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Review

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 (Omote 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

Abbreviations: ABA, abscisic acid; ABC, ATP-binding cassette; ALMT, aluminum-activated malate transporter; Cy3G, cyanidin-3-*O*-glucoside; E3′G, epicatechin 3′-*O*-glucoside; MATE, multidrug and toxic compound extrusion; MIA, monoterpene indole alkaloid; MITE, miniature inverted transposable elements; SA, salicylic acid.

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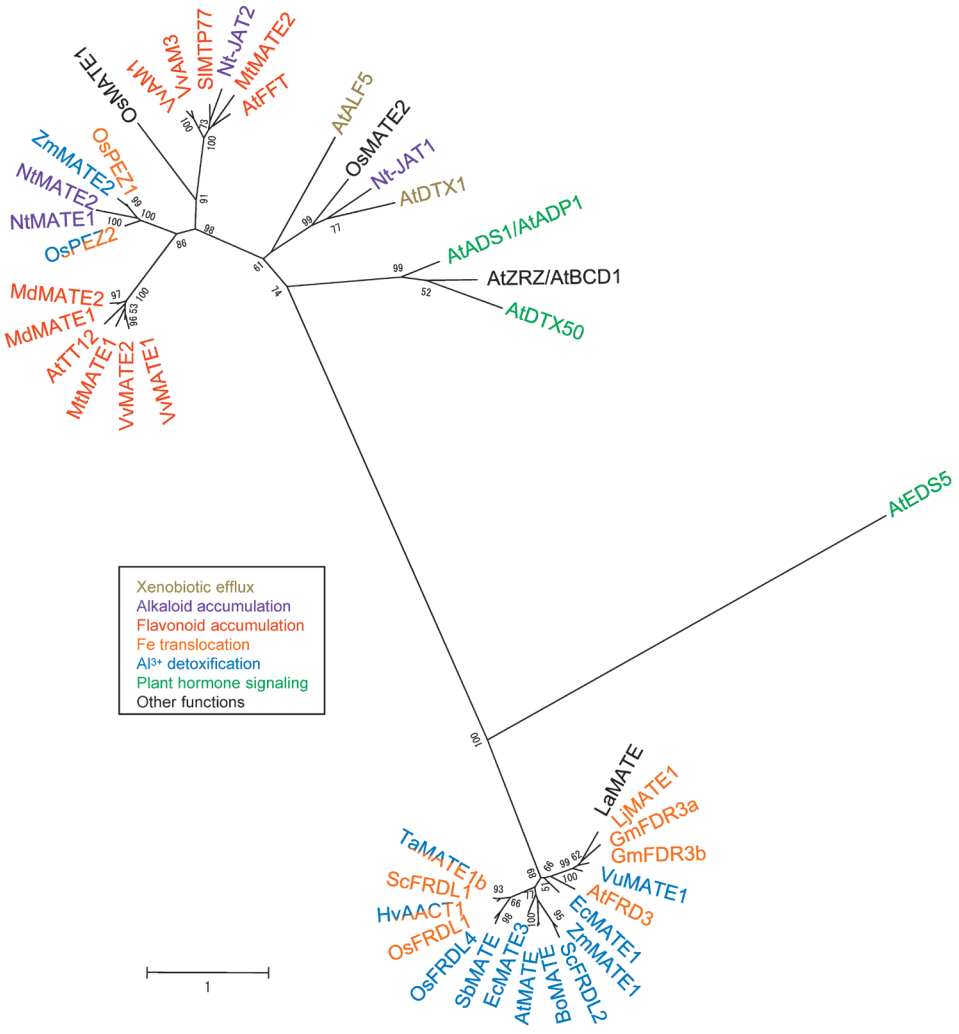

Figure 1. A phylogenetic analysis of plant MATE transporters. Reported plant MATE transporter sequences were aligned using the ClustalW program. The maximum likelihood method was used to construct a phylogenetic tree with 1,000 bootstrap replicates using MEGA 6.0 software (Tamura et al. 2013). MATE transporters were color-coded according to their physiological functions.

the substrate in a direction opposite that of Na^+ or H^+, 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 *AtDTX1* gene was found to confer resistance to norfloxacin during functional screening with *E. coli* (Li et al. 2002). Functional analysis using *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, 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 AtALF5. Mutations in the *AtALF5* gene lead to defects in lateral root formation, and increase the sensitivity of roots to various compounds. Physiologically, AtALF5, like AtDTX1, is thought to efflux xenobiotics, although the transport substrate of 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.

Gene name	AGI code/accession No.	Plant species	Tissue expression	Subcellurar localization	Function	Transport substrate	Driving force	References
AtALF5	At3g23560	*Arabidopsis thaliana*	Root		Xenobiotics efflux	Tetramethylammonium		Diener et al. (2001)
AtDTX1	At2g04070	*Arabidopsis thaliana*	Flower, Shoot	PM (GFP)	Xenobiotics efflux	Norfloxacin, Ethidium bromide, Berberine, Palmatine		Li et al. (2002)
NtMATE1	AB286961	*Nicotiana tabacum*	Root	VM (GFP, MF, IM)	Nicotine accumulation	Nicotine	H^+	Shoji et al. (2009)
NtMATE2	AB286963	*Nicotiana tabacum*	Root	VM (MF, IM)	Nicotine accumulation	Nicotine	H^+	Shoji et al. (2009)
Nt-JAT1	AM991692	*Nicotiana tabacum*	Leaf, Stem, Root	VM (MF)	Nicotine accumulation	Nicotine	H^+	Morita et al. (2009)
Nt-JAT2	AB922128	*Nicotiana tabacum*	Leaf	VM (GFP)	Nicotine accumulation	Nicotine		Shitan et al. (2014b)
AtFFT	At4g25640	*Arabidopsis thaliana*	Flower	VM (Proteome)	Flavonoid accumulation?			Thompson et al. (2010)
AtTT12	At3g59030	*Arabidopsis thaliana*	Seed	VM (GFP)	Proanthocyanidin accumulation	Cyanidin-3-*O*-glucoside Epicatechin 3′-*O*-glucoside	H^+	Debeaujon et al. (2001), Marinova et al. (2007) Zhao and Dixon (2009)
SlMTP77	BE354224	*Solanum lycopersicum*			Flavonoid accumulation?			Mathews et al. (2003)
MdMATE1	GU064954	*Malus*×*domestica* Borkh	Fruit		Proanthocyanidin accumulation			Frank et al. (2011)
MdMATE2	GU064956	*Malus*×*domestica* Borkh	Fruit		Proanthocyanidin accumulation			Frank et al. (2011)
MtMATE1	FJ858726	*Medicago truncatula*	Seed	VM (GFP)	Proanthocyanidin accumulation	Cyanidin-3-*O*-glucoside Epicatechin 3′-*O*-glucoside	H^+	Zhao and Dixon (2009)
MtMATE2	HM856605	*Medicago truncatula*	Flower, Leaf	VM (GFP)	Flavonoid accumulation	Malonylated flavonoid glucosides	H^+	Zhao et al. (2011)
VvAM1	FJ264202	*Vitis vinifera*	Fruit skin	VM (GFP) Small vesicle (GFP)	Anthocyanin accumulation	Acylated anthocyanins	H^+	Gomez et al. (2009), Gomez et al. (2011)
VvAM3	FJ264203	*Vitis vinifera*	Fruit skin	VM (GFP) Small vesicle (GFP)	Anthocyanin accumulation	Acylated anthocyanins	H^+	Gomez et al. (2009), Gomez et al. (2011)
VvMATE1	GSVIVP00018839001*	*Vitis vinifera*		VM (GFP)	Proanthocyanidin accumulation?			Pérez-Díaz et al. (2014)
VvMATE2	GSVIVP00018841001*	*Vitis vinifera*		Golgi(GFP)	Proanthocyanidin accumulation?			Pérez-Díaz et al. (2014)
AtFRD3	At3g08040	*Arabidopsis thaliana*	Flower, Seed, Root		Fe translocation	Citrate		Rogers and Guerinot (2002), Green and Rogers (2004) Durrett et al. (2007), Roschzttardtz et al. (2011)
GmFRD3a	EU591739	*Glycine max*	Root		Fe translocation	Citrate		Rogers et al. (2009)
GmFRD3b	EU591741	*Glycine max*	Root		Fe translocation	Citrate		Rogers et al. (2009)
LjMATE1	AB649311	*Lotus japonicus*	Nodule		Fe translocation	Citrate		Takanashi et al. (2013)
OsFRDL1	AK101556	*Oryza sativa*	Root	PM (GFP)	Fe translocation	Citrate		Inoue et al. (2004), Yokosho et al. (2009)
OsPEZ1	AK243209	*Oryza sativa*	Root	PM (GFP)	Fe translocation	Protocatechuic acid		Ishimaru et al. (2011)
OsPEZ2	AK102204	*Oryza sativa*	Root	PM (GFP)	Fe translocation/ Al^{3+} detoxification	Protocatechuic acid		Bashir et al. (2011)
ScFRDL1	AB571881	*Secale cereale*	Root		Fe translocation	Citrate		Yokosho et al. (2010)
AtMATE	At1g51340	*Arabidopsis thaliana*	Root		Al^{3+} detoxification	Citrate		Liu et al. (2009), Liu et al. (2012)
BoMATE	KF031944	*Brassica oleracea*	Root	PM (GFP)	Al^{3+} detoxification	Citrate		Wu et al. (2014)
EcMATE1	AB725912	*Eucalyptus camaldulensis*	Root	PM (GFP)	Al^{3+} detoxification	Citrate		Sawaki et al. (2013)
EcMATE3	AB725914	*Eucalyptus camaldulensis*	Root	PM (GFP)	Al^{3+} detoxification	Citrate		Sawaki et al. (2013)
HvAACT1	AB302223	*Hordeum vulgare*	Root	PM (GFP)	Al^{3+} detoxification/ Fe translocation	Citrate		Furukawa et al. (2007), Fujii et al. (2012) Zhou et al. (2013)
OsFRDL4	AB608020	*Oryza sativa*	Root	PM (GFP)	Al^{3+} detoxification	Citrate		Yokosho et al. (2011)
SbMATE	EF611342	*Sorghum bicolor*	Shoot, Root	PM (GFP)	Al^{3+} detoxification	Citrate		Magalhaes et al. (2007), Sivaguru et al. (2013) Caniato et al. (2014)
ScFRDL2	AB571882	*Secale cereale*	Root		Al^{3+} detoxification	Citrate		Yokosho et al. (2010)
TaMATE1b	KC152457	*Triticum aestivum*	Root, Shoot	PM (GFP)	Al^{3+} detoxification/ Fe translocation	Citrate	H^+	Tovkach et al. (2013)
VuMATE1	KM090855	*Vigna umbellata*	Root	PM (GFP)	Al^{3+} detoxification	Citrate	H^+	Yang et al. (2011), Liu et al. (2013)
ZmMATE1	FJ015155	*Zea mays*	Root	PM (GFP)	Al^{3+} detoxification	Citrate	H^+	Maron et al. (2010), Maron et al. (2013)
ZmMATE2	FJ873684	*Zea mays*	Root	PM (GFP)	Al^{3+} detoxification	Anion		Maron et al. (2010), Maron et al. (2013)
AtADS1/AtADP1	At4g29140	*Arabidopsis thaliana*	Meristematic regions	Post-Golgi endomembrane (GFP)	IAA/SA signaling?			Sun et al. (2011), Li et al. (2014)
AtDTX50	At5g52050	*Arabidopsis thaliana*	Leaf	PM (GFP)	ABA signaling	ABA		Zhang et al. (2014)
AtEDS5	At4g39030	*Arabidopsis thaliana*	Leaf	Chloroplast envelope (YFP)	SA signaling	SA		Nawrath et al. (2002), Ishihara et al. (2008) Serrano et al. (2013), Yamasaki et al. (2013)
AtZRZ/AtBCD1	At1g58340	*Arabidopsis thaliana*	Meristematic regions Shoot	MT? (GFP)	Organ initiation/Fe homeostasis			Burko et al. (2011), Seo et al. (2012)
LaMATE	AY631874	*Lupinus albus*	Root	PM (GFP)	P deficiency response			Uhde-Stone et al. (2005)
OsMATE1	AK242068	*Oryza sativa*		PM (GFP)	As response			Tiwari et al. (2014)
OsMATE2	AK073902	*Oryza sativa*		PM (GFP)	As response			Tiwari et al. (2014)

*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.

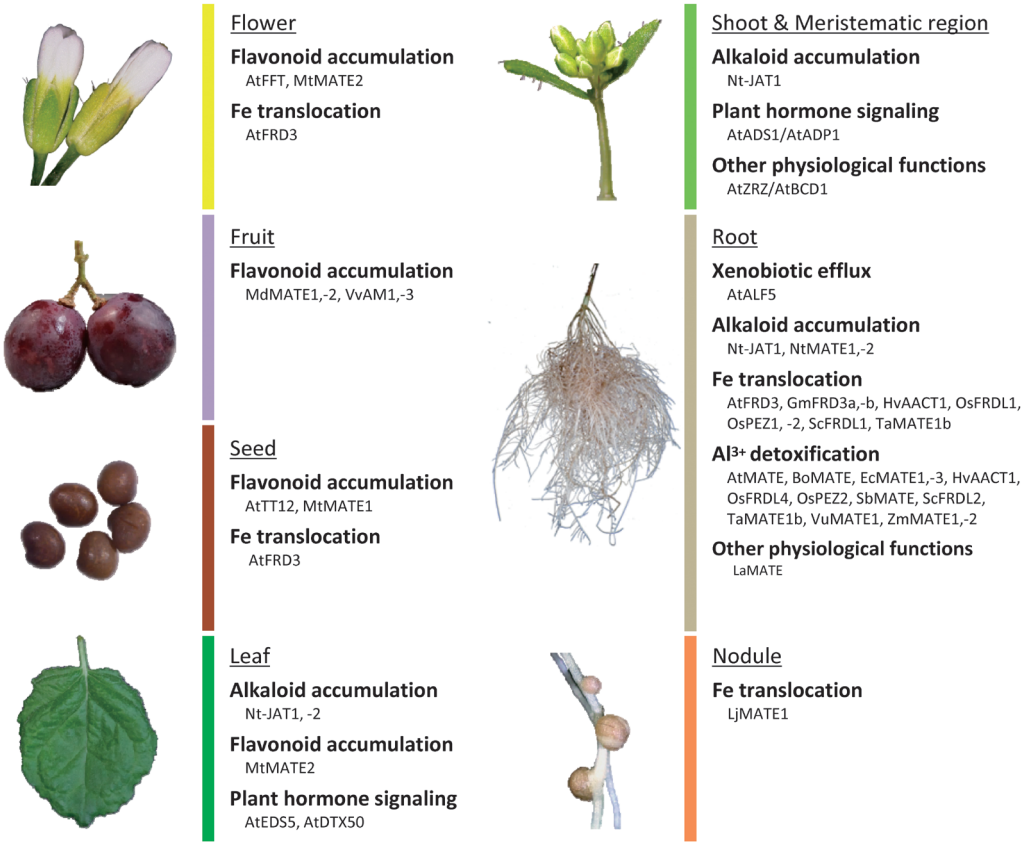

Figure 2. Tissue specificities of gene expression and physiological roles of plant MATE transporters. Each function is described in detail in the corresponding text.

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 AtDTX1 (49.6%) (Figure 1). NtMATE1 and NtMATE2 are closely related, having 96.4% 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

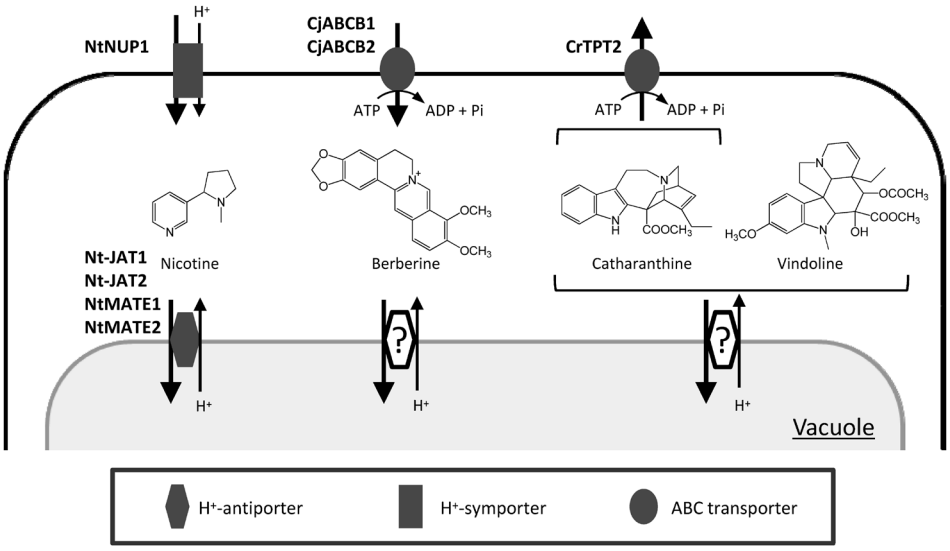

Figure 3. Plant alkaloids accumulated in vacuoles by MATE transporters. Nicotine accumulation is mediated by Nt-JAT1, Nt-JAT2, NtMATE1 and NtMATE2. MATE transporters may also be involved in the accumulation of berberine and two monoterpene indole alkaloids. Their transports across plasma membrane are mediated by members of the NUP and ABC transporter families.

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 Yazaki 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

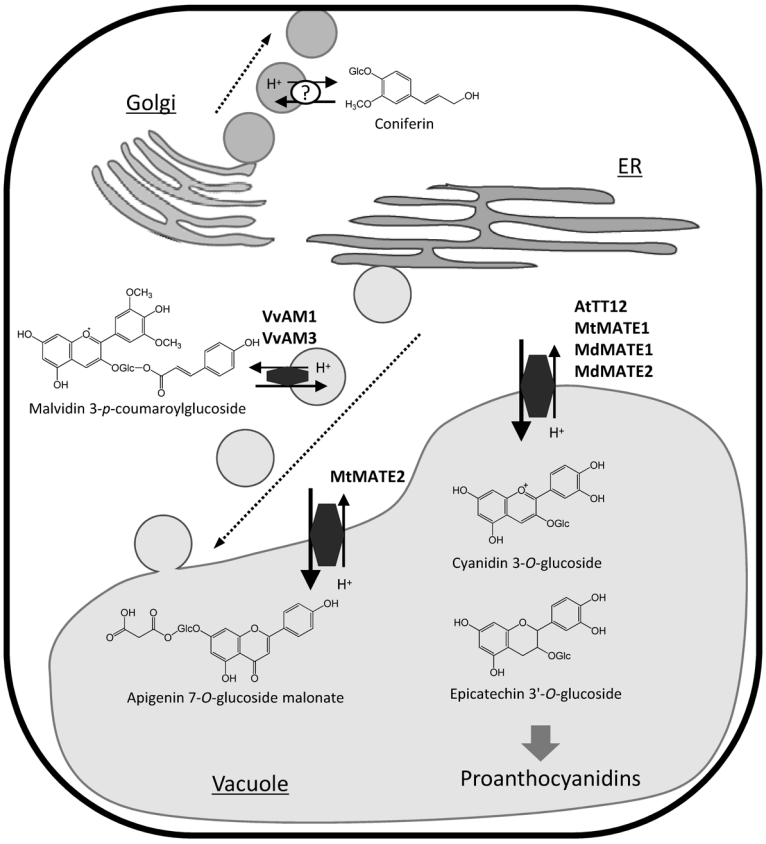

Figure 4. MATE transporters involved in flavonoid accumulation. AtTT12, MtMATE1 and MdMATEs transport protoanthocyanidin precursors, whereas MtMATE2 transports malonylated flavonoid glucosides. VvAMs load acylated anthocyanins into small vesicles around vacuoles. MATE may also load coniferin, a lignin precursor, into vesicles.

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 SlANT1, 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 (Figure 4) (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

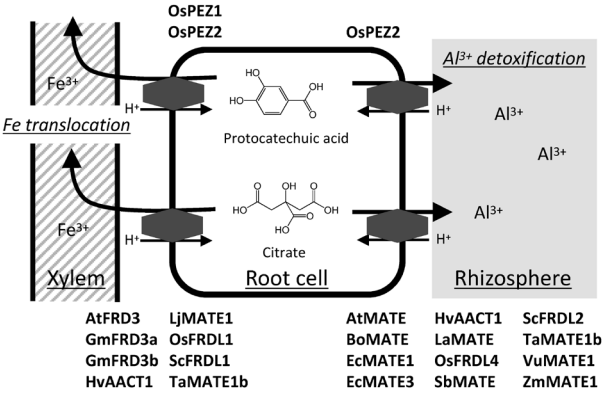

Figure 5. MATE transporters involved in Fe translocation and Al^{3+} detoxification. Fe translocation is mediated using citrate by AtFRD3, GmFRD3a, GmFRD3b, HvAACT1, LjMATE1, OsFRDL1, ScFRDL1, and TaMATE1b. Rice OsPEZ1 and OsPEZ2 also contribute to Fe translocation by effluxing protocatechuic acid. Al^{3+} detoxification is mediated by citrate release by AtMATE, BoMATE, EcMATE1, EcMATE3, HvAACT1, LaMATE, OsFRDL4, SbMATE, ScFRDL2, TaMATE1b, VuMATE1, and ZmMATE1. OsPEZ2 may also exude protocatechuic acid from root tissues for Al^{3+} detoxification.

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 (Roschzttardtz 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 (Roschzttardtz 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 HvAACT1 (*Hordeum vulgare* aluminum-activated citrate transporter1) and wheat TaMATE1b (*Triticum aestivum* MATE1b) from studies of Al^{3+} detoxification mechanisms (see "Al^{3+} 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 Al^{3+} tolerance, with the other being ZmMATE1 (see "Al^{3+} detoxification") (Maron et al. 2010). Al^{3+} treatment of maize was found to activate the release of phenolic compounds from the roots that may function as Al^{3+} chelators (Kidd et al. 2001), suggesting that ZmMATE2 as well as OsPEZ2 may be implicated in Al^{3+} tolerance by effluxing phenolic compounds into the rhizosphere (Figure 5).

Al^{3+} detoxification

Al^{3+}, 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 Al^{3+} sensitive plants, Al^{3+} tolerant plants can efflux high amounts of organic acids, which protect roots by chelating Al^{3+} 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 Al^{3+} 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 Al^{3+} detoxification were first identified in sorghum (*Sorghum bicolor*; SbMATE) and barley (HvAACT1) 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 Al^{3+}, indicating that high expression of these MATE transporters was necessary for Al^{3+} 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 Al^{3+} 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 Al^{3+} 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_{SB}, 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_{SB} 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 comparisn 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 AtAIT1 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 *Pst*DC3000. 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 was 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 Al^{3+} detoxification in maize, by transporting different substrates (Maron et al. 2010). Moreover, HvAACT1, TaMATE1b and probably OsPEZ2 are involved in two distinct physiological functions, Fe translocation and Al^{3+} 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 *HvAACT1*, *SbMATE*, 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

Alejandro S, Lee Y, Tohge T, Sudre D, Osorio S, Park J, Bovet L, Geldner N, Fernie AR, Martinoia E (2012) AtABCG29 is a monolignol transporter involved in lignin biosynthesis. *Curr Biol* 22: 1207–1212

Bashir K, Ishimaru Y, Shimo H, Kakei Y, Senoura T, Takahashi R, Sato Y, Sato Y, Uozumi N, Nakanishi H, et al. (2011) Rice phenolics efflux transporter 2 (PEZ2) plays an important role in solubilizing apoplasmic iron. *Soil Sci Plant Nutr* 57: 803–812

Boatwright JL, Pajerowska-Mukhtar K (2013) Salicylic acid: An old hormone up to new tricks. *Mol Plant Pathol* 14: 623–634

Brown MH, Paulsen IT, Skurray RA (1999) The multidrug efflux protein NorM is a prototype of a new family of transporters. *Mol Microbiol* 31: 394–395

Burko Y, Geva Y, Refael-Cohen A, Shleizer-Burko S, Shani E, Berger Y, Halon E, Chuck G, Moshelion M, Ori N (2011) From organelle to organ: ZRIZI MATE-Type transporter is an organelle transporter that enhances organ initiation. *Plant Cell Physiol* 52: 518–527

Burla B, Pfrunder S, Nagy R, Francisco RM, Lee Y, Martinoia E (2013) Vacuolar transport of abscisic acid glucosyl ester is mediated by ATP-binding cassette and proton-antiport mechanisms in Arabidopsis. *Plant Physiol* 163: 1446–1458

Caniato FF, Hamblin MT, Guimaraes CT, Zhang Z, Schaffert RE, Kochian LV, Magalhaes JV (2014) Association mapping provides insights into the origin and the fine structure of the sorghum aluminum tolerance locus, Alt_{SB}. *PLoS ONE* 9: e87438

Carqueijeiro I, Noronha H, Duarte P, Geros H, Sottomayor M (2013) Vacuolar transport of the medicinal alkaloids from *Catharanthus roseus* is mediated by a proton-driven antiport. *Plant Physiol* 162: 1486–1496

Cheng L, Bucciarelli B, Shen J, Allan D, Vance CP (2011) Update on lupin cluster roots. Update on white lupin cluster root acclimation to phosphorus deficiency. *Plant Physiol* 156: 1025–1032

Conn S, Curtin C, Bezier A, Franco C, Zhang W (2008) Purification, molecular cloning, and characterization of glutathione *S*-transferases (GSTs) from pigmented *Vitis vinifera* L. cell suspension cultures as putative anthocyanin transport proteins. *J Exp Bot* 59: 3621–3634

Croteau R, Kutchan T, Lewis N (2000) Natural Products (Secondary Metabolites). In: Buchanan B, Gruissem W, Jones R (eds) *Biochemistry & Molecular Biology of Plants*. American Society of Plant Physiologists, Maryland, pp 1250–1318

Cutanda-Perez MC, Ageorges A, Gomez C, Vialet S, Terrier N, Romieu C, Torregrosa L (2009) Ectopic expression of VlmybA1 in grapevine activates a narrow set of genes involved in anthocyanin synthesis and transport. *Plant Mol Biol* 69: 633–648

Debeaujon I, Peeters AJM, Leon-Kloosterziel KM, Koornneef M (2001) The *TRANSPARENT TESTA12* gene of Arabidopsis encodes a multidrug secondary transporter-like protein required for flavonoid sequestration in vacuoles of the seed coat endothelium. *Plant Cell* 13: 853–871

Delhaize E, Ma JF, Ryan PR (2012) Transcriptional regulation of aluminium tolerance genes. *Trends Plant Sci* 17: 341–348

Delhaize E, Ryan PR, Hebb DM, Yamamoto Y, Sasaki T, Matsumoto H (2004) Engineering high-level aluminum tolerance in barley

with the *ALMT1* gene. *Proc Natl Acad Sci USA* 101: 15249–15254

Diener AC, Gaxiola RA, Fink GR (2001) Arabidopsis *ALF5*, a multidrug efflux transporter gene family member, confers resistance to toxins. *Plant Cell* 13: 1625–1638

Durrett TP, Gassmann W, Rogers EE (2007) The FRD3-mediated efflux of citrate into the root vasculature is necessary for efficient iron translocation. *Plant Physiol* 144: 197–205

Falcone Ferreyra ML, Rius SP, Casati P (2012) Flavonoids: Biosynthesis, biological functions, and biotechnological applications. *Front Plant Sci* 3: 222

Frank S, Keck M, Sagasser M, Niehaus K, Weisshaar B, Stracke R (2011) Two differentially expressed *MATE* factor genes from apple complement the Arabidopsis *transparent testa12* mutant. *Plant Biol* 13: 42–50

Fujii M, Yokosho K, Yamaji N, Saisho D, Yamane M, Takahashi H, Sato K, Nakazono M, Ma JF (2012) Acquisition of aluminium tolerance by modification of a single gene in barley. *Nat Commun* 3: 713

Furukawa J, Yamaji N, Wang H, Mitani N, Murata Y, Sato K, Katsuhara M, Takeda K, Ma JF (2007) An aluminum-activated citrate transporter in barley. *Plant Cell Physiol* 48: 1081–1091

Gomez C, Conejero G, Torregrosa L, Cheynier V, Terrier N, Ageorges A (2011) *In vivo* grapevine anthocyanin transport involves vesicle-mediated trafficking and the contribution of anthoMATE transporters and GST. *Plant J* 67: 960–970

Gomez C, Terrier N, Torregrosa L, Vialet S, Fournier-Level A, Verries C, Souquet JM, Mazauric JP, Klein M, Cheynier V, et al. (2009) Grapevine MATE-type proteins act as vacuolar H^+-dependent acylated anthocyanin transporters. *Plant Physiol* 150: 402–415

Green LS, Rogers EE (2004) *FRD3* controls iron localization in Arabidopsis. *Plant Physiol* 136: 2523–2531

Hildreth SB, Gehman EA, Yang H, Lu RH, Ritesh KC, Harich KC, Yu S, Lin J, Sandoe JL, Okumoto S, et al. (2011) Tobacco nicotine uptake permease (NUP1) affects alkaloid metabolism. *Proc Natl Acad Sci USA* 108: 18179–18184

Inoue H, Mizuno D, Takahashi M, Nakanishi H, Mori S, Nishizawa NK (2004) A rice FRD3-like (*OsFRDL1*) gene is expressed in the cells involved in long-distance transport. *Soil Sci Plant Nutr* 50: 1133–1140

Ishihara T, Sekine KT, Hase S, Kanayama Y, Seo S, Ohashi Y, Kusano T, Shibata D, Shah J, Takahashi H (2008) Overexpression of the *Arabidopsis thaliana EDS5* gene enhances resistance to viruses. *Plant Biol* 10: 451–461

Ishimaru Y, Kakei Y, Shimo H, Bashir K, Sato Y, Sato Y, Uozumi N, Nakanishi H, Nishizawa NK (2011) A rice phenolic efflux transporter is essential for solubilizing precipitated apoplasmic iron in the plant stele. *J Biol Chem* 286: 24649–24655

Jaquinod M, Villiers F, Kieffer-Jaquinod S, Hugouvieux V, Bruley C, Garin J, Bourguignon J (2007) A proteomics dissection of *Arabidopsis thaliana* vacuoles isolated from cell culture. *Mol Cell Proteomics* 6: 394–412

Kaneda M, Rensing KH, Wong JC, Banno B, Mansfield SD, Samuels AL (2008) Tracking monolignols during wood development in lodgepole pine. *Plant Physiol* 147: 1750–1760

Kang J, Hwang JU, Lee M, Kim YY, Assmann SM, Martinoia E, Lee Y (2010) PDR-type ABC transporter mediates cellular uptake of the phytohormone abscisic acid. *Proc Natl Acad Sci USA* 107: 2355–2360

Kanno Y, Hanada A, Chiba Y, Ichikawa T, Nakazawa M, Matsui M, Koshiba T, Kamiya Y, Seo M (2012) Identification of an abscisic acid transporter by functional screening using the receptor complex as a sensor. *Proc Natl Acad Sci USA* 109: 9653–9658

Kidd PS, Llugany M, Poschenrieder C, Gunse B, Barcelo J (2001) The role of root exudates in aluminium resistance and silicon-induced amelioration of aluminium toxicity in three varieties of maize (*Zea mays* L.). *J Exp Bot* 52: 1339–1352

Kobayashi T, Nishizawa NK (2012) Iron uptake, translocation, and regulation in higher plants. *Annu Rev Plant Biol* 63: 131–152

Kochian LV, Hoekenga OA, Pineros MA (2004) How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorous efficiency. *Annu Rev Plant Biol* 55: 459–493

Kuroda T, Tsuchiya T (2009) Multidrug efflux transporters in the MATE family. *Biochim Biophys Acta* 1794: 763–768

Kuromori T, Miyaji T, Yabuuchi H, Shimizu H, Sugimoto E, Kamiya A, Moriyama Y, Shinozaki K (2010) ABC transporter AtABCG25 is involved in abscisic acid transport and responses. *Proc Natl Acad Sci USA* 107: 2361–2366

Lepiniec L, Debeaujon I, Routaboul JM, Baudry A, Pourcel L, Nesi N, Caboche M (2006) Genetics and biochemistry of seed flavonoids. *Annu Rev Plant Biol* 57: 405–430

Li L, He Z, Pandey GK, Tsuchiya T, Luan S (2002) Functional cloning and characterization of a plant efflux carrier for multidrug and heavy metal detoxification. *J Biol Chem* 277: 5360–5368

Li R, Li J, Li S, Qin G, Novak O, Pencik A, Ljung K, Aoyama T, Liu J, Murphy A, et al. (2014) *ADP1* affects plant architecture by regulating local auxin biosynthesis. *PLoS Genet* 10: e1003954

Liu J, Luo X, Shaff J, Liang C, Jia X, Li Z, Magalhaes J, Kochian LV (2012) A promoter-swap strategy between the AtALMT and AtMATE genes increased Arabidopsis aluminum resistance and improved carbon-use efficiency for aluminum resistance. *Plant J* 71: 327–337

Liu J, Magalhaes JV, Shaff J, Kochian LV (2009) Aluminum-activated citrate and malate transporters from the MATE and ALMT families function independently to confer Arabidopsis aluminum tolerance. *Plant J* 57: 389–399

Liu MY, Chen WW, Xu JM, Fan W, Yang JL, Zheng SJ (2013) The role of *VuMATE1* expression in aluminium-inducible citrate secretion in rice bean (*Vigna umbellata*) roots. *J Exp Bot* 64: 1795–1804

Ma JF, Ryan PR, Delhaize E (2001) Aluminium tolerance in plants and the complexing role of organic acids. *Trends Plant Sci* 6: 273–278

Magalhaes JV (2010) 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. *Ann Bot* (Lond) 106: 199–203

Magalhaes JV, Liu J, Guimaraes CT, Lana UGP, Alves VMC, Wang YH, Schaffert RE, Hoekenga OA, Pineros MA, Shaff JE, et al. (2007) A gene in the multidrug and toxic compound extrusion (MATE) family confers aluminum tolerance in sorghum. *Nat Genet* 39: 1156–1161

Marinova K, Pourcel L, Weder B, Schwarz M, Barron D, Routaboul JM, Debeaujon I, Klein M (2007) The *Arabidopsis* MATE transporter TT12 acts as a vacuolar flavonoid/H^+-antiporter active in proanthocyanidin-accumulating cells of the seed coat. *Plant Cell* 19: 2023–2038

Maron LG, Guimaraes CT, Kirst M, Albert PS, Birchler JA, Bradbury PJ, Buckler ES, Coluccio AE, Danilova TV, Kudrna D, et al. (2013) Aluminum tolerance in maize is associated with higher *MATE1* gene copy number. *Proc Natl Acad Sci USA* 110: 5241–5246

Maron LG, Pineros MA, Guimaraes CT, Magalhaes JV, Pleiman

JK, Mao C, Shaff J, Belicuas SNJ, Kochian LV (2010) Two functionally distinct members of the MATE (multi-drug and toxic compound extrusion) family of transporters potentially underlie two major aluminum tolerance QTLs in maize. *Plant J* 61: 728–740

Mathews H, Clendennen SK, Caldwell CG, Liu XL, Connors K, Matheis N, Schuster DK, Menasco DJ, Wagoner W, Lightner J, et al. (2003) Activation tagging in tomato identifies a transcriptional regulator of anthocyanin biosynthesis, modification, and transport. *Plant Cell* 15: 1689–1703

Miao YC, Liu CJ (2010) ATP-binding cassette-like transporters are involved in the transport of lignin precursors across plasma and vacuolar membranes. *Proc Natl Acad Sci USA* 107: 22728–22733

Morita M, Shitan N, Sawada K, Van Montagu MCE, Inze D, Rischer H, Goossens A, Oksman-Caldentey KM, Moriyama Y, Yazaki K (2009) Vacuolar transport of nicotine is mediated by a multidrug and toxic compound extrusion (MATE) transporter in *Nicotiana tabacum*. *Proc Natl Acad Sci USA* 106: 2447–2452

Morita Y, Kodama K, Shiota S, Mine T, Kataoka A, Mizushima T, Tsuchiya T (1998) NorM, a putative multidrug efflux protein, of *Vibrio parahaemolyticus* and its homolog in *Escherichia coli*. *Antimicrob Agents Chemother* 42: 1778–1782

Nawrath C, Heck S, Parinthawong N, Metraux JP (2002) EDS5, an essential component of salicylic acid-dependent signaling for disease resistance in Arabidopsis, is a member of the MATE transporter family. *Plant Cell* 14: 275–286

Nour-Eldin HH, Halkier BA (2013) The emerging field of transport engineering of plant specialized metabolites. *Curr Opin Biotechnol* 24: 263–270

Omote H, Hiasa M, Matsumoto T, Otsuka M, Moriyama Y (2006) The MATE proteins as fundamental transporters of metabolic and xenobiotic organic cations. *Trends Pharmacol Sci* 27: 587–593

Otani M, Shitan N, Sakai K, Martinoia E, Sato F, Yazaki K (2005) Characterization of vacuolar transport of the endogenous alkaloid berberine in *Coptis japonica*. *Plant Physiol* 138: 1939–1946

Otsuka M, Matsumoto T, Morimoto R, Arioka S, Omote H, Moriyama Y (2005) A human transporter protein that mediates the final excretion step for toxic organic cations. *Proc Natl Acad Sci USA* 102: 17923–17928

Perez-Diaz R, Ryngajllo M, Perez-Diaz J, Pena-Cortes H, Casaretto JA, Gonzalez-Villanueva E, Ruiz-Lara S (2014) VvMATE1 and VvMATE2 encode putative proanthocyanidin transporters expressed during berry development in *Vitis vinifera* L. *Plant Cell Rep* 33: 1147–1159

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

Rogers EE, Wu X, Stacey G, Nguyen HT (2009) Two MATE proteins play a role in iron efficiency in soybean. *J Plant Physiol* 166: 1453–1459

Römheld V (1987) Different strategies for iron acquisition in higher-plants. *Physiol Plant* 70: 231–234

Roschzttardtz H, Seguela-Arnaud M, Briat JF, Vert G, Curie C (2011) The FRD3 citrate effluxer promotes iron nutrition between symplastically disconnected tissues throughout *Arabidopsis* development. *Plant Cell* 23: 2725–2737

Ryan PR, Delhaize E (2010) The convergent evolution of aluminium resistance in plants exploits a convenient currency. *Funct Plant Biol* 37: 275–284

Ryan PR, Delhaize E, Jones D (2001) Function and mechanism of organic anion exudation from plant roots. *Annu Rev Plant Physiol Plant Mol Biol* 52: 527–560

Ryan PR, Tyerman SD, Sasaki T, Furuichi T, Yamamoto Y, Zhang WH, Delhaize E (2011) The identification of aluminium-resistance genes provides opportunities for enhancing crop production on acid soils. *J Exp Bot* 62: 9–20

Sasaki T, Yamamoto Y, Ezaki B, Katsuhara M, Ahn SJ, Ryan PR, Delhaize E, Matsumoto H (2004) A wheat gene encoding an aluminum-activated malate transporter. *Plant J* 37: 645–653

Sawaki Y, Kihara-Doi T, Kobayashi Y, Nishikubo N, Kawazu T, Koyama H, Sato S (2013) Characterization of Al-responsive citrate excretion and citrate-transporting MATEs in *Eucalyptus camaldulensis*. *Planta* 237: 979–989

Seo PJ, Park J, Park MJ, Kim YS, Kim SG, Jung JH, Park CM (2012) A Golgi-localized MATE transporter mediates iron homoeostasis under osmotic stress in *Arabidopsis*. *Biochem J* 442: 551–561

Serrano M, Wang B, Aryal B, Garcion C, Abou-Mansour E, Heck S, Geisler M, Mauch F, Nawrath C, Metraux JP (2013) Export of salicylic acid from the chloroplast requires the multidrug and toxin extrusion-like transporter EDS5. *Plant Physiol* 162: 1815–1821

Shitan N, Bazin I, Dan K, Obata K, Kigawa K, Ueda K, Sato F, Forestier C, Yazaki K (2003) Involvement of CjMDR1, a plant multidrug-resistance-type ATP-binding cassette protein, in alkaloid transport in *Coptis japonica*. *Proc Natl Acad Sci USA* 100: 751–756

Shitan N, Dalmas F, Dan K, Kato N, Ueda K, Sato F, Forestier C, Yazaki K (2013) Characterization of *Coptis japonica* CjABCB2, an ATP-binding cassette protein involved in alkaloid transport. *Phytochemistry* 91: 109–116

Shitan N, Kato K, Shoji T (2014a) Alkaloid transporters in plants. *Plant Biotechnol*, in press

Shitan N, Minami S, Morita M, Hayashida M, Ito S, Takanashi K, Omote H, Moriyama Y, Sugiyama A, Goossens A, et al. (2014b) Involvement of the leaf-specific multidrug and toxic compound extrusion (MATE) transporter Nt-JAT2 in vacuolar sequestration of nicotine in *Nicotiana tabacum*. *PLoS ONE* 9: e108789

Shitan N, Yazaki K (2007) Accumulation and membrane transport of plant alkaloids. *Curr Pharm Biotechnol* 8: 244–252

Shitan N, Yazaki K (2013) New insights into the transport mechanisms in plant vacuoles. *Int Rev Cell Mol Biol* 305: 383–433

Shoji T (2014) ATP-binding cassette and multidrug and toxic compound extrusion transporters in plants: A common theme among diverse detoxification mechanisms. *Int Rev Cell Mol Biol* 309: 303–346

Shoji T, Inai K, Yazaki Y, Sato Y, Takase H, Shitan N, Yazaki K, Goto Y, Toyooka K, Matsuoka K, et al. (2009) Multidrug and toxic compound extrusion-type transporters implicated in vacuolar sequestration of nicotine in tobacco roots. *Plant Physiol* 149: 708–718

Sirikantaramas S, Yamazaki M, Saito K (2008) Mechanisms of resistance to self-produced toxic secondary metabolites in plants. *Phytochem Rev* 7: 467–477

Sivaguru M, Liu J, Kochian LV (2013) Targeted expression of SbMATE in the root distal transition zone is responsible for sorghum aluminum resistance. *Plant J* 76: 297–307

Sun X, Gilroy EM, Chini A, Nurmberg PL, Hein I, Lacomme C, Birch PR, Hussain A, Yun BW, Loake GJ (2011) *ADS1* encodes a MATE-transporter that negatively regulates plant disease resistance. *New Phytol* 192: 471–482

Takanashi K, Yokosho K, Saeki K, Sugiyama A, Sato S, Tabata S, Ma JF, Yazaki K (2013) LjMATE1: A citrate transporter responsible for iron supply to the nodule infection zone of *Lotus japonicus*. *Plant Cell Physiol* 54: 585–594

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol* 30: 2725–2729

Terrier N, Torregrosa L, Ageorges A, Vialet S, Verries C, Cheynier V, Romieu C (2009) Ectopic expression of *VvMybPA2* promotes proanthocyanidin biosynthesis in grapevine and suggests additional targets in the pathway. *Plant Physiol* 149: 1028–1041

Thompson EP, Wilkins C, Demidchik V, Davies JM, Glover BJ (2010) An *Arabidopsis* flavonoid transporter is required for anther dehiscence and pollen development. *J Exp Bot* 61: 439–451

Tiwari M, Sharma D, Singh M, Tripathi RD, Trivedi PK (2014) Expression of OsMATE1 and OsMATE2 alters development, stress responses and pathogen susceptibility in *Arabidopsis*. *Sci Rep* 4: 3964

Tovkach A, Ryan PR, Richardson AE, Lewis DC, Rathjen TM, Ramesh S, Tyerman SD, Delhaize E (2013) Transposon-mediated alteration of *TaMATE1B* expression in wheat confers constitutive citrate efflux from root apices. *Plant Physiol* 161: 880–892

Tsuyama T, Kawai R, Shitan N, Matoh T, Sugiyama J, Yoshinaga A, Takabe K, Fujita M, Yazaki K (2013) Proton-dependent coniferin transport, a common major transport event in differentiating xylem tissue of woody plants. *Plant Physiol* 162: 918–926

Uhde-Stone C, Liu J, Zinn KE, Allan DL, Vance CP (2005) Transgenic proteoid roots of white lupin: A vehicle for characterizing and silencing root genes involved in adaptation to P stress. *Plant J* 44: 840–853

Wu X, Li R, Shi J, Wang J, Sun Q, Zhang H, Xing Y, Qi Y, Zhang N, Guo YD (2014) *Brassica oleracea* MATE encodes a citrate transporter, and enhances aluminum tolerance in *Arabidopsis thaliana*. *Plant Cell Physiol* 55: 1426–1436

Yamasaki K, Motomura Y, Yagi Y, Nomura H, Kikuchi S, Nakai M, Shiina T (2013) Chloroplast envelope localization of EDS5, an essential factor for salicylic acid biosynthesis in *Arabidopsis thaliana*. *Plant Signal Behav* 8: e23603

Yang XY, Yang JL, Zhou Y, Pineros MA, Kochian LV, Li GX, Zheng SJ (2011) A *de novo* synthesis citrate transporter, *Vigna umbellata* multidrug and toxic compound extrusion, implicates in Al-activated citrate efflux in rice bean (*Vigna umbellata*) root apex. *Plant Cell Environ* 34: 2138–2148

Yazaki K, Shitan N, Sugiyama A, Takanashi K (2009) Cell and molecular biology of ATP-binding cassette proteins in plants. *Int Rev Cell Mol Biol* 276: 263–299

Yokosho K, Yamaji N, Ma JF (2010) Isolation and characterisation of two MATE genes in rye. *Funct Plant Biol* 37: 296–303

Yokosho K, Yamaji N, Ma JF (2011) An Al-inducible MATE gene is involved in external detoxification of Al in rice. *Plant J* 68: 1061–1069

Yokosho K, Yamaji N, Ueno D, Mitani N, Ma JF (2009) OsFRDL1 is a citrate transporter required for efficient translocation of iron in rice. *Plant Physiol* 149: 297–305

Yu F, De Luca V (2013) ATP-binding cassette transporter controls leaf surface secretion of anticancer drug components in *Catharanthus roseus*. *Proc Natl Acad Sci USA* 110: 15830–15835

Zhang H, Zhu H, Pan Y, Yu Y, Luan S, Li L (2014) A DTX/MATE-type transporter facilitates abscisic acid efflux and modulates ABA sensitivity and drought tolerance in *Arabidopsis*. *Mol Plant* 7: 1522–1532

Zhao J, Dixon RA (2009) MATE transporters facilitate vacuolar uptake of epicatechin 3′-*O*-glucoside for proanthocyanidin biosynthesis in *Medicago truncatula* and *Arabidopsis*. *Plant Cell* 21: 2323–2340

Zhao J, Dixon RA (2010) The 'ins' and 'outs' of flavonoid transport. *Trends Plant Sci* 15: 72–80

Zhao J, Huhman D, Shadle G, He XZ, Sumner LW, Tang Y, Dixon RA (2011) MATE2 mediates vacuolar sequestration of flavonoid glycosides and glycoside malonates in *Medicago truncatula*. *Plant Cell* 23: 1536–1555

Zhou G, Delhaize E, Zhou M, Ryan PR (2013) The barley *MATE* gene, *HvAACT1*, increases citrate efflux and Al^{3+} tolerance when expressed in wheat and barley. *Ann Bot* (Lond) 112: 603–612

Zhou G, Pereira JF, Delhaize E, Zhou M, Magalhaes JV, Ryan PR (2014) Enhancing the aluminium tolerance of barley by expressing the citrate transporter genes *SbMATE* and *FRD3*. *J Exp Bot* 65: 2381–2390