Recent Advances on the Molecular Basis of Crop Aluminum Resistance

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The considerable genetic variability for aluminum (Al) resistance within many crop plant species has been utilized by plant breeders for a number of years to enhance Al resistance. But beyond this, genetic variability in Al resistance has been an excellent experimental resource that is being mined by researchers to elucidate the molecular basis for this trait. Because of the agronomic importance of Al toxicity, research on the identification of crop Al resistance genes has attracted significant interest from a number of laboratories around the world. There has been considerable recent research progress on identifying the genes underlying a primary mechanism of Al resistance: the release of Al chelating molecules such as low molecular weight organic acids from the root system, which consequently immobilize Al^{3+} at the root surface, preventing it from entering root cells. To date, the Al resistance genes that have been identified are from two different families of membrane transporters mediating organic acid efflux. For both types of transporters, Al-inducible regulation of transporter gene expression plays an important role in differential Al resistance. It is likely that differences in protein structure and function also play a role in differential resistance, although to date there is no data supporting this. The identification of genes conferring Al resistance now provides the necessary molecular tools to more effectively address a worldwide agronomic problem that is only exceeded by drought stress with regards to abiotic limitations to crop production (von Uexküll and Mutert, 1995).

The first Al resistance gene was identified in wheat, and was originally named *ALMT1*, for aluminum-activated membrane transporter (Sasaki et al., 2004). In this seminal publication, it was shown that *ALMT1* is preferentially expressed in wheat root tips and is expressed at constitutively higher levels in Al resistant wheat genotypes. Functional studies in *Xenopus* oocytes suggested that *TaALMT1* encodes a novel transporter that is activated in the presence of extracellular Al^{3+} and is permeable to malate (Sasaki et al., 2004; Piñeros et al., 2008a). Subsequently, studies in Arabidopsis, rape and maize identified *TaALMT1* homologues (*AtALMT1*, *BnALMT1* and *ZmALMT1*, respectively). The homologs in Arabidopsis and rape were also shown to encode Al-activated organic acid anion transporters when expressed in *Xenopus* oocytes (Hoekenga et al., 2006; Ligaba et al., 2006), while the maize homolog was shown to not be involved in maize Al resistance and did not transport organic acids (Piñeros et al., 2008b). Instead it appears to mediate mineral anion efflux and is involved in mineral nutrition.

Identification of a Second Al Resistance Gene

We have been using sorghum as a model system for Al resistance research as it is the fifth most important cereal crop grown worldwide (http://faostat.fao.org), is among the most tolerant of the major cereals to a number of abiotic stresses, and its genome has been fully sequenced. Of the major grain crops, it is the closest relative of maize and like maize, sorghum Al resistance is associated with Al-activated root citrate exudation. Magalhães et al. (2007) used high resolution mapping to clone the gene responsible for the major sorghum Al resistance locus, Alt_{SB}. This mapping approach identified two flanking markers that delineated a 25 kb region containing three putative open-reading frames (ORFs). Two of the ORFs (sucrose phosphate synthase and an unknown protein) have putative functions inconsistent with known physiological mechanisms of Al resistance, while the third ORF is a member of the MATE or multi-drug and toxic compound extrusion family of membrane transporters involved in the efflux of small organic solutes, which would be consistent with a role in root citrate release. Subsequent work confirmed that this transporter is in fact the sorghum Al resistance gene based on the following evidence: 1) The transporter gene is highly expressed in the root apices (the site of Al resistance) of a tolerant NIL derived from a cross between the tolerant and sensitive parents, but it is not expressed in the root apices of an Al sensitive NIL; 2) Expression of this gene is induced by Al only in the tolerant NIL; 3) Alt_{SB} expression increases significantly over a 3-6 day period of Al exposure, with a parallel increase of Al-activated root citrate exudation and Al resistance over the same time period; 4) The other 2 ORFs are expressed solely in the shoot, and thus are not expressed in the site where Al resistance mechanisms must operate (the root apex); 5) Transformation in Al sensitive *Arabidopsis* and wheat generated Al resistant transgenic plants that showed increased citrate release.

Due to the large difference in Alt_{SB} expression observed

between Al tolerant and Al sensitive NILs and the lack of polymorphisms in the coding sequence of the gene, it was hypothesized that the primary difference between the tolerant and sensitive lines was due to changes in regulatory regions of Alt_{SB}. Thus the entire 25 kb region within the flanking markers was scanned in these lines for polymorphisms. Several differences between these two alleles were identified, including a miniature inverted repeat transposable element insertion (MITE-type transposable element) in the promoter region of the gene at ~ 2.0 kb from the transcription initiation site, 6 single nucleotide polymorphisms (SNPs) and one insertion/deletion (indel) in the second intron of Alt_{SB}, and 2 SNPs/1 indel and a 19 bp indel located after the stop codon, in the 3 regions of the gene. It should be noted that the set of polymorphisms we described above was detected between two sorghum lines only, and that many more polymorphisms important for function might be found in a diverse and relatively large sorghum panel. In fact, we have recently identified another resistance source that has the most effective Alt_{SB} allele we have identified to date, exhibiting the largest Al-activated root citrate release and greatest Al resistance we have observed (Magalhães, unpublished results). This line harbors a non-conservative amino-acid substitution in the coding region of the gene, suggesting this amino acid may play a critical role in citrate transport. Altogether, these findings support the notion that identification of superior Alt_{SB} haplotypes can only be gathered through a comprehensive analysis on a relatively large and diverse sorghum panel.

Our sequence scanning was subsequently expanded into a broader investigation of Alt_{SB} diversity using a panel of 47 sorghum accessions of diverse origins. These studies indicate that the MITE containing region in the promoter is highly structured, with the MITE and flanking sequences repeated between 1 and 5 times. There is a significant and positive correlation between the number of repeats in this region, Alt_{SB} expression and Al resistance, with most of the Al tolerant lines harboring larger number of repeats. However, a number of interesting outliers have also been found using NILs generated with different sources of Alt_{SB}. Significant allelic variation was detected with these NILs within a single MITE insertion class, suggesting that the degree of phenotypic expression may depend on interactions among polymorphisms in the 25 kb region.

As with the discovery of *ALMT1* in wheat, the discovery of a second Al resistance gene from a different family of membrane transporters provides a new avenue of inquiry into candidate Al resistance genes in other species. It should be noted that the first member of the MATE family to be shown to be involved in plant Al resistance was *HvAACT1*, which was shown to be the Al-activated root citrate transporter in barley (Furukawa et al., 2007). Additionally, we have found a MATE family homolog that has the same function in Arabidopsis Al resistance (Liu et al., 2008), and we currently are investigating a MATE homolog in maize that appears to be involved in Al resistance (Maron et al., 2008). It is interesting that there is convergent evolution regarding a mechanism of Al resistance based on organic acid release in different plant species that involves genes from two completely unrelated families of transporters. This is depicted in Figure 1, where Al-activated malate and citrate release are shown to be mediated by members of the ALMT and MATE transporter families, respectively.

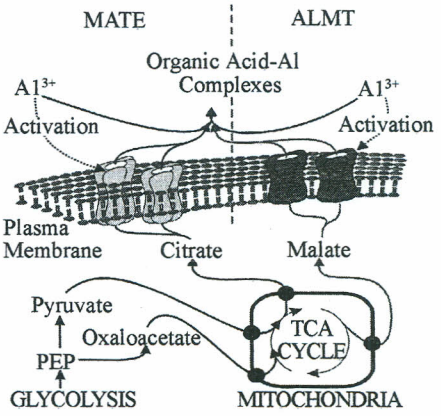

Figure 1 Model illustrating a major Al resistance mechanism based on Al-activated release of organic acids into the rhizosphere, where they chelate Al^{3+} ions and prevent their entry into the root tip. The model suggests that members of the ALMT family of membrane transporters mediate malate release in certain plant species, while MATE transporters are involved in Al-activated citrate exudation

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