How a microbial drug transporter became essential for crop cultivation on acid soils: aluminium tolerance conferred by the multidrug and toxic compound extrusion (MATE) family

Jurandir V. Magalhaes*
Embrapa Maize and Sorghum, MG 424, Km 65, 35701-970, Sete Lagoas, Minas Gerais, Brazil
*E-mail jurandir@cnpms.embrapa.br

INTRODUCTION

A mechanism of aluminium (Al) tolerance in plants, which is based on the release of organic acids into the rhizosphere, has long been hypothesized as a result of a substantial body of physiological evidence indicating that roots of Al-tolerant plants can release these organic compounds in response to Al. Organic acids have the ability to exclude this metal from sensitive sites in the root apex resulting in the formation of stable complexes with Al. However, it was only 6 years ago, in 2004, that the first major Al-tolerance gene, ALMT1, was cloned in wheat (Sasaki et al., 2004), lending molecular validation to the organic acid hypothesis. ALMT1 was found to be an Al-activated malate transporter, which belongs to a novel membrane protein family, and was later found to confer Al tolerance to other species in addition to wheat. In 2007, new insights into the molecular basis of Al tolerance in plants occurred with the publication of research findings implicating multidrug and toxic compound extrusion (MATE) proteins as major determinants of Al tolerance in plants. In sorghum, SbMATE, the gene underlying a major Al-tolerance locus, Al₈₃₁₈, was identified and shown to be a member in the MATE family. This transporter confers Al tolerance by a physiological mechanism based on Al-activated citrate release into the rhizosphere (Magalhaes et al., 2007). In barley, another MATE protein, HvAACT1 was found to be an Al-activated citrate transporter that confers barley Al tolerance (Furukawa et al., 2007).

Interestingly, FRD3, a MATE family member that was originally implicated in iron nutrition in arabidopsis (Green and Rogers, 2004) was also found to mediate citrate efflux into the root vascular tissue; citrate has thus been proposed to act as an iron chelator with a role in iron distribution in the plant (Durrett et al., 2007). The fact that FRD3 shares significant sequence identity with the Al-tolerance genes in sorghum and barley raises interesting questions concerning the nature and the evolution of the causative mutations that confer Al tolerance in plants. In the course of evolution, these mutations may conceivably have conferred very specific structural changes to some MATE proteins to create, for instance, Al-specific binding sites enabling Al-dependent transport activity, thus creating bona fide Al-tolerance genes. Alternatively, Al tolerance might merely be one of the many outcomes of a highly diverse and ubiquitous gene family, with Al tolerance resulting from the activity of polyspecific transporters. In the genomics era, association analysis in concert with detailed physiological and molecular investigations provides a means to dissect the fine structure of a locus into quantitative trait nucleotides. The nature of Al-tolerance quantitative trait nucleotides remains unknown
but it is nevertheless envisaged that novel molecular breeding strategies, based on MATE genes, will be developed as the relationship between the structure of these genes and their function in providing Al tolerance is elucidated. However, at the moment, some expectations can be formed based on the global knowledge of MATE proteins, which are by no means restricted to plants but rather are strikingly distributed throughout all domains of life. Here, we review the major characteristics of MATE genes and will discuss them with regards to the Al-tolerance phenotype in plants.

THE PHYLGENY OF THE MATE FAMILY AND NorM, THE PRIMORDIAL MATE

One of the most important mechanisms for drug resistance in prokaryotes and eukaryotes involves efflux of toxic compounds from the cell. These efflux systems belong to five transporter families: namely the major facilitator superfamily (MFS), the ATP-binding cassette (ABC) superfamily, the drug metabolite transporter (DMT) superfamily, the resistance/nodulation/division (RND) superfamily and the multidrug and toxin extrusion (MATE) family (Saier and Paulsen, 2001), with the last family of transporters functioning as secondary carriers that depend on an transmembrane electrochemical gradient for extrusion activity. MATE proteins range from approx. 400–700 amino acid residues in size and a typical characteristic of this family is the presence of 12 transmembrane helices (TM) (Omonte et al. , 2006). In addition, long N and C tails exposed to the cytoplasm suggest the possibility of interactions with other proteins (Moriyama et al. , 2008). Accordingly, no consensus sequences appear to be conserved across all MATE members, which share approx. 40% sequence similarity, although slightly conserved regions are found near TM1 and TM7, in extracellular loops between TM1 and TM2, and TM7 and TM8, in cytoplasmic loops between TM2 and TM3, and TM8 and TM9, and in loops between TM4 and TM5, and TM10 and TM11. MATEs are ubiquitously distributed in all domains of life and the family includes >1000 proteins (Moriyama et al. , 2008).

Accordingly, a phylogenetic analysis with MATE members highlighted three large families and 14 subfamilies. Class 1 comprises bacterial MATEs, class 2 comprises eukaryote MATEs and class 3 includes bacterial and archaeal MATEs. Thus, the clustering of MATE proteins is in general consistent with organisnal phylogeny. Furthermore, class 2 MATEs are divided into four subclasses: (1) yeast and fungi, (2) plant, (3) protozoan and (4) animal. A previous phylogenetic analysis undertaken by Hvorup et al. (2003) highlighted 15 subfamilies within which seven were bacterial specific, three comprised both archaeal and bacterial proteins, one was archaeal specific, one included bacterial and plant proteins and three subfamilies consisted of eukaryote proteins from yeast, animal and plants (Hvorup et al. , 2003). One of these subfamilies was composed largely of arabidopsis homologues and this plant species was found to possess 58 MATE paralogues (Li et al. , 2002; Hvorup et al. , 2003). A large number of MATE paralogues – exceeding 40 – has also been reported in Medicago truncatula (Zhao and Dixon, 2009). Sequence alignments in arabidopsis identified the conserved sequences as CGQA, located between TM2 and TM3, and RVSNXLGA in TM8 (Li et al. , 2002). Based on sequence similarity to the prokaryotic polysaccharide transporter (PST) family within the multidrug/oligosaccharide/lipid/polysaccharide (MOP) exporter superfamily, to which the MATE family was placed, MATE precursors were hypothesized to be complex carbohydrate exporters in prokaryotes with 6 TMs, which underwent internal duplication to generate a 12 TM protein (Hvorup et al. , 2003). Finally, symmetric repetition of conserved regions in the N- and C-terminal halves of MATE proteins suggests a common ancestry to the MATE family (Omonte et al. , 2006).

The first MATE gene isolated was NorM in Vibrio parahe- molyticus, and NorM was found to be a multidrug efflux protein that conferred resistance to norfloxacin and other antimicrobial agents by Na+/drug antiport (Morita et al. , 1998, 2000). On the basis of 12 TMs present in NorM, the encoding gene was first hypothesized to be a member of the Major Facilitator Superfamily. However, because neither NorM nor its homologue in Escherichia coli, YdhE, were found to share significant similarity with MFS members, Brown et al. (1999) concluded NorM was a prototype of a previously unidentified family, thereby designated the MATE family.

FUNCTIONAL DIVERSITY IN THE MATE FAMILY

In addition to NorM, which confers resistance to structurally unrelated compounds, several proteins in the MATE family have been implicated in a variety of functions related to cell protection from toxins. Two DinF proteins from E. coli and S. pneumoniae, whose expression is DNA damage-inducible, were also found to be related to NorM (Brown et al. , 1999) and DinF proteins have also been shown to be involved with bacterial virulence in tomato (Brown et al. , 2007), presumably due to a protection mechanism based on the efflux of antimicrobial compounds. Also, ERC1 in yeast confers resistance to the methionine analogue, ethionine (Shiomi et al. , 1991; Brown et al. , 1999), which is likely to result from the sequestration of S-adenoslythioneine into the vacuole (Diener et al. , 2001). The eds5 mutant in arabidopsis does not accumulate salicylic acid in response to biotic or abiotic stresses (Nawrath et al. , 2002). EDS5 was found to be homologous to DinF of E. coli and plays an important role in the salicylic acid-dependent pathway of plant defence against pathogens. The arabidopsis MATE gene, ALF5, is expressed in the root epidermis and cortex, and was found to restore normal lateral root growth in seedlings cultivated in bacto agar (Diener et al. , 2001). The alf5 mutant plants were sensitive to contaminants present in the media, and ALF5 was thus thought to act in cellular detoxification by means of vacuolar sequestration or cellular efflux of toxic compounds.

In arabidopsis, loss-of-function mutations in genes belonging to the core flavonoid or proanthocyanidin branch convert the dark-brown wild-type seed colour into transparent testa mutants (Chai et al. , 2009). From this mutant the TRANSPARENT TESTA12 (TT12) gene was cloned and found to encode a MATE family member that epistatically interacts with other genes in the flavonoid pathway, being expressed in ovules and developing seeds (Debeaujon et al. , 2001). Marinova et al. (2007) showed that the TT12
transporter is expressed in the tonoplast and based on transport analysis in heterologous systems, is a vacuolar flavonoid/H\textsuperscript{+} antiporter. In addition, tight regulation of TT12 expression was observed as TT12 promoter activity (Marinova et al., 2007) was observed specifically in proanthocyanidin-synthesizing cells. These authors raised the possibility that the functional uniqueness of TT12 as a flavonoid transporter in arabidopsis is related to this highly specific expression pattern. Interestingly, activation tagging in tomato identified an MYB transcription factor involved in anthocyanin accumulation (Mathews et al., 2003). In this work the MYB gene was also found to up-regulate a putative permease similar to TT12 in tomato, and in arabidopsis TT12 was found to be regulated by three regulatory loci, TT2 encoding a R2R3-MYB protein, TT8 encoding a bHLH protein, and TTG1 corresponding to a WDR protein, which play a role in proanthocyanidin biosynthesis (Lepiniec et al., 2006; Zhao and Dixon, 2009). This suggests that at least part of the specificity of function for MATE genes may arise from the activity of possibly complex regulatory networks. Two MATE genes in grapevine are also likely to promote vacuolar transport of anthocyanins, and, analogous to the aforementioned cases, the grape MATEs are expressed more specifically in anthocyanin-synthesizing tissues (Gomez et al., 2009). Another example of strong regulatory effects affecting the expression of MATE genes was found in tobacco roots, where NiMATE1 and NiMATE2, along with genes in the nicotine biosynthetic pathway, are co-ordinately regulated by NIC regulatory loci to sequester the alkaloid compound in the vacuole (Shoji et al., 2009). Similar co-regulation was found by Morita et al. (2009) for Ni-JAT1, which was nonetheless expressed ubiquitously in tobacco. Interestingly, the microbial MATEs, NorM and YdhE, appear to confer resistance to berberine, a common plant alkaloid (Morita et al., 1998). This function appears to be conserved in plants at AtDTX1 in arabidopsis was also found to mediate efflux of berberine (Li et al., 2002) and a similar mechanism based on H\textsuperscript{+} antiport is possibly encoded by a MATE gene in the medicinal plant, Coptis japonica (Iton et al., 2005; Moriyama et al., 2008).

Mammalian MATEs function as H\textsuperscript{+} antiporters to transport organic cations and are involved in a variety of functions (Moriyama et al., 2008). Otsuka et al. (2005) identified the MATE1 gene in humans, which is predominantly expressed in kidney and liver and was proposed to be the final step of organic cation efflux by means of H\textsuperscript{+} antiport. Accordingly, cis-inhibition studies with a wide variety of metabolites indicated MATE1-mediated transport of organic cations to be polyspecific in nature. The mouse orthologue, mMATE1, showed characteristics very similar to MATE1 in humans, but its expression was widely distributed in various tissues in addition to kidney and liver, suggesting involvement in various biological functions (Hiasa et al., 2006).

AI TOLERANCE SPECIFICITY AND THE MATE FAMILY

Although MATE proteins have been initially reported to show preference for hydrophobic cations (Eckardt, 2001), the rather polyspecific nature of MATE transporters has now become clear. For instance, mammalian MATEs have been shown to transport zwitterionic and anionic compounds in addition to cationic compounds (Tanihara et al., 2007). Plant MATEs that have been implicated in citrate transport are FRD3 in arabidopsis (Durrett et al., 2007), SbMATE in sorghum (Magalhaes et al., 2007), HvAACT1 in barley (Furukawa et al., 2007), OsFRDL1 in rice (Yokosho et al., 2009), AtMATE in arabidopsis (Liu et al., 2009), ZmMATE1 in maize (Maron et al., 2009) and a MATE gene was also implicated in citrate efflux in wheat (Ryan et al., 2009). In addition, SbMATE is similar to LaMATE that is expressed in proteoid roots in white lupin under phosphorus deficiency (Uhde-Stone et al., 2005; Magalhaes et al., 2007), suggesting a possible role in citrate transport for LaMATE. It should be noted, however, that these studies were largely hypothesis-driven and did not include a thorough examination of substrate specificity for the respective MATE proteins. Therefore, at this point there are no reasons to rule out the possibility that plant MATEs could transport substrates other than citrate, which may also play a role in Al tolerance. In fact, several indirect reasons point in that direction. For instance, phenolic compounds can form stable complexes with Al\textsuperscript{3+}, possibly playing a role in Al tolerance (reviewed by Kochian et al., 2004; Tolrà et al., 2009). However, given the documented involvement of MATE proteins in the transport of compounds such as alkaloids and flavonoids, it is possible that citrate-transporting MATEs may have acquired dual transport functions, also enabling transport of phenolic compounds. Alternatively, other members of the MATE family may have specifically diverged to confer Al tolerance by a physiological mechanism based on phenolics. In fact, a bacterial mutant complemented with AtDTX1 from arabidopsis, which has been shown to mediate efflux of plant alkaloids among other toxic compounds, became resistant to Cd\textsuperscript{2+}, although no effect was observed in the presence of Al\textsuperscript{3+} (Li et al., 2002). In addition, for two MATE homologues in maize, ZmMATE1 and ZmMATE2, which colocalize to two major Al-tolerance quantitative trait loci, only the former is a citrate transporter whereas ZmMATE2 may be involved in a novel Al-tolerance mechanism (Maron et al., 2009), possibly mediating transport of a different compound.

Some plant species appear to utilize internal mechanisms of Al detoxification. Buckwheat can accumulate Al and it has been shown that Al is present in leaves and roots as an Al:oxalate complex (Ma et al., 1998). In addition, Al in the xylem sap was found to be complexed with citrate (Ma and Hiradate, 2000), suggesting that Al undergoes a ligand exchange from oxalate to citrate when it is transported to the xylem, and exchanged back into oxalate when transported to the leaves (Kochian et al., 2004). It is interesting that two MATE homologues appear to be involved in citrate efflux into vascular tissue. Those are FRD3 in arabidopsis (Durrett et al., 2007) and OsFRDL1 in rice (Yokosho et al., 2009), which are both localized to the pericycle cells of roots and appear to be necessary for efficient Fe translocation to the shoots. Therefore, considering various examples of tonoplast MATEs, it will not be surprising if MATE homologues involved with internal transport of citrate are found to play a role in plant Al tolerance by an internal detoxification mechanism involving Al sequestration into the vacuoles. Indeed, most of the Al:oxalate complex in buckwheat was found to be
sequestered into vacuoles (Shen et al., 2002), which is an accepted mechanism of internal detoxification (Ma, 2007). In addition, an ABC transporter has been suggested to play a role in Arabidopsis Al tolerance and has been proposed to mediate Al redistribution from sensitive tissues (Larsen et al., 2005). Subsequently, the same researches identified ALS1, a half-type ABC transporter, which is localized to the tonoplast and thought to be involved in Al sequestration in Arabidopsis (Larsen et al., 2007).

Examples have been presented indicating that the expression patterns of MATE genes involved with flavonoid transport are influenced by regulatory loci. Expression of AtMATE, which confers Al tolerance in Arabidopsis by a mechanism based on Al-activated root citrate exudation, has been found to depend on STOP1 (Liu et al., 2009), a zinc finger transcription factor involved with tolerance to protons in Arabidopsis (Iuchi et al., 2007). In fact, STOP1 appears to be involved in signal transduction pathways regulating a number of Al- and H⁺-responsive genes in Arabidopsis (Sawaki et al., 2009), revealing a broader effect that goes beyond that in AtMATE and AtALMT1 (Liu et al., 2009). In rice, another transcription factor similar to AtSTOP1, ART1, regulates the expression of several genes possibly involved with Al tolerance. In addition, ART1 has been shown to interact with the promoter of the region of STAR1, which is involved with Al tolerance in rice (Yamaji et al., 2009).

CONCLUSIONS

The MATE family has been shown to be ubiquitously distributed and extremely flexible in function. Although the study of plant MATEs is in its infancy, the importance of regulatory loci controlling MATE function appears pivotal. FRD3 in Arabidopsis and OsFRDL1 in rice are both localized to the pericycle cells of roots and are likely to be involved with Fe translocation to the shoot (Durrett et al., 2007; Yokosho et al., 2009). In a study with a knockout mutant, OsFRDL1 was not found to confer Al-induced citrate release in rice (Yokosho et al., 2009). However, when ectopically expressed under the control of the 35S promoter, Arabidopsis FRD3 was found to confer Al tolerance by a mechanism involving citrate release from the roots (Durrett et al., 2007), although apparently not in an Al-activated fashion. Therefore, it is possible that Al-tolerance mutations may have arisen to drive expression of functional transporters to Al-sensitive sites in the plant, which is widely accepted to be the root apex. Nevertheless, at this point it is still not possible to rule out the existence of specific structural features that tie together MATE activity and Al tolerance. For example, in Arabidopsis, MATE members other than AtMATE and AtFRD3 lack a large intracellular loop, which led Liu et al. (2009) to speculate on a specific role for this loop in citrate binding and transmembrane transport.

In sorghum, the correlation between SbMATE expression and Al tolerance in a panel including an allelic series at the underlying locus was extremely high (Magalhaes et al., 2007), indicating that allelic effects are likely to be regulatory in nature. Similarly in barley cultivars differing in Al tolerance, HvAACT1 expression was correlated with citrate release (Furukawa et al., 2007). Considering that a transcription factor has been identified that modulates the expression of plant MATEs, further research should be directed towards identification and characterization of possible regulatory networks within a breeding perspective in crop species. This effort could reconcile a long-standing question in plant Al tolerance: does the rather quantitative inheritance of Al tolerance observed in species such as maize and rice necessarily translate into diversity of physiological mechanisms of Al tolerance? It is tempting to speculate that multiple ‘Al-tolerance’ genes may act by modulating a few sets of physiological mechanisms, which have been fine tuned along the evolutionary history of plant species, providing Al tolerance without undesirable genetic load, thus escaping purifying selection. Clearly, the perspective to find additional functional variation that can lead to transgressive segregation in plant breeding is quite large in the MATE family itself, which furthermore is present as many paralogues that might have acquired divergent functions within some plant species. Therefore, efforts should now be directed to the identification of the molecular determinants of this variation, both within and outside loci encoding organic acid transporter families.

LITERATURE CITED


resistance in higher plants.

The Plant Journal

Ma JF. 2007.


APPENDIX

Two relevant papers were published after this review was accepted: Ryan and Delhaize (Functional Plant Biology 37: 275–284, 2010) discuss evolutionary aspects of aluminum resistance in plants whilst Yokosho et al. (Functional Plant Biology 37: 296–303, 2010) describe the isolation of MATE homologues involved with citrate efflux in rye.