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A gene in the multidrug and toxic compound extrusion (MATE) family confers aluminum tolerance in sorghum

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Crop yields are significantly reduced by aluminum toxicity on highly acidic soils, which comprise up to 50% of the world's arable land[1–3]. Candidate aluminum tolerance proteins include organic acid efflux transporters, with the organic acids forming non-toxic complexes with rhizosphere aluminum[1,4]. In this study, we used positional cloning to identify the gene encoding a member of the multidrug and toxic compound extrusion (MATE) family, an aluminum-activated citrate transporter, as responsible for the major sorghum (*Sorghum bicolor*) aluminum tolerance locus, Alt_{SB}[5]. Polymorphisms in regulatory regions of Alt_{SB} are likely to contribute to large allelic effects, acting to increase Alt_{SB} expression in the root apex of tolerant genotypes. Furthermore, aluminum-inducible Alt_{SB} expression is associated with induction of aluminum tolerance via enhanced root citrate exudation. These findings will allow us to identify superior Alt_{SB} haplotypes that can be incorporated via molecular breeding and biotechnology into acid soil breeding programs, thus helping to increase crop yields in developing countries where acidic soils predominate.

The large areas of acidic soils in the tropics and subtropics are critical food-producing regions for developing countries, but agriculture therein is seriously limited by aluminum toxicity. On highly acidic soils (pH < 5.0), the rhizotoxic aluminum species, Al^{3+}, is solubilized, inhibiting root growth and function[1]. Therefore, aluminum toxicity is a primary limitation for crop production on 38% of the farmland in Southeast Asia, 31% of Latin America and 20% of the arable lands in East Asia, sub-Saharan Africa and North America[2], thus constituting a worldwide food security problem that is exceeded only by drought stress with regard to abiotic limitations on crop production[3].

A major physiological mechanism of plant aluminum tolerance involves aluminum activation of membrane transporters that mediate organic acid release from the root apex[4], the site of aluminum phytotoxicity[6], with the released organic acids forming stable, non-toxic complexes with Al^{3+} in the rhizosphere[7]. In wheat (*Triticum aestivum*), the aluminum-activated malate transporter encoded by the aluminum tolerance gene *ALMT1* (ref. 8) is a member of a new family of membrane proteins and most likely corresponds to Alt_{BH}, a major aluminum tolerance locus in wheat and other members of the Triticeae tribe[8,9]. In sorghum (*Sorghum bicolor*), we have recently mapped the major aluminum tolerance locus, Alt_{SB}, to the terminal region of chromosome 3 (ref. 5), and comparative mapping indicated that Alt_{SB} represents a previously unknown aluminum tolerance gene in the grass family. Given that Alt_{SB} is a major gene that is responsible for 80% of the aluminum tolerance phenotype in our sorghum mapping population[5], and that elite Alt_{SB} alleles cause a marked (over tenfold) increase in sorghum aluminum tolerance[10], we set out to isolate Alt_{SB} by positional cloning in sorghum.

We undertook high-resolution mapping of Alt_{SB} by screening 4,170 gametes from an SC283 (aluminum-tolerant) × BR007 (aluminum-sensitive) F_2 population for recombination events within the interval delimited by two sequence-tagged site (STS) markers at 0.2 and 0 cM from Alt_{SB} (**Fig. 1a**). Ultimately, we localized the Alt_{SB} locus to a 24.6-kb region of sorghum BAC 181g10 (**Fig. 1b**), where only three predicted candidate ORFs (ORFs 7, 8 and 9) were found.

ORFs 8 and 9 (highly similar to a hypothetical protein and a sucrose phosphate synthase gene, respectively) were highly expressed in shoots of near-isogenic lines (NILs) contrasting in aluminum tolerance at Alt_{SB}, but expression in roots, where the aluminum tolerance mechanism must function, was extremely low and near the limit of detection (**Fig. 1c**). Conversely, TBLASTX searches with ORF 7 identified highly similar sequences in *Arabidopsis thaliana* (At1g51340) and rice (Os01g69010) (**Supplementary Fig. 1a** online), which represent members of the multidrug and toxic compound extrusion (MATE) transporter family[11]. MATE proteins have been implicated in the efflux of small organic molecules[12–14], consistent with the physiological

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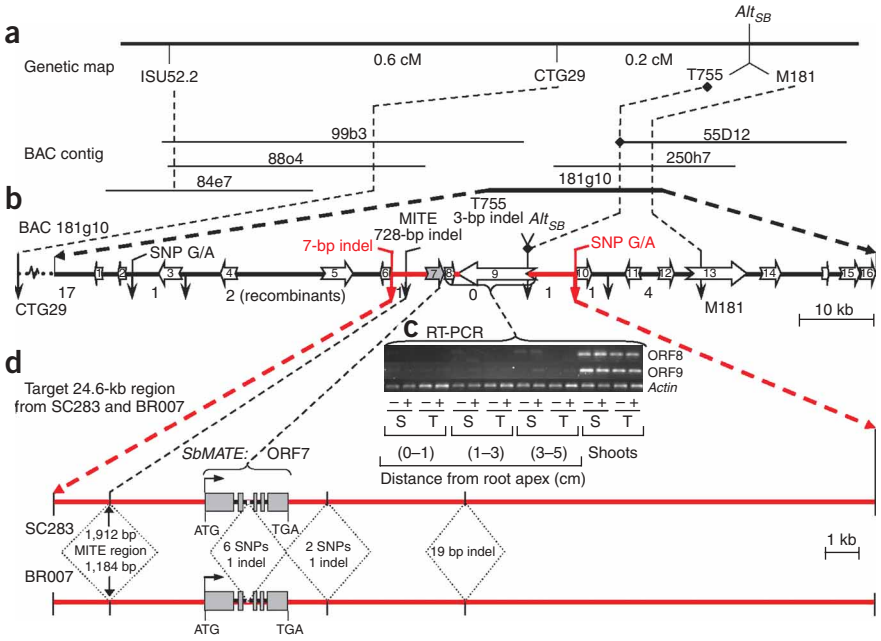

Figure 1 Positional cloning of Alt_{SB}. (**a**) Genetic and physical map of the Alt_{SB} region on chromosome 3. The marker T755 corresponds to the leftmost end of BAC 55D12. Dashed lines indicate the approximate physical position of genetic markers in the sorghum BAC contig. (**b**) High-resolution map of Alt_{SB} on BAC 181g10 (bold line). Broad horizontal arrows indicate ORFs (1 to 16) and their predicted transcriptional orientations. Numbers between downward arrows below 181g10 indicate the distribution of the 27 single recombination events detected by high-resolution mapping. The target 24.6-kb region that contains ORFs 7, 8 and 9 (candidates for Alt_{SB}) is marked in red along with the flanking markers (7-bp indel and a G/A SNP). (**c**) Semiquantitative RT-PCR of ORFs 8 and 9 in roots and shoots of the aluminum-tolerant (T) and aluminum-sensitive (S) NILs in response to the presence (+) or absence (–) of {27} μM Al^{3+}. (**d**) Target 24.6-kb region from the aluminum-tolerant (SC283) and aluminum-sensitive (BR007) parents with polymorphisms shown inside dotted diamonds. The genomic *SbMATE* (ORF7) is 2,407 bp long and contains five exons (filled gray boxes) and four introns (bold black lines).

mechanism for sorghum aluminum tolerance based on aluminum-activated root citrate exudation[15].

Sequence comparison showed that the MATE homolog isolated from *Sorghum bicolor* (*SbMATE*) is not related to the ALMT family of membrane proteins and thus is a new candidate tolerance gene. The *SbMATE* coding region was identical between the aluminum-tolerant (SC283) and aluminum-sensitive (BR007) parents, with polymorphisms found only within one of the introns (**Fig. 1d**). There were only three polymorphic regions elsewhere in the 24.6-kb region; by far, the most divergent was a 728-bp indel ~1.4 kb upstream of the predicted TATA box for *SbMATE*. This polymorphic region harbored a *Tourist*-like miniature inverted repeat transposable element (MITE)[16,17].

Quantitative RT-PCR analysis showed that *SbMATE* was expressed only in roots of the aluminum-tolerant NIL in an aluminum-inducible fashion; expression was highest in the first centimeter of the root (**Fig. 2a**), consistent with an exclusion mechanism acting to prevent Al^{3+} from reaching sensitive sites in the root apex. Aluminum tolerance increased substantially in the tolerant NIL as root exposure time to aluminum increases (**Fig. 2b**); aluminum exposure inhibited root growth in the tolerant NIL by 40%–50% on days 1 and 2, whereas we did not see any inhibition by days 5 and 6. This induction of tolerance correlates closely with the observed increase in root citrate exudation over time in aluminum (**Fig. 2b**) and the incremental increase in *SbMATE* expression in response to aluminum (**Fig. 2c**).

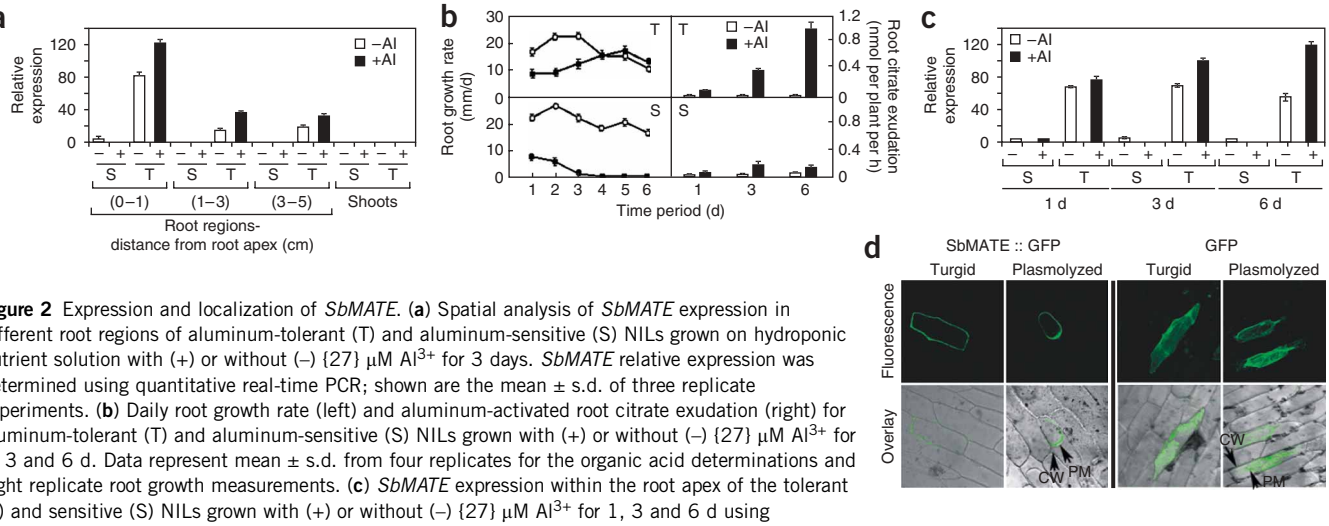

Figure 2 Expression and localization of *SbMATE*. (**a**) Spatial analysis of *SbMATE* expression in different root regions of aluminum-tolerant (T) and aluminum-sensitive (S) NILs grown on hydroponic nutrient solution with (+) or without (–) {27} μM Al^{3+} for 3 days. *SbMATE* relative expression was determined using quantitative real-time PCR; shown are the mean ± s.d. of three replicate experiments. (**b**) Daily root growth rate (left) and aluminum-activated root citrate exudation (right) for aluminum-tolerant (T) and aluminum-sensitive (S) NILs grown with (+) or without (–) {27} μM Al^{3+} for 1, 3 and 6 d. Data represent mean ± s.d. from four replicates for the organic acid determinations and eight replicate root growth measurements. (**c**) *SbMATE* expression within the root apex of the tolerant (T) and sensitive (S) NILs grown with (+) or without (–) {27} μM Al^{3+} for 1, 3 and 6 d using quantitative real-time PCR. The data are the mean ± s.d. from three replicate experiments. (**d**) Membrane localization of the SbMATE protein in epidermal onion cells. The upper panels show the GFP fluorescence patterns for SbMATE::GFP (left panels) and cytoplasmic GFP (right panels). The lower set of images show the overlay of bright-field and GFP fluorescence images for the same specimens. The images were acquired before (first and third columns) and after (second and fourth columns) cell plasmolysis (onion epidermal strips exposed to 1 M sucrose). The fluorescence associated with SbMATE is localized to the plasma membrane (first column); after cell plasmolysis, the SbMATE fluorescence signal is associated with the retracted plasma membrane (second column). Scale bar = 50 μm. Images are representative of three independent replicate experiments. PM: plasma membrane, CW: cell wall.

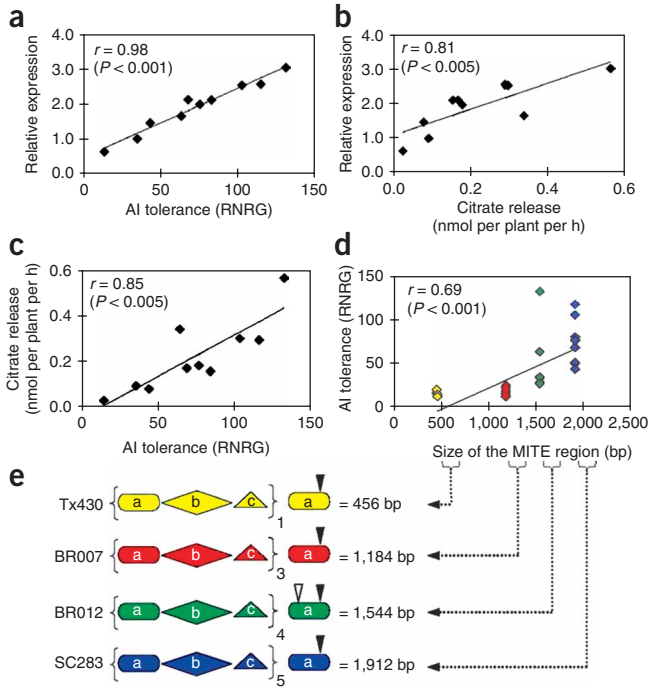

Figure 3 Correlation of *SbMATE* expression, root citrate exudation and aluminum tolerance in ten sorghum lines that harbor an allelic series at Alt_{SB} (BR012, BR007, IS8577, SC549, 3DX, SC175, 9DX, CMS225, SC283, SC566)[10] exposed to {27} μM Al^{3+} in nutrient solution. (**a**) *SbMATE* expression relative to that of the *Actin* gene (assessed by semiquantitative RT-PCR) versus aluminum (Al) tolerance, in terms of relative net root growth (RNRG). (**b**) *SbMATE* relative expression versus root citrate exudation. (**c**) Root citrate exudation versus aluminum tolerance. (**d**) Aluminum tolerance versus the size of the region within the putative *SbMATE* promoter harboring the MITE insertion. Correlation coefficients (*r*) and probability (*P*) values are shown. (**e**) Structure and size of the MITE insertion region in four sorghum lines that are representatives for each of the four size classes shown in **d**.

Altogether, these parallel, time-dependent inductive responses to aluminum support our contention that SbMATE is an organic acid transporter that underlies Alt_{SB}. Note that there is a significant constitutive *SbMATE* expression that does not seem to be involved in aluminum tolerance; the increase in expression in response to longer-term aluminum exposure is what is best correlated with increased aluminum tolerance and root citrate exudation over the 6-d period. One possible explanation for this response is that citrate release mediated by SbMATE is regulated at multiple levels—not only by changes in gene expression, but also by a direct effect of Al^{3+} on transporter activity (as was shown with the electrogenic *ALMT1*- and *AtALMT1*-mediated transport in oocytes[8,18]) and/or by aluminum-mediated post-translational modifications of SbMATE.

Localization of the SbMATE::GFP fusion protein in plasmolyzed onion epidermal cells allowed us to differentiate between localization within the cell wall versus localization within the plasma membrane (**Fig. 2d**), substantiating a plasma membrane localization of the protein. Although this type of expression assay is commonly used for plant plasma membrane proteins (see, for example, ref. 19), we cannot entirely rule out a possible localization of SbMATE to the tonoplast in these highly vacuolated cells. However, current-voltage analysis of *Xenopus laevis* oocytes expressing *SbMATE* complementary RNA (cRNA) under ionic conditions similar to those used to previously characterize the malate efflux transporters AtALMT1 and ALMT1 (refs. 8,18) yielded inward ion currents consistent with SbMATE mediating anion efflux at the plasma membrane of these cells (**Supplementary Fig. 2** online). These preliminary data clearly indicate that SbMATE functions as a plasma membrane anion efflux transporter responsible for citrate release into the rhizosphere.

We also examined *SbMATE* expression in a genetically diverse sorghum panel that includes BR007 and SC283, where we have shown that the large difference in aluminum tolerance are largely due to an allelic series at the Alt_{SB} locus[10]. Differences in *SbMATE* expression explained over 95% of the phenotypic variation for aluminum tolerance in this panel (**Fig. 3a**), providing strong evidence that *SbMATE* underlies Alt_{SB} and that differences in gene expression constitute the basis for allelic variation at this aluminum tolerance locus. Similarly, we found significant correlation ($P < 0.005$) between *SbMATE* expression and aluminum-activated root citrate release (**Fig. 3b**) and between citrate release and aluminum tolerance (**Fig. 3c**), suggesting that differences in gene expression condition the aluminum tolerance phenotype primarily by modulating root citrate exudation.

We amplified the highly polymorphic MITE insertion region upstream of the *SbMATE* gene by PCR in an expanded sorghum panel, and the resulting variation in size for this region was significantly and positively correlated with aluminum tolerance (**Fig. 3d**). Notably, others[8] have found that amplification of a repeat region upstream of the *ALMT1* gene in wheat lines of non-Japanese origin was also positively correlated with *ALMT1* expression and aluminum tolerance, although transposons were not involved in that case. Analysis of the MITE-insertion region in selected sorghum accessions uncovered a highly structured and repeated pattern composed of the *Tourist*-like MITE (unit b in **Fig. 3e**) and sequences that flanked the MITE insertion site (units a and c). This a-b-c structure is a singlet in the smallest insertion found in Tx430 and is repeated three, four and five times in the representatives examined from the next three size classes, BR007, BR012, and SC283 (for the complete sequences, see **Supplementary Fig. 3** online).

We subsequently carried out genetic complementation experiments in the *A. thaliana* ecotype Columbia (Col) and in the highly aluminum-sensitive T-DNA knockout mutant, *AtALMT1*-KO, in which an *A. thaliana* homolog of the wheat *ALMT1* gene is disrupted in the first exon. We screened ten T-DNA *A. thaliana* insertion lines in which the six *A. thaliana* genes that are the most closely related to *SbMATE* homologs were disrupted and did not observe any reduction of aluminum tolerance in comparison to the Col-0 wild type (**Supplementary Table 1** online). This indicates that functional MATE alleles are either rare in *A. thaliana* or not present in the Columbia ecotype. Conversely, the disruption of *AtALMT1* caused a strong reduction in aluminum tolerance compared with the wild type (**Fig. 4a**) as the result of a lack of AtALMT1 function, which leads to a nearly complete loss of aluminum-activated root malate efflux[18]. Homozygous T3 lines expressing *SbMATE* driven by the CaMV 35S promoter were significantly more aluminum tolerant than control seedlings in both backgrounds (**Fig. 4a**). Four transgenic lines in the wild-type background significantly outperformed the wild-type Columbia with regard to aluminum tolerance, with an average relative net root growth (RNRG) of 88% ± 4%, compared with an RNRG of 65% ± 5% for the wild-type line. Expression of *SbMATE* in the highly aluminum-sensitive *AtALMT1* background increased the sensitivity of the complementation test, as eight transgenic lines in the knockout (KO) background showed a significant increase in aluminum tolerance compared with the parental line (**Fig. 4b**). In a separate experiment, we selected the best-performing transgenic line as well

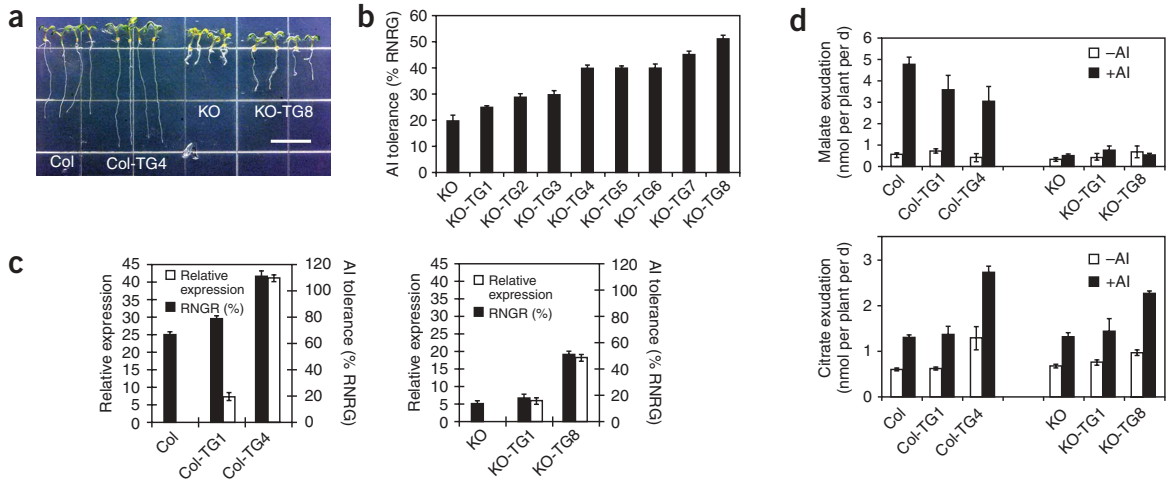

Figure 4 Expression of *SbMATE* in transgenic *A. thaliana* plants. (**a**) Aluminum (Al) tolerance (root growth in nutrient solution + 1.5 μM Al^{3+}) for control and T3 homozygous *A. thaliana* lines expressing *SbMATE* under the CaMV 35S promoter. *SbMATE* was expressed in the Columbia ecotype (Col: nontransgenic; Col-TG: transgenic lines expressing *SbMATE*) and in a very aluminum-sensitive *AtALMT* knockout line (KO: nontransgenic knockout line; KO-TG: transgenic knockout lines expressing *SbMATE*). Scale bar, 1 cm. (**b**) Aluminum tolerance as measured by percentage of relative net root growth (% RNRG) in eight independent KO-TG lines. The data represent mean ± s.d. ($n = 20$). (**c**) Relationship between *SbMATE* expression and aluminum tolerance (% RNRG) in control and selected T3 transgenic lines. (**d**) Root malate and citrate exudation in control and selected T3 transgenic plants grown with (+) or without (–) aluminum. For **c** and **d**, the data represent mean ± s.d. ($n = 20$).

as a poorly performing transgenic line in both backgrounds and found that aluminum tolerance increased proportionally with the level of *SbMATE* expression (**Fig. 4c**). The most tolerant transgenic (TG) lines in both backgrounds, Col-TG4 and KO-TG8, showed the greatest *SbMATE* expression and aluminum-activated root citrate release, but we did not observe any increase in malate exudation (**Fig. 4d**). We also are in the process of generating transgenic wheat lines in the aluminum-sensitive cultivar Bobwhite, where *SbMATE* driven by the maize ubiquitin promoter is stably expressed. We identified four transgenic T1 lines with substantially increased aluminum tolerance compared with nontransgenic Bobwhite (**Supplementary Fig. 4** online). These results with *A. thaliana* and wheat provide experimental support that a member of the MATE family from *Sorghum bicolor*, *SbMATE*, is an aluminum-activated citrate efflux transporter that confers aluminum tolerance via the Alt_{SB} locus.

MATE proteins are members of a large and complex family of transporters; functional members of this family were found first in prokaryotic organisms and later in eukaryotic organisms and are generally involved in the efflux of small organic solutes[12,20,21]. In proteoid (or cluster) roots of white lupin (*Lupinus albus*), *LaMATE* is highly expressed under phosphorus deficiency[22] and might have a role in the transport of citrate as an adaptive response to increase phosphorous availability in low-phosphorous soils. If so, considering that both aluminum toxicity and phosphorous deficiency are the two most important agricultural constraints on acidic soils, the MATE family may have a more general role in plant adaptation to low-pH soils. The involvement of MATE genes in citrate transport is supported by an extensive phylogenetic analysis of more than 70 different transporter families, where substrate transport specificity was found to be a well conserved trait that typically correlates with phylogeny[20]. In addition, in *A. thaliana*, FRD3 (ferric reductase defective 3) is a MATE protein involved in iron nutrition, and its localization to the stele led to speculation that it may be involved in citrate efflux into the xylem, with concomitant iron chelation for transport to the shoot[23]. Very recently, it was shown that when *FRD3* was ectopically expressed in *A. thaliana*, it confers enhanced aluminum tolerance, presumably owing to an increase in root citrate release[24]. Based on the hypothesis that the MATE family arose in prokaryotes and that some family members were then transmitted to eukaryotes[20], it is possible that aluminum tolerance encoded by Alt_{SB} originated from mutation(s) in the gene encoding a pre-existing MATE family member that already had the ability to transport small organic molecules.

Our data from genetically diverse sorghum accessions indicate that these aluminum tolerance–related mutations are located in regulatory regions of Alt_{SB} and act to enhance gene expression in the root apex. Because it is the only polymorphism near the Alt_{SB} promoter of the aluminum-tolerant and aluminum-sensitive parents, the repeated region harboring the MITE insertion stands out as a candidate for this role. MITEs have been identified in noncoding regions of genes and have a role in altering gene expression[17,25]. Furthermore, the terminal inverted repeats from a maize *Mu* transposon have also been shown to contain pollen- or gamete-specific enhancer sequences[26]. The regular $\{abc\}_{1-5}\{a\}$ structure that is positively correlated with aluminum tolerance in the different sorghum accessions raises the possibility that *cis*-acting elements within this repeated region and possibly within the MITE are acting multiplicatively to enhance Alt_{SB} expression specifically in root apices. However, this possibility must be viewed with caution, as the MITE insertion, which could be in linkage disequilibrium with polymorphisms elsewhere in the 24.6-kb region, could also reflect the insertion bias of the *Tourist* elements in genic regions[16]. In addition, particularly for the sorghum lines within the two largest size classes, we observed a significant variation in aluminum tolerance, and our recent study using NILs[10] for Alt_{SB} indicates that significant allelic variation at the Alt_{SB} locus occurs for lines that are in the 1.9-kb MITE insertion size class. NILs for 3DX and CMS225, which are both in the 1.9 kb MITE insertion size class, have been shown to possess alleles that encode significantly different aluminum tolerance levels[10]. Therefore, although our current data indicate that a minimum MITE insertion size in the promoter region is needed for aluminum tolerance, as encoded by *SbMATE*, the

phenotypic expression may depend to a certain extent on interactions of this region with other polymorphisms. In addition, we have also shown that significant gene diversity exists for aluminum tolerance in this sorghum diversity panel[10], and other novel aluminum tolerance genes elsewhere in the sorghum genome may also account for part of the phenotypic variation observed for lines within the 1.9-kb insertion class.

Our previous comparative mapping studies indicated that the major aluminum tolerance loci in sorghum and wheat, Alt_{SB} and Alt_{BH}, are located in nonconserved positions and are probably nonorthologous loci, whereas a major aluminum tolerance quantitative trait locus (QTL) that had been repeatedly detected on rice chromosome 1 is possibly orthologous to Alt_{SB}[5,27]. Our analysis using *SbMATE* as a query showed that a highly similar homolog lies in a syntenic position in the rice genome (**Supplementary Methods** online). Although the functionality of this rice homolog has not yet been verified, these findings implying that there is a conservation of aluminum tolerance genes over a long evolutionary continuum suggest a broader role for the MATE family in providing aluminum tolerance not only in sorghum but also in the grass family as a whole.

We find it interesting that the two major aluminum tolerance genes identified to date, *ALMT1* in wheat and Alt_{SB} in sorghum, are distinctly different genes that evolved independently to encode similar physiological mechanisms of aluminum tolerance involving aluminum exclusion from root apices based on organic acid release. Domestication of sorghum, rice and maize seems to have resulted from mutations at orthologous loci[28]. However, the apparently independent origins and evolution of *ALMT1* and Alt_{SB}, which nonetheless have converged to be responsible for similar traits, suggest that possibly only a few physiologically amenable solutions exist for highly specific phenotypes such as tolerance to aluminum toxicity. As other aluminum tolerance QTLs are isolated, it remains to be seen whether it is possible to explore the additive effects provided by nonorthologous aluminum tolerance genes that control convergent physiological mechanisms of aluminum tolerance.

METHODS

Phenotypic analysis of sorghum aluminum tolerance. Sorghum aluminum tolerance based on root growth inhibition elicited by {27} μM Al^{3+} (brackets denote free Al^{3+} activity) was assessed in nutrient solution according to the methods detailed in ref. 10. For more details, see **Supplementary Methods**.

Positional cloning of Alt_{SB}. A recombinant inbred line (RIL) map of Alt_{SB} was developed with 354 RILs derived from a cross of highly aluminum-tolerant SC283 and aluminum-sensitive BR007. The subsequent high-resolution map was constructed by screening 2,085 F_2 individuals from a BR007×SC283 cross and identifying individuals with single recombination events between the markers CTG29 and M181. Recombinant F_2 individuals were self-pollinated, and aluminum tolerance was assessed in $F_{2:3}$ families as described in the previous section. See **Figure 1** and **Supplementary Methods** for more details.

Determination of gene expression via semiquantitative RT-PCR. Sorghum seedlings were grown as described in ref. 10 and in **Supplementary Methods**. Briefly, seedlings were grown in nutrient solution with or without {27} μM Al^{3+} for 3 d. Root apices were collected and frozen in liquid nitrogen, and total RNA was extracted using the RNeasy Plant Mini Kit (Qiagen). The Alt_{SB} and *Actin* (internal control) cDNAs were amplified via RT-PCR with the primer pairs Alt_{SB}-F, Alt_{SB}-R, Actin-F and Actin-R (see **Supplementary Table 2** online for primer sequences).

Determination of gene expression via quantitative real-time RT-PCR. Sorghum seedlings were grown as described previously in nutrient solution with or without {27} μM Al^{3+}. Root and shoot tissues were collected after 1, 3 and 6 d either with or without aluminum treatment. Total RNA was extracted from individual tissue samples using the RNeasy Plant Mini Kit (Qiagen).

SbMATE and 18S RNA (internal reference) transcripts were quantified using the ABI Prism 7900 Sequence Detection System (Applied Biosystems) using the TaqMan Gene Expression Assay (Applied Biosystems) as described in **Supplementary Methods**. See **Supplementary Table 2** for the sequences for the primers used to quantify *SbMATE* expression, including ORF7-forward, ORF7-reverse, and ORF7-probe. Levels of endogenous 18S RNAs were determined using TaqMan Ribosomal RNA Control Reagents (Applied Biosystems).

Subcellular localization of SbMATE. The membrane localization of *SbMATE* was determined by examining the transient expression of the *SbMATE* coding region tagged with GFP in onion (*Allium cepa*) epidermal cells. Transient expression of the *SbMATE::GFP* chimera was achieved by particle bombardment of onion epidermal cells. Imaging of GFP fluorescence was carried out using confocal microscopy (Leica TCS SP2 system). For more details, see **Supplementary Methods**.

Determination of aluminum-activated root citrate exudation in sorghum near-isogenic lines (NILs). Seeds for the aluminum-tolerant (ATF10B) and aluminum-sensitive (ATF8B) NILs were grown as described previously, and root exudates were collected after 1, 3 and 6 d of growth on nutrient solution containing {0} or {27} μM Al^{3+}. Analysis of organic acids in root exudates was performed using a capillary electrophoresis system as described in ref. 29. For more details, see **Supplementary Methods**.

Expression of sorghum *SbMATE* in transgenic *A. thaliana* seedlings and analysis of *A. thaliana* aluminum tolerance and root organic acid exudation. Both the empty transformation vector and the vector carrying the *SbMATE* construct were individually electroporated into the *Agrobacterium tumefaciens* strain GV3101 (Invitrogen) and used for *Arabidopsis thaliana* transformation (in both the Columbia and *AtALMT* knockout backgrounds). The presence of the transgene was confirmed by Basta herbicide resistance of the transgenic plants and by PCR confirmation of T-DNA insertions.

Individual T2 lines with enhanced root growth rate in the presence of aluminum indicating increased aluminum tolerance as compared with corresponding controls were selfed and the segregating T3 progeny analyzed to identify transgenic and nontransgenic homozygous T3 progenies, which were confirmed by progeny testing. Corresponding transgenic and nontransgenic homozygous T3 lines were then used for determination of aluminum tolerance (root growth) and root organic acid exudation as described in ref. 18 and in **Supplementary Methods**.

Accession codes. GenBank: *SbMATE* nucleotide sequence data, EF611342.

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Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

J.V.M. and L.V.K. shared equally in the work, including the direction and oversight of the research and writing of the paper and, thus, are both equally contributing first authors and corresponding authors. J.L., C.T.G., U.G.P.L., Y.-H.W. and P.E.K. contributed to the positional cloning of Alt_{SB}, and J.L. conducted the real-time PCR analysis and generation and characterization of transgenic *A. thaliana*. U.G.P.L. and C.M.C. conducted the RT-PCR analysis of Alt_{SB} expression in the diversity panel. V.M.C.A. and J.E.S. conducted the analysis of sorghum root organic acid exudation, and J.E.S. conducted the *A. thaliana* root organic acid analysis. R.E.S. generated the mapping populations and NILs. O.A.H. contributed to the analysis of *A. thaliana* Al tolerance and organic acid exudation, and M.A.P. conducted the Alt_{SB}-GFP protein localization and expression and electrophysiological characterization of SbMATE in *Xenopus laevis* oocytes.

COMPETING INTERESTS STATEMENT
The authors declare no competing financial interests.

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