 is activated in a complex manner, which is controlled by both transcriptional and post-translational regulation.5 Recent studies have shown that AtALMT1 expression is induced by root treatments with Al; phytohormones such as indole-3-acetic acid (IAA) and abscisic acid (ABA); and chemical inducers such as hydrogen peroxide (H2O2), low pH, or flagellin 22 (flg22; a kind of microbe-associated molecular pattern [MAMP]).7 Furthermore, it is also inducible in response to bacterial challenge, and by flg22 treatment to the shoots.8 These multiple regulations likely fit to its pleiotropic roles in stress tolerance. Modification of AtALMT1 expression patterns could be one approach for the transgenic breeding of crops that have multiple enhanced stress tolerances.
Previously, this concept was tested in barley (Hordeum vulgare) to confer Al-tolerance by the introduction of wheat (Triticum aestivum) ALMT1 (TaALMT1). Growth of TaALMT1-overexpressing barley (cv Golden Promise) in acid soil showed the same Al tolerance level as the wheat Al-tolerant accessions. In the study, transgenic barley with ectopic expression of TaALMT1 developed the capacity for malate excretion in plants that originally lacked the ability to excrete malate. It is of interest to analyze whether a similar approach can be used for the enhancement of malate excretion, and its beneficial effects, in other host plants with malate excreting capacity, such as Arabidopsis. In the present study, we introduced AtALMT1 to the Arabidopsis accession Columbia-0 (Col-0) and analyzed beneficial effects.

A cDNA of AtALMT1 (At1g08430) was introduced into a T-DNA region of the mini Ti-plasmid vector pBE2113. This was then used to overexpress AtALMT1 via a cauliflower mosaic virus (CaMV) 35S promoter with an Ω enhancer. The CaMV35S-Ω-AtALMT1 was introduced into Col-0 by Agrobacterium-mediated floral dip transformation using hyper-virulence Agrobacterium strain GV3101. Transgenic Arabidopsis plants were selected using kanamycin resistance as the selection marker, and T2 seed progenies obtained by controlled self-pollination of T1 plants. These procedures were identical to those described by Sawaki et al. Transgenic lines were examined for the integration and expression of the transformed gene by reverse transcription (RT)-PCR. Al-activated malate excretion was examined in progeny 5-d-old × 15 seedlings (3 biological replicates) by incubating them in an Al-containing solution (Al 10 μM, pH 5.0; containing nutrients and sucrose; as described in Kobayashi et al.) for 12 h. Malate was quantified using the enzyme reaction coupled with NADH cycling method as described in Kobayashi et al.14 Histochemical analysis of the attraction of the beneficial rhizobacterium strain (Bacillus subtilis strain...
FB17) was performed as previously described by Lakshmanan et al. 

The examination of phenotypes was performed using 2 independent transgenic lines. Control treatment of no Al did not induce AtALMT1 in Col-0, whereas the transgenic lines expressed the gene at much greater levels. AtALMT1 expression was induced by Al in Col-0, but the expression levels were greater in the transgenic lines (Fig. 1A). These results indicated that AtALMT1 was constitutively expressed in the transgenic plants in both Al and control treatments. When the transgenic plants were incubated in Al solutions, they excreted about 5–10 times greater levels of malate than observed in wild type Col-0 (Fig. 1B). Therefore, Al-activated malate excretion can be enhanced in the naturally malate releasing Arabidopsis Col-0 accession. Although excretion levels were much lower in the control solutions of both genotypes, a similar level of increase (approximately 3-fold) was detected in the transgenic lines. Under these conditions, the recruitment of beneficial bacterium was enhanced in transgenic plants overexpressing AtALMT1 (Fig. 1C). The colonization of FB17 at the root surface was greater in the transgenic line than in Col-0 (observe the brighter green color at the root surface; Fig. 1C). These results indicated that the overexpression of AtALMT1 could be beneficial for the enhancement of Al-activated malate excretion, and the recruitment of beneficial bacteria.

Under our conditions, gain of Al tolerance, and the suppression of disease symptoms in transgenic plants were limited. When plants overexpressing AtALMT1 were grown in Al toxic medium containing 6 μM Al (pH 5.0), one of the transgenic line significantly grew better than Col-0 (Fig. 1D). However, the other transgenic line (Line 11) did not show increased Al-tolerance; this line released a lower level of malate than the other line (Line 5). The severity of the visible disease symptom of Pseudomonas syringae pv tomato (Pst) DC3000 infection in the presence of FB17 colonization to the roots was slightly repressed in the transgenic lines in comparison to Col-0 (Fig. 1E). Both phenotypes (i.e., Al tolerance by root growth and suppression of disease by root colonization of FB17) were suppressed in the AtALMT1-KO (the SALK Arabidopsis T-DNA insertion mutant; SALK_009629), which clearly indicated that this gene is essential for obtaining these phenotypes. AtALMT1-KO showed hypersensitivity to Al, and no suppression of the severity of the visible disease symptom of Pst DC3000 by root colonization of FB17 that triggers ISR. These observations confirm our previous investigation in which we identified essential roles of AtALMT1 in these responses. 4,6 Overexpression resulted in enhanced malate excretion to the Al medium, and enhanced colonization of FB17 (Fig. 1B and C). It induced additional small gains of Al tolerance or suppression of disease symptoms in line 5 that released malate by greater levels of AtALMT1 expression (Fig. 1A, D, and E). This indicates that overexpression technology of ALMT1 would be useful to have additional gain of beneficial phenotypes in the host plant releasing malate. However, the gain of positive phenotypes is negligible in another line (Line 11) that has lower capacity of malate excretion than the Line 5. This may be explained by the characteristics of the background levels of both traits in the Col-0 accession. Col-0 is one of the most Al tolerant accessions of Arabidopsis, and it has a greater ability for malate excretion than other accessions. 6 This suggests that Al tolerance due to malate excretion might be nearly saturated in this host accession, and improvement of Al-tolerance require very high levels of malate excretion (e.g., the level observed with Line 5). Similarly, ISR induced by FB17 could be saturated by the basal level of malate excretion in Col-0. These possibilities can be analyzed by introducing the same gene into other Arabidopsis accessions that have a lower ability to excrete malate, such as Ler-0. In conclusion, the overexpression of AtALMT1 is thought to be a reasonable technology to enhance Al-activated malate excretion and the recruitment of beneficial bacterium. This would be useful in the improvement of phenotypes of accessions or species that have an insufficient ability for malate excretion.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Author's Note

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