

# Identification of the *trans*-acting factors controlling expression of SbMATE, an aluminum-activated citrate transporter that underlies aluminum tolerance in sorghum

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Aluminum (Al) toxicity is a major cause of grain yield reduction in crops cultivated on acid soils. In sorghum, the major Al tolerance locus, *Alt<sub>SB</sub>*, is located on chromosome 3. Within *Alt<sub>SB</sub>*, *SbMATE*, a member in the multidrug and toxic compound extrusion family, encodes an Al-activated citrate transporter that underlies Al tolerance. We have previously observed a reduction in Al-tolerance and *SbMATE* expression in near-isogenic lines (NILs) generated by introgressing the *Alt<sub>SB</sub>* locus from donors showing different levels of Al tolerance into the genetic background of BR012, an Al sensitive line. This incomplete transfer of Al tolerance to NILs indicates the importance of regulatory loci acting in *trans* on *SbMATE* expression and the identification of these *trans*-factors is the aim of this study. Sequencing of the *SbMATE* coding region in different Al tolerance donors revealed its highly monomorphic nature. However, a single SNP was identified in the first exon of SC566, a highly tolerant line, which shows high *SbMATE* expression and citrate exudation. Hybrids between SC566 and lines differing to their degree of Al tolerance and *SbMATE* expression were generated. In addition, a NIL harboring the SC566 allele was crossed to other NILs on the same Al sensitive genetic background belonging to BR012. The SNP identified in SC566 was used to develop an allele-specific expression assay in order to simultaneously analyze the expression of each *SbMATE* allele in hybrid combinations. The expression of the allele coming from the Al sensitive parent was induced in the hybrids, complementing the low level of expression in the Al sensitive parents. However, a reduction in expression of the Al sensitive allele was observed in hybrids between the SC566-NIL and other NILs. These results indicate that SC566 harbors *trans*-acting factors located out of the *Alt<sub>SB</sub>* locus, which can complement the low expression observed in alleles derived from Al sensitive lines. Therefore, maximum levels of Al tolerance can be achieved by the combination of alleles in the *Alt<sub>SB</sub>* locus and *trans*-acting regulatory loci. We performed expression quantitative trait loci (eQTL) mapping in SC283 (Al-tolerant) x BR007 (Al-sensitive) recombinant inbred line population. For that, genotyping by sequencing technology allowed us to screen 68,878 SNPs for association with Al tolerance and *SbMATE* expression. A major eQTL colocalized with *SbMATE* on chromosome 3, which is consistent with the dominance of *cis*-effects in controlling the expression of the Al tolerance gene. However, a second eQTL was detected on chromosome 9, which is candidate for harboring *trans*-factors regulating *SbMATE* expression. Genome-wide association mapping and RNA-seq analysis are now being conducted to define an array of candidate genes responsible by *SbMATE* regulation. The results generated here will contribute to the formulation of advanced strategies of molecular breeding of Al tolerance in sorghum. Financial Support: CNPq and Generation Challenge Programme (GCP).