The multidrug and toxic compound extrusion (MATE) family in plants

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Abstract  Multidrug and toxic compound extrusion (MATE) transporters are a family of cation antiporters occurring in most organisms from prokaryotes to eukaryotes. This family constitutes one of the largest transporter families in plants, with, for example, more than 50 MATE genes in the Arabidopsis genome. Moreover, MATE transporters are involved in a wide variety of physiological functions throughout plant development, transporting a broad range of substrates such as organic acids, plant hormones and secondary metabolites. This review categorizes plant MATE transporters according to their physiological roles and summarizes their tissue specificity, membrane localization, and transport substrates. We also review the molecular evolutionary development of plant MATE transporters.

Key words:  Aluminum tolerance, iron translocation, multidrug and toxic compound extrusion, secondary metabolite, transporter.

Introduction

Living organisms produce a vast number of metabolites for growth, communication with other species, and adaptation to the environment. Since optimal use of these metabolites requires that they function at an appropriate time in an appropriate tissue, their biosynthesis and transport must be coordinately regulated. The transport mechanism of metabolites in plants can be classified into three types: transporter-independent trapping, transporter-mediated transport, and vesicle-mediated transport (Shitan and Yazaki 2013). Of these, transporter-mediated transport has been well investigated at the molecular level, and several transporter families characterized to date, including the ATP-binding cassette (ABC) transporters, major facilitator superfamily (MFS), and multidrug and toxic compound extrusion (MATE) transporters (Nour-Eldin and Halkier 2013; Shoji 2014; Yazaki et al. 2009). Most of these proteins transport their substrates in only one direction in vivo, and several transporters were shown to be specifically involved in the transport of a single metabolite.

MATE transporters were first identified in Vibrio parahaemolyticus and Escherichia coli as multidrug efflux proteins, and were designated MATE due to their lack of sequence homology with other transporters (Brown et al. 1999; Morita et al. 1998). Shortly afterward, this novel transporter family was found to be widely conserved in living organisms, including higher plants (Omot et al. 2006). The first plant MATE transporter, AtALF5 (Arabidopsis thaliana aberrant lateral root formation 5) was isolated in 2001 and shown to be involved in multidrug resistance (Diener et al. 2001). The first multi-specific MATE transporter, AtDTX1 (A. thaliana detoxification 1), was characterized in 2002 (Li et al. 2002). Because these Arabidopsis MATE transporters were found to be involved in the efflux of xenobiotics, plant MATEs were thought to function as multidrug resistance proteins, similar to MATE transporters found in microorganisms. However, further research on more than 40 plant MATEs found that these proteins have many physiological functions, showing rather restricted substrate specificities in plants (Figure 1, Table 1). MATE transporters mediate secondary transport, utilizing an electrochemical gradient of either Na⁺ or H⁺ across the localized membrane as the driving force. Since MATE transporters transport...
MATE transporters in plant

Most MATE transporters function as substrate efflux transporters from cytoplasm to apoplasts or vacuoles. The physiological functions of plant MATEs reported to date are xenobiotic efflux, accumulation of secondary metabolites including alkaloids and flavonoids, iron (Fe) translocation, aluminum (Al\(^{3+}\)) detoxification, and plant hormone signaling, indicating that MATE transporters are involved in a wide range of biological events during plant developments (Figure 2). This review will provide an overview of plant MATE transporters according to their physiological function.

**Xenobiotic efflux**

The first MATE transporters isolated from plants were involved in xenobiotic detoxification. In Arabidopsis, two MATEs were reported to mediate this function. The \(\text{AtDTX1}\) gene was found to confer resistance to norfloxacin during functional screening with \(E.\ coli\) (Li et al. 2002). Functional analysis using \(\text{AtDTX1}\)-transformed \(E.\ coli\) cells showed that this transporter effluxes toxic compounds such as antibiotics and cadmium (Cd), as well as two plant alkaloids, berberine and palmatine. Due to the absence of these alkaloids from Arabidopsis, \(\text{AtDTX1}\) probably exports these molecules from plant cells as xenobiotics, as other bacterial and mammalian MATE transporters efflux plant alkaloids (Kuroda and Tsuchiya 2009; Li et al. 2002; Morita et al. 1998; Otsuka et al. 2005). Another MATE transporter identified in Arabidopsis is \(\text{AtALF5}\). Mutations in the \(\text{AtALF5}\) gene lead to defects in lateral root formation, and increase the sensitivity of roots to various compounds. Physiologically, \(\text{AtALF5}\), like \(\text{AtDTX1}\), is thought to efflux xenobiotics, although the transport substrate of \(\text{AtALF5}\) has not been well characterized (Diener et al. 2001).

**Alkaloid accumulation**

Alkaloids are nitrogen-containing low-molecular weight compounds, constituting a major class of
Table 1. MATE transporters in plants.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>AGI code/accession No.</th>
<th>Plant species</th>
<th>Tissue expression</th>
<th>Subcellular localization</th>
<th>Function</th>
<th>Transport substrate</th>
<th>Driving force</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>AtALF5</td>
<td>At2g23560</td>
<td>Arabidopsis thaliana</td>
<td>Root</td>
<td>PM (GFP)</td>
<td>Xenosolactone efflux</td>
<td>Tetrastigmellamyosin</td>
<td>Nicotinoxam, Fru-based monoxide, Berberine, Palmitate</td>
<td>Duan et al. (2001)</td>
</tr>
<tr>
<td>AtDIT1</td>
<td>At2g40470</td>
<td>Arabidopsis thaliana</td>
<td>Root and Shoot</td>
<td>PM (GFP)</td>
<td>Xenosolactone efflux</td>
<td>Nicotinoxam, Fru-based monoxide, Berberine, Palmitate</td>
<td>Duan et al. (2001)</td>
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<tr>
<td>NtMATE1</td>
<td>AB286963</td>
<td>Nicotiana tabacum</td>
<td>Root</td>
<td>V (GFP, MF, IM)</td>
<td>Nicotinoxam accumulation</td>
<td>Nicotinoxam H+</td>
<td>Shoji et al. (2009)</td>
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<tr>
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<td>Nicotiana tabacum</td>
<td>Root</td>
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<td>NtJAT1</td>
<td>AM191992</td>
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<td>Nicotinoxam accumulation</td>
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<td>Morita et al. (2009)</td>
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<td>Leaf</td>
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<td>Nicotinoxam accumulation</td>
<td>Nicotinoxam H+</td>
<td>Shim et al. (2014)</td>
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<td>At5g25640</td>
<td>Arabidopsis thaliana</td>
<td>Flower</td>
<td>V (Proton-exchange)</td>
<td>Proanthocyanid acid accumulation</td>
<td>Malonylated flavonoid glucosides</td>
<td>Acylated anthocyanins H+ Shoji et al. (2009)</td>
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<td>V (GFP)</td>
<td>Proanthocyanid acid accumulation</td>
<td>ATP-dependent H+</td>
<td>Shoji et al. (2009)</td>
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<td>SIMT1</td>
<td>BE354224</td>
<td>Solanum lycopersicum</td>
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<td>V (GFP)</td>
<td>Proanthocyanid acid accumulation</td>
<td>Malonylated flavonoid glucosides</td>
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<td>At3g59030</td>
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* Identities of Genoscope database (http://www.genoscope.cns.fr). Notes: Subcellular localization: IM, Immunoelectron microscopy; MF, Microsomal fractionation; MT, Mitochondria; PM, Plasma membrane; VM, Vacuolar membrane. Function: ABA, Abscisic acid; As, Arsenic; IAA, Indole-3-acetic acid; SA, Salicylic acid.
MATE transporters in plant secondary metabolites. Because many of these metabolites have diverse chemical structures and strong biological activities, some alkaloids have been used pharmaceutically, as, for example, anticancer agents and analgesics (Croteau et al. 2000). These metabolites, however, may also be toxic to humans and to plant cells. Therefore, alkaloid-producing plant cells have several detoxification strategies (Sirikantaramas et al. 2008). Of these, excretion to apoplasts or vacuoles is a widely conserved strategy in plants, and largely contributes to alkaloid detoxification (Shitan et al. 2014a; Shitan and Yazaki 2007; 2013).

MATE transporters responsible for the accumulation of endogenous alkaloids were first isolated from tobacco plants (Nicotiana tabacum), which produce nicotine as a defensive toxin against herbivores. Nicotine is biosynthesized in the root tissue, translocated via xylem transport to aerial tissues, and finally accumulates in leaf vacuoles. Genes encoding three MATE transporters, Nt-JAT1 (N. tabacum jasmonate-inducible alkaloid transporter 1), NtMATE1 and NtMATE2 (N. tabacum MATE1 and 2), were isolated as genes co-regulated with alkaloid biosynthesis (Morita et al. 2009; Shoji et al. 2009). Nt-JAT1 has a relatively high amino acid identity with each other, and both have lower (31–33%) amino acid identity with Nt-JAT1 (Figure 1). Although all these tobacco MATE transporters are localized to tonoplasts, they clearly show distinct tissue-specific expression patterns. Specifically, Nt-JAT1 is expressed in leaves, stems and roots (Morita et al. 2009), whereas NtMATE1 and NtMATE2 are expressed only in roots (Shoji et al. 2009). Nt-JAT1 has transport activity for nicotine and other alkaloids such as anabasine and scopolamine, but not for flavonoids (Morita et al. 2009), suggesting the involvement of this MATE transporter in nicotine accumulation in leaf vacuoles. NtMATE1 also showed H+/nicotine antiport activity when expressed in yeast cells. However, suppression of both NtMATEs by RNAi reduced root tolerance to nicotine exogenously added to the medium, indicating that the NtMATEs function in nicotine sequestration into vacuoles for detoxification in roots, in which nicotine biosynthesis occurs (Figure 3) (Shoji et al. 2009). Very recently, a MATE transporter Nt-JAT2 has been identified as a nicotine transporter in leaves (Shitan et al. 2014b). Nt-JAT2 has lower amino acid identity with the three Nicotiana MATE transporters (32–38%), suggesting that Nicotiana recruited and developed different MATE transporters for nicotine translocation (Figure 1). It is of interest to find a conserved domain and/or structure of

Figure 2. Tissue specificities of gene expression and physiological roles of plant MATE transporters. Each function is described in detail in the corresponding text.
nicotine recognition in these nicotine transporters.

Plant MATE transporters have also been implicated in the transport of other alkaloids. Biochemical analysis of *Coptis japonica* vacuoles suggested that berberine was transported across the tonoplast via an H⁺/berberine antiporter (Otani et al. 2005). Similarly, two monoterpene indole alkaloids (MIAs), vindoline and catharanthine, are taken up by mesophyll vacuoles via an H⁺/MIAs antiport system in *Catharanthus roseus* (Carqueijeiro et al. 2013). These antiport properties suggested the involvement of putative MATE transporters in vacuolar transport of these alkaloids. Indeed, we recently identified a *C. japonica* MATE transporter that localizes to tonoplasts and shows berberine transport activity (our unpublished data) (Figure 3).

In contrast to vacuolar accumulation via H⁺/alkaloid antiporters, ABC transporters mediate berberine influx (CjABCB1 and 2) at the plasma membranes of *C. japonica* cells and catharanthine efflux (CrTPT2) at the plasma membranes of *C. roseus* cells (Shitan et al. 2003; Shitan et al. 2013; Yu and De Luca 2013). Another type of transporter, the purine uptake permease NUP1 (nicotine uptake permease 1), takes up nicotine as a proton symporter at the plasma membrane of tobacco root cells (Hildreth et al. 2011) (Figure 3). These findings indicate that different transporter families function cooperatively to transport a single alkaloid in a plant cell and/or body. Isolation and characterization of additional new transporters will clarify the overall mechanisms of alkaloid transport in plants.

**Flavonoid accumulation**

Flavonoids, which are biosynthesized from an aromatic amino acid, phenylalanine, have two C6 aromatic rings and a C3 alkyl-chain as their main chemical structure. Due to their importance in plant development, many plants biosynthesize and accumulate flavonoids in a species-specific manner (Falcone Ferreyra et al. 2012). To date, four major pathways of flavonoid transport have been proposed: vesicle-mediated, glutathione S-transferase (GST)-mediated, ABC transporter-mediated, and MATE transporter-mediated transport. These pathways co-exist in the same cells, and their combined functions were suggested from the results of several studies. For example, ABC transporters may transport GST-flavonoid conjugates, whereas MATE transporters may mediate the loading of flavonoids into intracellular vesicles (Shitan and Yázaki 2013; Shoji 2014; Zhao and Dixon 2010). A phylogenetic analysis showed that MATE transporters involved in flavonoid accumulation form two distinct clades, apparently reflecting their transport substrates, proanthocyanidin precursors and acylated anthocyanins (Figures 1, 4).

*Arabidopsis* TT12 (transparent testa 12), the first MATE transporter found to transport flavonoids, was originally isolated during screening of mutants with altered seed coloration (Debeaujon et al. 2001). The accumulation of proanthocyanidins and flavonols was reduced in mutant seeds, similar to findings in other *tt* mutants, which encode the genes related to flavonoid biosynthesis (Lepiniec et al. 2006). Using microsomal vesicles from yeast expressing AtTT12, the AtTT12 was found to transport two flavonoids, cyanidin-3-O-glucoside (Cy3G) and epicatechin 3′-O-glucoside (E3′G) (Marinova et al. 2007; Zhao and Dixon 2009). Since Cy3G levels are similar in *tt12* mutant and wild-type seeds, and since E3′G is effluxed by AtTT12 with higher
affinity and velocity than Cy3G. E3’G was regarded as a native transport substrate of AtTT12 (Zhao and Dixon 2009). Arabidopsis proanthocyanidins are epicatechin-type, indicating that the reduction of proanthocyanidins in tt12 seeds is caused by the lack of E3’G transport into vacuoles, which is mediated by AtTT12 (Figure 4).

Subsequently, an ortholog of AtTT12 was isolated from a legume, Medicago truncatula, and designated MtMATE1, with 69.6% amino acid identity to AtTT12 (Zhao and Dixon 2009). Similar to AtTT12, MtMATE1 localizes at the tonoplast and transports E3’G and Cy3G. This transporter, however, had a greater preference for E3’G, relative to Cy3G, than AtTT12. The tt phenotype of the Arabidopsis tt12 mutant was complemented by MtMATE1, and the knockout mutant of MtMATE1 showed the tt phenotype in M. truncatula as well, indicating that MtMATE1 is a functional ortholog of AtTT12 (Zhao and Dixon 2009) (Figure 4). Sequence similarity with AtTT12 led to the isolation of two apple MATEs (Malus×domestic MATE1 and 2, with 75.1% and 72.0% amino acid identity to AtTT12, respectively) (Frank et al. 2011). Similar to MtMATE2, the apple MATE transporters were able to complement the seed phenotype of the Arabidopsis tt12 mutant, indicating that these four MATE transporters are functional orthologs of each other (Frank et al. 2011). Two grapevine MATE transporters, Vitis vinifera MATE1 and 2, with 71.3% and 72.8% to AtTT12, respectively, were also isolated, VvMATE1 as a gene co-regulated with proanthocyanidin biosynthesis genes during the overexpression of VvMYBPA, a transcriptional regulator of the proanthocyanidin pathway (Terrier et al. 2009), and VvMATE2 from its sequence similarity with VvMATE1 (Perez-Diaz et al. 2014). These VvMATEs differ in localization, with VvMATE1 localizing to tonoplasts and VvMATE2 to the golgi, indicating that these MATE transporters are involved in the accumulation of proanthocyanidins via different pathways (Perez-Diaz et al. 2014).

A Medicago MATE transporter, MtMATE2, was also identified as an ortholog of AtTT12 with lower amino acid identity (38.5%) than MtMATE1 (Zhao et al. 2011). MtMATE2 has higher similarity with the tomato (Solanum lycopersicum) MATE transporter SlMTP77 (65.7%), the expression of which is co-regulated with anthocyanin biosynthesis genes by SIANT1, a MYB transcription factor (Mathews et al. 2003). Transport assays using yeast membrane vesicles revealed that MtMATE2 had broad substrate specificity for flavonoid glycosides, e.g. Cy3G and delphinidin-3-O-glucoside,
and showed more efficient transport activity when malonylated flavonoid glycosides were used as substrates. The MtMATE2 knockout mutant showed a reduction in pigmentation of flowers and leaves, in which MtMATE2 was expressed, indicating that this transporter protein mediates flavonoid accumulation in the tissues (Zhao et al. 2011).

Two MATE transporters, VvAM1 and VvAM3 (Vitis vinifera anthoMATE1 and 3), were also isolated from grapevine as orthologs of SlMTP77, with 67.1% and 68.1% amino acid identity, respectively (Gomez et al. 2009). The expression of these MATEs is almost specific to berry skin, and is induced during the ripening stage in Syrah, a dark-skinned grape cultivar. Analysis of VvAM3 expression in 15 cultivars showed that its level of expression correlated with anthocyanin content. By contrast, the expression level of VvAM1 was independent of anthocyanin content. Both MATE transporters showed transport activity for an acylated anthocyanin mixture extracted from Syrah grape berries, which primarily consists of 3-p-coumaroylglucosylated anthocyanins. In contrast, these MATE transporters showed no transport activity for non-acylated anthocyanins, such as Cy3G (Gomez et al. 2009). Subcellular localization analysis using MYBA1 transformed hairy roots revealed that both MATE transporters are localized to small vesicles (Cutanda-Perez et al. 2009; Gomez et al. 2011). Analysis of these hairy roots showed that anthocyanins accumulated not only in vacuoles, but in small vesicles that actively moved around the tonoplast, suggesting that VvAM1 and VvAM3 are involved in the vesicle-mediated transport of anthocyanins into vacuoles (Figure 4). Because grapevine GST is also a candidate protein for anthocyanin transport (Conn et al. 2008), and because it was the most up-regulated gene when VvMYBA1 was overexpressed in hairy roots (Cutanda-Perez et al. 2009), its involvement in vesicle trafficking was also investigated. Suppression of GST reduced anthocyanin accumulation in vacuoles, while having no effect on the colors of small vesicles. In contrast, reduction of VvAMs decreased the number of small vesicles, but did not affect vacuolar anthocyanin, suggesting that MATE transporters and GST function independently in regulating anthocyanin accumulation in grapevines (Gomez et al. 2011).

A H⁺-antiporter may also be involved in the vesicle-mediated transport of phenol glucosides into apoplasts (Tsuyama et al. 2013). A vesicle transport assay using microsomal fraction of hybrid poplar (Populus sieboldii × Populus grandidentata) found that coniferin (coniferyl alcohol glucoside) is transported in a proton-dependent manner, with the highest degree of transport activity present in the fraction, in which tonoplasts and endomembranes are highly concentrated. Because coniferyl alcohol is a major component of lignin, and lignin monomer (monolignol) should be transported into apoplasts, coniferin is likely loaded into small vesicles or golgi, and then delivered to the outside via secretory vesicles (Tsuyama et al. 2013). Moreover, ABC transporters were also found to be involved in monolignol transport into apoplasts (Alejandro et al. 2012; Kaneda et al. 2008; Miao and Liu 2010), suggesting the existence of coordinated transport mechanisms of monolignol into apoplasts by MATE transporters and ABC transporters.

An ortholog of SlMTP77, Arabidopsis FFT (flower flavonoid transporter) has also been characterized (Thompson et al. 2010). Vacuole proteome analysis had previously shown that AtFFT localized to tonoplasts (Jaquinod et al. 2007). AtFFT is expressed in almost all tissues, including floral tissues, and its suppression altered flavonoid levels in flowers, as well as germination rate, suggesting that the role of AtFFT in seed development involves its regulation of flavonoid levels (Thompson et al. 2010).

**Fe translocation**

Since Fe is an essential mineral for plant growth, plants have developed highly sophisticated systems for Fe acquisition and translocation. Some MATE transporters are involved in Fe translocation by effluxing citrate or protocatechuic acid, which can chelate Fe to increase its solubility. MATE transporters that efflux citrate are similar to each other, but are dissimilar to other MATE transporters (<20% amino acid identify), and thus form a distinct clade in phylogenetic analysis (Figure 1, Table 1). Of these, an Arabidopsis MATE transporter AtFRD3 (A. thaliana ferric reductase defective 3) was identified from mutants that lack ferric reductase.

![Figure 5](image-url)
activity and exhibit chlorosis (Rogers and Guerinot 2002). AtFRD3 protein shows citrate efflux activity when expressed in *Xenopus* oocytes, and localizes to the plasma membranes of pericycle and vascular tissue cells of Arabidopsis. Moreover, abnormal Fe has been detected in *frd3* mutants, indicating that AtFRD3 is responsible for loading citrate, a chelator of Fe, into root xylem for efficient Fe translocation (Figure 5) (Durrett et al. 2007; Green and Rogers 2004). The rice ortholog of AtFRD3, OsFRDL1 (*Oryza sativa* frd-like 1) has a similar mechanism of action, indicating that this mechanism is widely conserved in angiosperm (Inoue et al. 2004; Yokosho et al. 2009). In contrast, Fe acquisition mechanisms from soil have been found to differ, with gramineous plants using mugineic acid to chelate Fe (strategy II) and other plants reducing ferric ion with ferric reductase (strategy I) to increase Fe solubility (Kobayashi and Nishizawa 2012; Römheld 1987).

Recently, AtFRD3 was found to function in seeds and flowers, as well as in roots (Roschztattardtz et al. 2011). Using novel *frd3* loss-of-function mutants, Fe loading on pollen grains and embryo mediated by AtFRD3 was found to be necessary for pollen development and germination, respectively, suggesting that AtFRD3 mediates Fe transport between symplastically disconnected tissues throughout development (Roschztattardtz et al. 2011). Similarly, a legume MATE transporter, *LjMATE1* (*Lotus japonicus* MATE1), was found to assist in the translocation of Fe from the roots to the nodules (Takanashi et al. 2013). The citrate-mediated Fe translocation systems with MATE transporters in root xylem have also been reported in soybean GmFRD3a and GmFRD3b (*Glycine max* FRD3a and 3b) and rye ScFRDL1 (*Secale cereal* FRDL1) using reverse genetic approaches (Rogers et al. 2009; Yokosho et al. 2010), and also in barley HvAAC T1 (*Hordeum vulgare* aluminum-activated citrate transporter1) and wheat TaMATE1b (*Triticum aestivum* MATE1b) from studies of Al3+ detoxification mechanisms (see “Al3+ detoxification”) (Fujii et al. 2012; Tovkach et al. 2013).

In 2011, two rice MATE transporters, OsPEZ1 and OsPEZ2 (*O. sativa* phenolix efflux zero 1 and 2) were shown to assist in Fe translocation by effluxing a phenolic compound, protocatechuic acid (Bashir et al. 2011; Ishimaru et al. 2011). These MATE transporters were originally isolated as genes involved in Cd accumulation, because suppression mutants of these *MATE* genes accumulated more Cd than wild-type rice. Both OsPEZ1 and OsPEZ2 were expressed in stele and localized at the plasma membrane. Analysis of xylem sap showed that the levels of protocatechuic acid and caffeic acid, as well as Fe content, were lower in these suppression mutants. The ability of OsPEZ1 and OsPEZ2 to transport protocatechuic acid was confirmed using *Xenopus* oocytes. These findings indicated that OsPEZ1 and OsPEZ2 control the efflux of protocatechuic acid and possibly caffeic acid, which chelate apoplasmic Fe and thus increase its solubility in stele (Bashir et al. 2011; Ishimaru et al. 2011). Interestingly, OsPEZ2 was also found to be expressed in the root elongation zone, and phenolic compounds in root exudates were decreased in ospez2 mutants (Bashir et al. 2011). A maize ortholog of OsPEZ2, ZmMATE2 (*Zea mays* MATE2), was identified as one of two major quantitative trait loci (QTLs) for Al3+ tolerance, with the other being ZmMATE1 (see “Al3+ detoxification”) (Maron et al. 2010). Al3+ treatment of maize was found to activate the release of phenolic compounds from the roots that may function as Al3+ chelators (Kidd et al. 2001), suggesting that ZmMATE2 as well as OsPEZ2 may be implicated in Al3+ tolerance by effluxing phenolic compounds into the rhizosphere (Figure 5).

### Al3+ detoxification

Al3+, the most abundant metal ion in the Earth's crust, inhibits root elongation in acidic soil, although it is non-toxic at neutral pH. Compared with Al3+ sensitive plants, Al3+ tolerant plants can efflux high amounts of organic acids, which protect roots by chelating Al3+ in the apoplasts and rhizospheres around the root tips (Kochian et al. 2004; Ma et al. 2001; Ryan et al. 2001). Malate and citrate are major contributors to Al3+ detoxification in rhizospheres, with their release mediated mainly by ALMT (aluminum-activated malate transporter) and MATE, respectively (Ryan and Delhaize 2010; Ryan et al. 2011).

MATE transporters involved in Al3+ detoxification were first identified in sorghum (*Sorghum bicolor*; SbMATE) and barley (*HvAAC T1*) by map-based cloning. Both MATE transporters efflux citrate in assay systems using *Xenopus* oocytes as host organisms, and both were found to localize to plasma membranes in the root tips. Their levels of expression were found to correlate with the amount of citrate released from roots, and also with the root elongation rate in the presence of Al3+, indicating that high expression of these MATE transporters was necessary for Al3+ tolerance (Figure 5) (Furukawa et al. 2007; Magalhaes et al. 2007; Sivaguru et al. 2013). Similarly, ZmMATE1 and ZmMATE2 were isolated from microarrays and QTL analyses as the Al3+ tolerance genes in maize (Maron et al. 2010). Both ZmMATEs localize to plasma membranes, whereas only ZmMATE1 showed citrate efflux activity with *Xenopus* oocytes. Although ZmMATE2 mediated anion efflux, its endogenous substrate remains unknown. Its high sequence similarity to both OsPEZ1 and OsPEZ2 (Figure 1) suggests that ZmMATE2 may efflux phenolic compounds for Al3+ tolerance (Bashir et al. 2011). Homologs isolated to date from other plant species by reverse genetic approaches include AtMATE from...
Arabidopsis, OsFRDL4 from rice, ScFRDL2 from rye, VuMATE1 from rice beans (Vigna umbellata), TaMATE1b from wheat, BoMATE from sprouts (Brassica oleracea) and EcMATE1 and EcMATE3 from Eucalyptus (Eucalyptus camaldulensis). These findings suggest that, similar to the Fe translocation system, this MATE-mediated Al^{3+} detoxification system is widely conserved among angiosperms (Liu et al. 2012; Liu et al. 2009; Sawaki et al. 2013; Tovkach et al. 2013; Wu et al. 2014; Yang et al. 2011; Yokosho et al. 2010, 2011).

The MATE transporter-associated processes by which plants adapt to Al^{3+} differ among species (Delhaize et al. 2012). SbMATE was localized to the major Al^{3+} tolerance locus, Alt_{5β}, in which a variety of genome polymorphisms was detected. One polymorphism was a repeat number of miniature inverted transposable elements (MITE) in a putative promoter region, with the repeat number of MITEs correlating with the level of expression of SbMATE, suggesting that these repeat insertions is required for Al^{3+} tolerance in sorghum (Magalhaes et al. 2007). Of the 21 polymorphism in the Alt_{5β} locus, nine, including the MITE region, were highly correlated with Al^{3+} tolerance (Caniato et al. 2014). Moreover, a haplotype network analysis suggested that all nine polymorphisms originated in West Africa.

One polymorphism, a 1-kb transposon insertion in a promoter region of the HvAACT1 gene, has been found to be involved in Al^{3+} tolerance. This insertion was found only in Al^{3+}-tolerant barley cultivars from East Asia, and functioned as a promoter, which enhanced the level of expression of HvAACT1 and spatially altered its expression to the root tips (Fujii et al. 2012). In the absence of the insertion, HvAACT1 expression was detected only in the central cylinder, and suppression of HvAACT1 expression caused leaf chlorosis in the presence of low Fe, suggesting that HvAACT1 originally functioned in Fe translocation by effluxing citrate into the xylem. A similar transposon insertion, 11.1-kb in length, has also been detected in the promoter region of TaMATE1b. This insertion extended the expression of TaMATE1b from root pericycles to root apices, enabling the release of higher amounts of citrate into rhizospheres. Because these insertions differ in length and location, they likely occurred independently in barley and wheat (Tovkach et al. 2013).

By contrast, the expression level of ZmMATE1 is associated with the copy number of the ZmMATE1 gene in the maize genome. Three tandemly arrayed ZmMATE1s were detected in three Al^{3+} tolerant cultivars, all of which originated in tropical regions of South America (Maron et al. 2013). A genetic polymorphism may also be present in rice OsFRDL4, because its level of expression was found to correlate with citrate secretion and Al^{3+} tolerance among cultivars (Yokosho et al. 2011).

The transport activities of MATE transporters are also regulated in several ways. BoMATE, HvAACT1, OsFRDL4, and SbMATE require Al^{3+} to activate citrate efflux (Furukawa et al. 2007; Magalhaes et al. 2007; Wu et al. 2014; Yokosho et al. 2011), whereas VuMATE1 requires phosphorylation for its activation (Liu et al. 2013). These differences in the regulation of MATE transporters, at both the transcriptional and post-transcriptional levels, indicated the existence of multiple evolutionary processes of MATE transporters that enabled plants to acquire Al^{3+} tolerance. It will be interesting to determine whether these differences in regulation mechanisms correlate with differences in environmental conditions.

Al^{3+} tolerance was recently investigated in three transgenic barley lines, which express AtFRD3, HvAACT1, or SbMATE under the regulation of a constitutive maize ubiquitin promoter (Zhou et al. 2013; Zhou et al. 2014). All three transgenic barley plants showed a more Al^{3+} tolerant phenotype than control plants, with the levels of Al^{3+} tolerance of the three transgenic lines being almost identical (Zhou et al. 2014). These results indicate that AtFRD3, which was originally reported to function in Fe translocation, may also be involved in Al^{3+} tolerance when the regulation of AtFRD3 is altered, e.g. by insertion of transposons in the promoter region, as in HvAACT1 and TaMATE1b (Fujii et al. 2012; Tovkach et al. 2013). A comparison of the three transgenic barley lines with barley expressing TaALMT1 showed that TaALMT1 conferred a much greater Al^{3+} tolerant phenotype than AtFRD3, HvAACT1, or SbMATE (Zhou et al. 2014). TaALMT1 is an Al^{3+}-activated malate channel localized to the plasma membranes of root cells, and is primarily responsible for Al^{3+} tolerance in wheat (Delhaize et al. 2004; Sasaki et al. 2004). Because all four transporters were regulated by the same ubiquitin promoter, this result indicated that TaALMT1 was the protein most able to confer Al^{3+} tolerance in the test conditions. The relationships between MATE and ALMT, especially regarding Al^{3+} toxicity and their evolutionary development, must be further clarified to better understand Al^{3+} detoxification mechanisms. Indeed, further investigations of individual MATE transporters are also necessary.

Plant hormone signaling

Recent analyses have revealed that MATE transporters are also involved in plant hormone signaling. The Arabidopsis activation tagging mutant adp1-D (altered development program 1-Dominant), which overexpresses a MATE transporter, AtADP1, was found to display various phenotypes, including accelerated growth rate of rosette leaves, early flowering and increased numbers of lateral roots (Li et al. 2014). This phenotype was caused by reductions in the level of indole-3-acetic acid (IAA) due to the suppression of auxin biosynthetic
genes in meristematic regions, in which AtADP1 is expressed. A quadruple mutant, in which AtADP1 and its putative functional paralogs (At5g19700, At2g38510 and At5g52050) were simultaneously down-regulated, showed growth retardation, a lower number of lateral organs and slightly elevated auxin levels, suggesting that AtADP1 and its paralogous MATE transporters maintain plant architecture by regulating local auxin biosynthesis (Li et al. 2014). A MATE transporter identical to AtADS1 has also been associated with plant disease resistance (Sun et al. 2011). The activated disease susceptibility1-Dominant (ads1-D) gene was found to be a mutant that increases susceptibility to various pathogens, such as Pseudomonas syringae pv. tomato (Pst) DC3000 and P. syringae pv. phaseolicola (Psp) NPS3121. This overexpression mutant was found to have a reduced salicylic acid (SA) level. In contrast, depletion of ads1/adp1 resulted in enhanced basal disease resistance. These findings suggested that AtADS1/AtADP1 is a negative regulator of plant disease resistance that acts through the modulation of SA accumulation and SA-dependent signaling (Sun et al. 2011). The relationships of these phenotypes caused by AtADS1/AtADP1 will be determined when its native transport substrate is identified.

SA is a plant hormone that plays a crucial role in plant defenses against pathogens (Boatwright and Pajerowska-Mukhtar 2013). The Arabidopsis enhanced disease susceptibility 5 (eds5) mutant displayed impaired SA accumulation and reduced basal disease resistance (Nawrath et al. 2002). In contrast, overexpression of AtEDS5 in Arabidopsis enhanced SA accumulation and resistance to viruses (Ishihara et al. 2008). Recent genetic and biochemical analyses showed that this MATE transporter functioned in epidermal cells by exporting SA from its site of biosynthesis in the chloroplast to the cytosol (Serrano et al. 2013; Yamasaki et al. 2013). This intracellular SA transport by AtEDS5, leading to SA accumulation, plays an essential role in plant innate immune signaling and in disease resistance.

More recently, a comprehensive mutant analysis of all Arabidopsis MATE members found that AtDTX50 acts as an abscisic acid (ABA) efflux transporter (Zhang et al. 2014). Greater ABA accumulation in leaves, and higher drought tolerance with lower stomatal conductance, were observed in atdtx50 mutants. The efflux activity of ABA was confirmed using three different systems, E. coli, Xenopus oocytes, and atdtx50 mutant protoplasts, indicating that AtDTX50 functions as an ABA regulator in guard cells. Two ABCs, AtABCG25 and AtABCG40, and the nitrate transporter AtAAT1 have been identified as ABA transporters, with each transporter showing distinct physiological functions in ABA signaling (Kang et al. 2010; Kanno et al. 2012; Kuromori et al. 2010). Moreover, abscisic acid glucosyl ester, a conjugate of ABA, was found to accumulate in Arabidopsis vacuoles by a mechanism involving proton antiporters, which may be MATE transporters (Burla et al. 2013). Additional new transporters involved in local ABA movement may be identified in the future.

Other physiological functions

Plant MATE transporters also function in other physiological roles, including in plant development and phosphorus (P) acquisition. Screening of enhancer trap mutants identified an Arabidopsis MATE transporter ZRZ (‘zariz’ means agile in Hebrew) as having an altered developmental phenotype (Burko et al. 2011). Overexpression of ZRZ in initiating leaves resulted in reduced apical dominance, early flowering, and a bushy phenotype, together with a dramatically increased leaf initiation rate. Another screening of overexpressing mutant lines identified this ZRZ gene as BCD1 (bush-and chorotic-dwarf 1) (Seo et al. 2012). In addition to the phenotype observed in atzrz-overexpressing mutants, bcd1 mutants exhibited pale green leaves with reductions in chlorophyll and Fe contents. This phenotype was rescued by Fe feeding, and excess Fe induced the expression of AtBCD1, indicating the involvement of AtBCD1 in the maintenance of cellular Fe homeostasis by secreting excess Fe (Seo et al. 2012). Future studies seek to characterize its transport substrate(s) and biochemical transport function.

Morphological changes resulting from the mutation of MATE genes or the overexpression of MATE transporters have also been observed in transgenic Arabidopsis plants. OsMATE1 and OsMATE2 are genes up-regulated by arsenic treatment, with relatively low amino acid sequence identity with each other (36%) and belonging to different phylogenetic clades (Figure 1) (Tiwari et al. 2014). Overexpression of either OsMATE1 or OsMATE2 in Arabidopsis plants resulted in pleiotropic phenotypes, such as longer leaf size, early flowering, and enhanced susceptibility to PstDC3000. In addition, various Arabidopsis genes involved in developments, circadian clocks, and defense responses were modulated. Both OsMATEs localize to the plasma membranes of shoot apex and reproductive organs. These results indicate that OsMATEs are involved in plant growth and development, and negatively regulate disease resistance (Tiwari et al. 2014).

In P deficient conditions, white lupin (Lupinus albus) was found to develop cluster roots, in which a MATE transporter, LaMATE, was highly expressed (Uhde-Stone et al. 2005). Findings showing that Lupinus releases high amounts of malate and citrate under P stress (Cheng et al. 2011), and that LaMATE shows high sequence similarity with citrate-transporting MATEs (Figure 1), suggested that LaMATE functions as a citrate efflux carrier in cluster roots. However, LaMATE could not
complement an atfrd3 mutant with a native LaMATE promoter (Uhde-Stone et al. 2005).

**Conclusion and future indications**

Genetic and biochemical approaches have revealed the high diversity of plant MATE transporters. Despite their importance in plant development and environmental adaptation, there have been few comprehensive reviews of plant MATEs (Magalhaes 2010). The present review provides a current inventory of plant MATE transporters, including their physiological functions.

Molecular evolution of plant MATE transporters is of high interest. Some MATE transporters, such as AtZRZ/AtBCD1, AtADP1/AtADS1, OsMATE1 and OsMATE2, were identified and characterized due mainly to their overexpression (Burko et al. 2011; Li et al. 2014; Seo et al. 2012; Sun et al. 2011; Tiwari et al. 2014). The phenotypes of single knockout mutants of AtZRZ/AtBCD1 and AtADP1/AtADS1 were similar to the phenotype of wild-type plants, indicating that several MATE transporters function redundantly, like ABC proteins (Yazaki et al. 2009). Similarly, OsFRDL1 and OsPEZ1 play the same physiological role in Fe translocation with different transport substrates (Ishimaru et al. 2011; Yokosho et al. 2009), and ZmMATE1 and ZmMATE2 display a similar functional redundancy in Al³⁺ detoxification in maize, by transporting different substrates (Maron et al. 2010). Moreover, HvAAC1T1, TaMATE1b and probably OsPEZ2 are involved in two different physiological functions, Fe translocation and Al³⁺ detoxification, with a single transport substrate (Bashir et al. 2011; Fujii et al. 2012; Tovkach et al. 2013), indicating that plant MATE transporters are often recruited for environmental adaptation due to the broad utility of their transport substrates. As shown by genetic association studies of HvAAC1T1, ShMATE, and ZmMATE1, the evolutionary processes of other MATE transporters will be also revealed using collections of both wild plants and cultivars (Caniato et al. 2014; Fujii et al. 2012; Maron et al. 2013). A recently identified MATE transporter in *L. japonicus* showed expression patterns in wild accessions that seemed to correlate with the latitude of the sampled location (our unpublished data). Future studies will reveal additional novel physiological roles as well as environmental adaptation strategies by plant MATE transporters, which will contribute to a better understanding of this large transporter family in plants.

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**References**


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ALF5, a al. (2009) Grapevine MATE-type proteins act as vacuolar H+-anthoMATE transporters and GST.

Plant J

Ageorges A (2011) grapevine anthocyanin transport In vivo

Plant Cell Physiol


Maron LG, Pineros MA, Guimaraes CT, Magalhaes JV, Pleiman with the ALMT1 gene. Proc Natl Acad Sci USA 101: 15249–15254


Rogers EE, Guerinot ML (2002) FRD3, a member of the multidrug and toxin efflux family, controls iron deficiency responses in Arabidopsis. *Plant Cell* 14: 1787–1799


