Brassica oleracea MATE Encodes a Citrate Transporter and Enhances Aluminum Tolerance in Arabidopsis thaliana

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The secretion of organic acid anions from roots is an important mechanism for plant aluminum (Al) tolerance. Here we report cloning and characterizing BoMATE (KF031944), a multidrug and toxic compound extrusion (MATE) family gene from cabbage (Brassica oleracea). The expression of BoMATE was more abundant in roots than in shoots, and it was highly induced by Al treatment. The 14C-citrate efflux experiments in oocytes demonstrated that BoMATE is a citrate transporter. Electrophysiological analysis and SIET analysis of Xenopus oocytes expressing BoMATE indicated BoMATE is activated by Al. Transient expression of BoMATE in onion epidermal cells demonstrated that it localized to the plasma membrane. Compared with the wild-type Arabidopsis, the transgenic lines constitutively overexpressing BoMATE enhanced Al tolerance and increased citrate secretion. In addition, Arabidopsis transgenic lines had a lower K⁺ efflux and higher H⁺ efflux, in the presence of Al, than control wild type in the distal elongation zone (DEZ). This is the first direct evidence that MATE protein is involved in the K⁺ and H⁺ flux in response to Al treatment. Taken together, our results show that BoMATE is an Al-induced citrate transporter and enhances aluminum tolerance in Arabidopsis thaliana.

Keywords: aluminum tolerance • BoMATE • cabbage • citrate • H⁺ flux • K⁺ flux.

Abbreviations: MATE, multidrug and toxic compound extrusion; SIET, the non-invasive scanning ion-selective electrode technique; DEZ, the distal elongation zone.

Introduction

Cabbage (Brassica oleracea) is one of the most important vegetable crops around the world. Low pH is a major soil constraint to agricultural production, reducing yield on almost 25% of the world’s arable lands (Wood et al. 2000). High-input farming practices, including the excessive use of ammonia fertilizers in developed countries, also acidify previously neutral soils (Jackson and Reisenauer 1984). On highly acidic soils (pH < 5.0), the rhizotoxic aluminum species, Al³⁺, is solubilized, and inhibits root growth and function (Kochian 1995). The primary symptom of Al toxicity is retarded root growth, but Al³⁺ can also disrupt many other functions including root hair elongation, nutrient uptake (especially Ca²⁺ and K⁺), oxidative stress induction, the cytoskeleton and apoplastic processes, and intracellular transport (Ryan et al. 2011). A major physiological mechanism of plant aluminum tolerance involves aluminum-mediated activation of membrane transporters that mediate organic acid release from the root apex (Kochian et al. 2004). Organic anions such as citrate, malate and oxalate protect roots by chelating and detoxifying Al³⁺ in the apoplast and rhizosphere around sensitive root apices (Delhaize et al. 1993, Ryan et al. 2001, Delhaize et al. 2012). This mechanism has been correlated with differential Al tolerance in a large number of monocot and dicot species (Kochian et al. 2004).

The first isolated organic anion transporter was ALMT1 gene responsible for Al-induced malate secretion from wheat (Sasaki et al. 2004). The ALMT1 homologous genes have been identified in Arabidopsis (Hoekenga et al. 2006), oilseed rape (Ligaba et al. 2006), rye (Collins et al. 2008) and soybean (Liang et al. 2013). Al-induced citrate transporter named multidrug and toxic compound extrusion (MATE) gene was first isolated in sorghum (Magalhaes et al. 2007) and barley (Furukawa et al. 2007). The MATE homologous genes have been characterized in Arabidopsis (Liu et al. 2009), maize (Maron et al. 2010), rice (Yokosho et al. 2011), rice bean (Yang et al. 2011), Eucalyptus (Sawaki et al. 2013) and wheat (Tovkach et al. 2013). Expression of those genes (ALMT and MATE) conferred Al³⁺-activated malate or citrate efflux and enhanced their Al tolerance. For example, heterologous expression of TaALMT1 in tobacco-suspension cells, barley and wheat conferred Al-activated malate efflux and enhanced their resistance to Al stress (Delhaize et al. 2004, Sasaki et al. 2004, Pereira et al. 2010). Heterologous expression of ShMATE in Arabidopsis plants and HvMATE in Xenopus oocytes, tobacco plants, wheat and barley established that they encode transport proteins which facilitate...
the Al-activated efflux of citrate and enhanced their resistance to Al stress (Furukawa et al. 2007, Magalhaes et al. 2007, Zhou et al. 2013).

Al can significantly inhibit the activity of the plasmamembrane H^+-ATPase, impeding formation and maintenance of the trans-membrane H^+ gradient (Kochian et al. 2004). Electrophysiological approaches were subsequently used to demonstrate that A1^{3+} interacts directly with several different plasma-membrane channel proteins, blocking the uptake of K^+ (Gassmann and Schroeder 1994, Pineros and Kochian 2001). Therefore, to elucidate the relationship of expression of MATE and net K^+ and H^+ fluxes, the scanning ion-selective electrode technique (SIET) was used to monitor the fluxes of K^+ and H^+ at the root DEZ (Chen et al. 2013).

In the current study, BoMATE was cloned from cabbage roots and expression of gene was determined to be induced by Al treatment. Overexpressing in Xenopus oocytes and Arabidopsis, BoMATE mediated a large constitutive citrate efflux, one of the important components of high tolerance to Al in cabbage. In transgenic Arabidopsis, expression of BoMATE could facilitate H^+ efflux and diminish K^+ efflux under Al stress. We directly showed that MATE proteins participate in the Al-induced K^+ and H^+ transport.

**Results**

**Cloning of BoMATE**

Using Arabidopsis AtMATE as a query, we performed BALSTN search at the National Center for Biotechnology Information (NCBI), and identified two cabbage EST sequences (DK545931 and DK498585) similar to AtMATE. We then aligned the two sequences into one sequence and amplified the full-length cDNA of BoMATE using PCR. The coding region of BoMATE (NCBI GenBank accession no. KF031944) was 1554 bp in length, with the deduced protein consisting of 517 amino acid residues. The predicted protein sequence was 57% identical to TaMATE from Triticum aestivum, 51% identical to SbMATE from Sorghum bicolor, and 89% identical to AtMATE from Arabidopsis thaliana. Similar to other citrate-transporting MATE proteins, BoMATE consisted of 12 predicted transmembrane domains and a characteristic highly conserved amino acid sequence in the loop between the second and third transmembrane domains (Supplementary Fig. S1). Phylogenetic analysis of BoMATE and other MATEs with known functions in plant species showed that BoMATE was most closely clustered with AtMATE from Arabidopsis thaliana (Supplementary Fig. S2).

**Functional expression of BoMATE in Xenopus Oocytes**

Heterologous expression of BoMATE in Xenopus laevis oocytes supports its characterization as an Al-activated citrate efflux transporter. To elucidate the identity of the BoMATE as a citrate efflux transporter, we measured the efflux of radioactively labeled citrate from cells loaded with ^14C-labeled citrate in BoMATE expressing cells. The ^14C efflux from cells expressing BoMATE showed a higher efflux activity for citrate than that observed in control cells (Fig. 1a). This result indicates that BoMATE can mediate citrate efflux.

Electrophysiological analysis of Xenopus oocytes expressing BoMATE was used to examine whether BoMATE was activated by Al. The two-electrode voltage clamp analysis showed that cells expressing BoMATE not exposed to Al had a significantly larger inward current than that of control cells (Fig. 1b). The current of oocytes that expressed BoMATE was two-fold higher in the presence of Al than that in the absence of Al, while the control cells had no significant change under the same condition (Fig. 1b). The increase in current magnitude and the positive shift in the reversal potential indicate that BoMATE is capable of mediating an Al-activated citrate efflux from the cell. To further validate this result, the non-invasive scanning ion-selective electrode technique (SIET) was used to measure the net flux of H^+ in oocytes expressing BoMATE both in the absence and presence of Al. As seen in Fig. 1c, cells expressing BoMATE had a lower H^+ influx than those of control cells in the absence of Al. However, with addition of Al to the bathing media, a significant decrease in H^+ influx in control cells was observed while cells expressing BoMATE were not significantly changed (Fig. 1c, d). These results demonstrated that BoMATE could diminish H^+ efflux caused by Al. Therefore BoMATE, like other MATEs in this group, is an Al-activated citrate efflux transporter.

**Expression pattern of BoMATE**

BoMATE gene expression patterns were monitored by quantitative real-time PCR (Fig. 2). BoMATE is primarily expressed in the root tip (1 cm) and the rest of the root. It can also be detected in shoots but at a very low level (Fig. 2a). Al treatment up-regulates BoMATE expression in the root tip (1 cm) and particularly, the rest of the root (Fig. 2a). To determine the specificity of Al-induced BoMATE gene expression, we examined the effect of other metals on BoMATE expression. As shown in Fig. 3b, exposure to trivalent cations lanthanum (La), cadmium (Cd), zinc (Zn) or copper (Cu) did not induce BoMATE expression, these results indicate that BoMATE is specifically induced by elevated Al levels, but not other metals. To further dissect BoMATE expression in response to Al treatment, the effect of Al concentration and time of Al exposure was examined in cabbage root. BoMATE gene was induced after 2 h exposure to Al with no further increased occurring after 6 h (Fig. 2c) and BoMATE expression was no significant difference between different Al concentrations (Fig. 2d).

**Pattern of citrate secretion**

To examine whether the expression pattern of BoMATE was consistent with the organic acid exudation pattern, we characterized citrate exudation from cabbage roots. Citrate exudation

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from cabbage roots was low under normal growth conditions. After 3 h treatment, citrate exudation was significantly induced exposure to 50 mM Al (Fig. 3).

Subcellular localization of BoMATE

The cellular localization of BoMATE protein was investigated using transient expression assays of translational fusions with green fluorescent protein (GFP) in onion epidermal cell using particle bombardment (Fig. 4). The GFP signal was observed only at the outer layer of the cells expressing BoMATE::GFP, whereas the signal was observed in the nuclei and cytoplasm when GFP was expressed alone. To further distinguish localization in the plasma membrane from that in the cell wall, cell plasmolysis was induced by adding 0.8 M mannitol. In the plasmolysed cells, the fluorescence of BoMATE::GFP was observed exclusively in the plasma membrane. Together, these results strongly confirm that BoMATE is localized to the plasma membrane.

Overexpression of BoMATE in transgenic Arabidopsis confers citrate efflux and Al tolerance

The coding region of BoMATE, driven by the CaMV 35S promoter, was stably introduced into Arabidopsis. Two homozygous T3 lines (OX1 and OX2) were identified by RT-PCR (Fig. 5a) and used in further investigation of root elongation and root citrate exudation under Al treatment. Citrate efflux rates in transgenic Arabidopsis were observed higher than those
of wild type (Fig. 5b). In the absence of Al, the root elongation of two BoMATE overexpression lines and wild type was similar (Fig. 5d). However, in the presence of Al, root elongation of wild type seedlings was significantly inhibited while two BoMATE overexpression lines showed less inhibition (Fig. 5c, d). These results demonstrated that heterologous overexpression of BoMATE in Arabidopsis increases root citrate release, and significantly enhances Al tolerance.

**BoMATE alters net K⁺ and H⁺ fluxes**

Aluminum has the potential to affect plant-cell membranes by blocking K⁺ channels (Gassmann and Schroeder 1994) or by inhibiting the H⁺-ATPase in the plasma membrane (Ahn et al. 2002). To determine whether BoMATE participates in K⁺ and H⁺ transport, the scanning ion-selective electrode technique (SIET) was used to monitor the fluxes of K⁺ and H⁺ at the root DEZ (Fig. 6). When low-pH (pH 4.5) stress was imposed, the pattern of K⁺ efflux in the BoMATE transgenic lines was similar to that of wild type (Fig. 5b).
significantly altered compared to the wild type. The 50 μM Al treatment induced lower K⁺ efflux in the BoMATE transgenic line than that of wild type (Fig. 6a). The pattern of H⁺ influx had no significant difference in low-pH condition between the wild type and the BoMATE transgenic line. Al treatment significantly reduced H⁺ influx in wild type, but surprisingly facilitated H⁺ efflux in the BoMATE transgenic line (Fig. 6b).

Fig. 4 Subcellular localization of BoMATE. The fusion protein BoMATE::GFP (e–h) and GFP alone (a–d) were transiently expressed in onion epidermal cells. (a, c, e, g) GFP fluorescence; (b, d, f, h) merged image; (c, d, g, h) plasmolysed the cells with 0.8 M mannitol. PM, plasma membrane; CW, cell wall.
Discussion

Multidrug and toxic compound extrusion (MATE) proteins represent a large family in bacteria, fungi, plants and mammals and are able to transport numerous substrates (Omote et al. 2006). Plant MATE proteins capable of transporting citrate characterized to date appear to be involved in several physiological processes including Al tolerance (Furukawa et al. 2007, Magalhaes et al. 2007, Liu et al. 2009), iron translocation (Durrett et al. 2007, Yokosho et al. 2009), and phosphorus efficiency (Uhde-Stone et al. 2003). Here, we cloned and characterized the functional properties of BoMATE from cabbage to address its potential role in plants.

AtMATE, VuMATE and EcMATE have been identified and contain 12 transmembrane domains and a unique cytoplasmic loop between the 2nd and 3rd transmembrane domains (Liu et al. 2009, Yang et al. 2011, Sawaki et al. 2013), BoMATE shared the consensus features of citrate-transporting MATEs (Supplementary Fig. S1). The expression of BoMATE was moderately and rapidly up-regulated by Al treatment in both the root tip and basal root regions (Fig. 2a), which is different from other homolog members. For example, the expression of HvMATE was not induced by Al and the expression of SbMATE induced by Al required a longer exposure time while the induction enhancement appeared to be greater for
OsFRDL4 (Furukawa et al. 2007, Magalhaes et al. 2007, Yokosho et al. 2011). It has been reported that VuMATE has no expression in the absence of Al (Yang et al. 2011). Kobayashi et al. (2013) reported the complex regulation of AtALMT1 expression during the adaptation to abiotic and biotic stresses. Therefore, BoMATE may be required not only for the external Al detoxification but also for other uncharacterized functions in the absence of Al.

We analyzed the function of BoMATE by heterologous expression in oocytes and Arabidopsis using the SIEt method (Figs. 1, 6). In oocytes, Al could inhibit H⁺ influx in control cells. Similar results were reported in roots of squash, wheat and Arabidopsis (Ahn et al. 2001, Ahn et al. 2004, Bose et al. 2010). In Arabidopsis, the pattern of H⁺ efflux of the transgenic lines was greatly enhanced by Al in the elongation zone compared to wild type (Fig. 6b). The result is similar to the schematic diagram illustrating a possible mechanism linking the Al-induced exudation of malate and enhancement of H⁺-ATPase activity in Al-tolerant plants (Ahn and Matsumoto 2006). While in BoMATE expression cells, the pattern of H⁺ influx was no different in the presence or absence of Al (Fig. 1). The results indicated that BoMATE maybe mediate citrate transport, instead of directly mediate H⁺ flux across the PM. And in Fig. 6b, Al treatment significantly reduced H⁺ influx in wild type, but surprisingly facilitated H⁺ efflux in the BoMATE transgenic line. These different patterns are observed because the protein might behave differently in different transgenic systems. Attempts to express ALMTs in yeast and bacteria were unable to detect function, despite confirming protein expression in yeast (Ryan et al. 2011).

Previous studies have shown that Al³⁺ induces K⁺ efflux or reduces K⁺ uptake (Gassmann and Schroeder 1994, Liu and Luan 2001). The pattern of K⁺ efflux in the BoMATE transgenic lines was significantly lower than that of the wild type (Fig. 6a), suggesting that BoMATE could increase Al tolerance. Along with the citrate release, H⁺ efflux and K⁺ influx enhanced or alternatively, H⁺ influx and K⁺ efflux was inhibited in the presence of Al (Fig. 7). The mechanism of Al stress affected OAA secretion and K⁺ and H⁺ fluxes was verified by Bose et al. (2010). AI-induced inhibition of H⁺-ATPase activity and consequent disruption of the H⁺ gradient has been reported both in vitro (e.g. membrane vesicle studies) and in intact roots of Arabidopsis (Bose et al. 2010).
several plant species (Ahn et al. 2001, Ahn et al. 2002). In the present study, we directly demonstrated that MATE was involved in the process of Al induced K⁺ and H⁺ transport. The transgenic lines also increased root citrate release and root elongation (Fig. 5). Therefore, overexpression of BoMATE could confer Al tolerance.

Recently, two important C₂H₂-type zinc finger transcription factors for Al tolerance had been characterized. The transcription factor ART1 had been reported to regulate multiple genes implicated in Al resistance in rice, including the members from the MATE transporter family (Yaami et al. 2009). Iuchi et al. (2007) and Liu et al. (2009) also reported that in Arabidopsis, a zinc finger transcription factor STOP1 was involved in AtMATE expression and Al-activated citrate exudation. Further analysis should be carried out to find STOP1- or ART1-homolog regulatory genes in cabbages.

In conclusion, we have identified a citrate transporter BoMATE in cabbage. Functional analysis in Xenopus oocytes and transgenic Arabidopsis indicates that the cabbage Al-resistant protein mediates root citrate efflux and is responsible for external detoxification of Al. Additionally, BoMATE regulates K⁺ and H⁺ homeostasis under Al treatment.

Materials and Methods

Plant materials and growth conditions

Cabbage (Brassica oleracea cv. Zhonggan-11) seeds were pre-germinated in the dark on moist filter paper at room temperature. After germination, seedlings were grown hydroponically at pH 5.0 as described by Ligaba et al. (2006). Five-day-old uniform seedlings were subjected to the nutrient solution (pH 4.5) for 0, 2, 4 or 6 h. For the Al concentration dependence experiment, seedlings were subjected to 0, 10, 50 or 100 μM AlCl₃ (pH 4.5) for 6 h. For other metal treatments, the seedlings were subjected to the nutrient solution described earlier supplemented with 25 μM CdCl₂, 0.5 μM CuCl₂, 10 μM LaCl₃, 20 μM ZnCl₂ or 50 μM AlCl₃ for 6 h.

Gene cloning and sequencing

Total RNA was isolated from 50 μM Al-treated root tips of cabbage. To identify cabbage BoMATE gene, a BLAST search was performed with the known Arabidopsis (Arabidopsis thaliana) AtMATE sequence (accession no. At1g51340) at the NCBI website (http://www.ncbi.nlm.nih.gov/). Among the obtained expressed sequence tags (ESTs), two mRNA sequences (DK545931 and DK498585) with similarity to AtMATE were chosen for further amplification. The two nucleotide sequences were aligned into one sequence. According to the 5’- and 3’-end of this sequence, the full-length cDNA of BoMATE was amplified with PCR forward primer ‘ATGATGTCTGAAGATGGCTACA’ and reverse primer ‘TTATCTTCTGAGAAAAGACCAAGG’, cloned into pMD18-T vector (Takara, Japan) and fully sequenced using internal primers. Multi-sequence alignment was conducted using ClustalX (version 1.83; Thompson et al. 1997) with default parameters. Phylogenetic analysis was performed with MEGA4.1. Transmembrane proteins were predicted by HMMTOP.

Characterization of BoMATE expression

The expression of BoMATE was determined by semi-quantitative RT-PCR. Total RNA was isolated using Trizol reagent (Biomed, Beijing) according to the manufacturer’s protocol. One microgram of total RNA was synthesized into the first-strand cDNA using the Primescript reverse transcriptase (Takara, Japan) following the manufacturer’s instructions. The qRT-PCR analysis was performed using the Applied Biosystems 7500 Real-Time PCR System (ABI). Semi-quantitative RT-PCR by a SYBR Premix Ex Taq™ (perfect real time) kit (Takara, Japan) was performed with one-fifth of the first-strand cDNA as template using internal primers for BoMATE (5’- ATTTGGTCTC TTAGAGCTTCTGCTG -3’ and 5’- GGAAGTTGTGATGCTAAC GAG -3’) and for Actin as internal control (5’- TAAACAGGAGA AGATGACTCAGATCA -3’ and 5’- AAGATCAAGAGCAAGGA TAGCAGTAG -3’). The reaction procedures were as follows: denatured at 95°C for 3 min, followed by 40 cycles of 95°C for 10 s, 60°C for 30 s and 72°C for 30 s. The expression of BoMATE was calculated from the relative expression levels of BoMATE and the expression levels of the reference gene Actin using arbitrary units.

Subcellular localization of BoMATE

The ORF of the BoMATE cDNA fragment was amplified with PCR forward primer with a BglII restriction site ‘GCAGATCTAT GATGTCAGGAGCATGCTACA’ and reverse primer with a SpeI restriction site ‘GGACTAGTTCTTCTGAGAAAAGACCAAGG’. The BoMATE fragment was ligated to the 3’ end of GFP and placed under the control of the CaMV 35S promoter in pCAMBIA1302. The resulting plasmid was fully sequenced to ensure sequence accuracy. Transient expression of the BoMATE::GFP translational fusion protein in onion epidermal cells was carried out as described by Yang et al. (2011). 1.5 mg gold particles with a diameter of 1 μm, and coated with 1–5 μg BoMATE::GFP or GFP alone, were bombarded into onion epidermal cells. To induce plasmolysis, cells were incubated with 0.8 M mannitol for 3–5 min. GFP fluorescence was observed with a confocal laser scanning microscopy (Leica DMI 6000B-CS, Germany) with a 488 nm excitation wavelength.

In vitro transcription and cRNA injection

The cRNA was prepared by using the Message Machine (Ambion) Kits according to the manufacturer’s instructions with Ascl-digested pCS107 plasmid DNA, which contained the BoMATE coding region cloned between the BamH1 and Stul unique site of pCS107 plasmid DNA and flanked by the 3’ and 5’ UTRs of a Xenopus β-globin gene. The construct was fully sequenced for confirmation. Harvesting of stage V–VI
Xenopus laevis oocytes was performed as described (Golding 1992, Hoekenga et al. 2006). Defolliculated oocytes were maintained overnight before injections. The oocyte culture medium (OCM) (1L OCM contains 600 ml L-15 (Sigma L4386), 400 mg BSA (Sigma A4919), 5 ml Penicillin-Streptomycin (Gibco 15140-122) and 400 ml H2O) used in this study was prepared as described by Kofron et al. (2002) with some modification. The Xenopus laevis oocytes were injected with 50 nl of water containing 20 ng of cRNA encoding BoMATE (or 50 nl of water as control) and incubated in OCM at 18°C for 2–4 d. The Xenopus laevis oocytes were preloaded with citrate by injecting 50 nl of 0.1 M sodium citrate 2 h before flux measurements of H+ and electrophysiological assay. For the electrophysiological measurements, oocytes were transferred to OCM, pH 4.5, containing 0 or 100 μM AlCl3. Two-electrode voltage clamp system was used to measure net current across the oocyte membrane with the amplifier (MEZ-7200 and CEZ-1200, Nihon Kohden) at different test voltages (Furukawa et al. 2007). The electrical potential difference across the membrane was clamped from -140 mV to 0 mV, in 20 mV steps. The 14C-labeled citrate efflux experiment was performed according to Maron et al. (2010) using OCM bath solution adjusted to pH 4.5.

**Flux measurements of H+ and K+**

Net fluxes of H+ and K+ were measured using the non-invasive Scanning Ion-selective Electrode Technique (SIET) (Xuyue Science and Technology Co., Ltd., Beijing, China) as described by Bose et al. (2010). In the first set of experiments, 4- to 5-day-old Arabidopsis seedlings expressing BoMATE were equilibrated in a solution (0.1 mM CaCl2, 0.1 mM KCl, 0.3 mM MES, pH 4.5) with or without 50 μM Al for 5–10 min. H+ and K+ fluxes were measured 200 μm from the root tip for 6–10 min to ensure that steady-state conditions were reached. In both experiments, 4- to 5-day-old wild type Arabidopsis seedlings were used as a control and received the same treatments as BoMATE expressing seedlings. The H+ and K+ fluxes were calculated by using the JCal V3.1 (a free MS Excel spreadsheet, youngerusa.com or ifluxes.com). In the second set of experiments, H+ fluxes were measured 30 μm away from Xenopus laevis oocytes expressing BoMATE pretreated with citrate. Recordings were performed in a solution consisting of 96 mM NaCl, 2 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2, 0.3 mM MES with or without 0.1 mM AlCl3 and with the pH adjusted to 4.5. The ion flux assay around each type was replicated independently 4–6 times and the data were averaged.

**Overexpression of BoMATE in Arabidopsis**

The coding region of the BoMATE was amplified using a forward primer with a XbaI restriction site at the 5' end (GCTCTAGAATGTGCTGAAGATGGCTACA) and a reverse primer with a SmaI restriction site (TCCCCCGGTTATCTCTGAGAAAAAG ACCAAGG) at the 3' end. The coding region of BoMATE was subcloned into the pBI121 vector under the control of the cauliflower mosaic virus 35S promoter. The construct was introduced into Agrobacterium tumefaciens strain EHA 105 and subsequently transformed into Arabidopsis ecotype via the floral dip method (Clough and Bent 1998). The expression of BoMATE in the transgenic plant roots was confirmed by RT-PCR.

Two independent transgenic homozygous T3 lines were selected for Al tolerance. Al tolerance was determined by measuring relative root elongation according to Liang et al. (2013). In brief, seeds of wild-type Arabidopsis and two transgenic lines were surface sterilized, stratified at 4°C for 3 days, and then germinated for 4 days on MS medium. After germination, uniform seedlings were transferred to 0.5 mM CaCl2-agar plates containing 0 or 400 μM AlCl3 (pH 4.5). Plates were then placed in a near vertical position so that the lines of seedlings were horizontal. After a 2-day treatment, roots were scanned, and primary root length was recorded using the ImageJ program. Relative root elongation (% RRE) was calculated as described (Liu et al. 2009).

**Detection of root organic acid exudation**

For cabbage, 2-week-old seedlings cultured in the nutrient solution were exposed in 0.5 mM CaCl2 (pH 4.5) and 50 μM Al for 0, 1, 3, 6, 12 or 24 h. After that, root exudates from each treatment were collected. Before collection of root exudates in Arabidopsis, seeds of WT and two BoMATE overexpression lines were surface sterilized and germinated on solid MS medium for 7 days. The seedlings were then transferred to a 25 ml solution consisting of 0.5 mM CaCl2 (pH 4.5) and free Al for 1 day, subsequently the solution was replaced by the same CaCl2 solution containing 50 μM Al for 24 h. The collecting solution was sampled for citrate assay by capillary electrophoresis as described by Hoekenga et al. (2006).

**Supplementary data**

Supplementary data are available at PCP online.

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**Disclosures**

The authors have no conflict of interest to declare.
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