The toxicity caused by Aluminum (Al) is one of the most limiting factors for crop growth and development on acid soils. In sorghum, allelic variation at the AltSB locus accounts for nearly 80% of the phenotypic variation for the trait in some mapping populations, even though other loci controlling Al tolerance do exist. Our previous positional cloning effort led to the identification of a gene belonging to the multidrug and toxic compound extrusion (MATE) family that underlies the AltSB locus. Attempting to identify the molecular determinants of allelic effects at AltSB, we completely sequenced a 24.6kbp region defined by high resolution mapping of AltSB in the parents of a sorghum RIL population, SC283 (Al tolerant) and BR007 (Al sensitive). Six candidates regions containing polymorphisms were subsequently selected for association studies. These candidates regions were genotyped or sequenced in 254 sorghum accessions comprising our association panel. Association tests were performed using a mixed linear model that accounts for multiple levels of relatedness for appropriate Type I error control. Highly significant associations were detected for traits related to Al inhibition of root growth in nutrient solution. An important role in Al tolerance is possibly played by a polymorphism located in the second intron of the AltSB gene, which had a major contribution to an overall Al tolerance index created by principal component analysis. However, this polymorphism is in strong linkage disequilibrium with others in the region, which prevented us to definite the causative nature of the associated loci. Highly associated SNPs are being used to develop tag markers for marker-assisted selection, which can be used for allele mining in diverse sorghum germplasm.