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Expression profile analysis of *MATE* gene family in rice

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Abstract

Multidrug and toxic compound extrusion (MATE) proteins is a newly characterized transporter family in plants. However, knowledge of this family in systematic classification, molecular evolution, and expression patterns in plants is limited. In this study, *MATE* gene sequence, structure, and names as well as MATE protein size and subcellular localization in rice were analyzed using bioinformatics tools, chromosome localizations, and gene clusters. The function of MATE proteins was further elucidated on a basis of phylogenetic relationships. Using available transcriptomic data, the expression pattern and function of *MATE* were different in the selected organs and developments stages of rice. In addition, the relative abundance of *OsMATE1* transcripts increased 3 h after copper treatment and so it was identified as a candidate gene for Cu tolerance in rice. This research provided basic data for further studies on *MATE* genes in rice and theoretical information about the biological function of MATE proteins.

Additional key words: copper, bioinformatics, *Oryza sativa*, phylogenetic tree, transcriptom.

Introduction

Multidrug and toxic compound extrusion (MATE) family is a new secondary transporter family distinct from any other known multidrug resistance transporters because of the unique topology of the 12 predicted transmembrane (TM) helices (He *et al.* 2010, Shoji 2014, Takanashi *et al.* 2014). Their activities depend on the electrochemical gradient of cations (such as H⁺ or Na⁺ ions) to translocate substrates across the membrane (Kuroda and Tsuchiya 2009). MATE transporters are widely distributed in prokaryotic and eukaryotic cells and implicated in various processes of the growth and development.

Multiple studies have shown that the diverse functions of MATE are consistent with the broad range of the transportation of substrates (Zhao and Dixon 2009), such as secondary metabolites, alkaloids, organic acids, flavonoids, and plant hormones (Diener *et al.* 2001, Zhu *et al.* 2016, Li *et al.* 2017). The functions of additional MATE transporters have been clarified and divided into three classes based on their established functions in plants. The first group of MATE transporters is involved in disease resistance. The molecular mechanism of pathogen resistance is illustrated by analyzing some *Arabidopsis* mutants, such as *Ateds5*, which encodes AtDTX47 that is localized in the chloroplast and involved in export of

salicylic acid out of cells to promote disease tolerance (Serrano *et al.* 2013, Yamasaki *et al.* 2013). The second group of MATE proteins generally transports small organic molecules out of cells and acts as ligands or chelating agents. For instance, roots secrete citrate out of cell to chelate aluminum and avoid damage to the plants. The Al is different from other metals. In acidic soil, Al³⁺ can produce toxic effects and then inhibit root development. As such, the mechanism of Al toxicity has been widely studied (Yokosho *et al.* 2016b, Pereira *et al.* 2019). The first aluminum-activated citrate transporter, namely, *SbMATE*, in sorghum has been cloned, and SbMATE can secrete citrate, thereby chelating Al to form nontoxic complexes in soil solutions (Magalhaes *et al.* 2007). Since then, MATE genes encoding citrate transporter proteins participating in Al tolerance have been cloned in *Triticum*, *Hordeum*, *Secale*, *Oryza*, *Phaseolus*, *Arabidopsis*, *Zea mays*, and *Nicotiana tabacum* (Furukawa *et al.* 2007, Liu *et al.* 2009, Ryan *et al.* 2009, Maron *et al.* 2010, Yang *et al.* 2011, Yokosho *et al.* 2010, 2011, Tovkach *et al.* 2013.). The third group of MATE proteins is located in vacuoles and involved in the transport of flavonoids and secondary metabolites. Vacuoles are the main storage components of most conjugated forms of flavonoids, including anthocyanins, flavonols, and flavone glycoside. A kinetic study on vesicles has revealed that the MATE family

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Abbreviations: MATE - multidrug and toxic compound extrusion; pI - isoelectric point; PM - plasma membrane; TM - transmembrane.

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member AtTT12 can specifically transport glycosidic forms, such as cyanidin 3-O glucoside⁵ and epicatechin 3-O-glucoside *via* AtTT12 expressed in yeast (Debeaujon *et al.* 2001, Zhao and Dixon 2009). The homologous genes of *TT12* are cloned from *Vitis* and *Medicago*, and the expressed proteins in cell organelles are implicated in the mobilization of flavonoids (Marinova *et al.* 2007, Gomez *et al.* 2009, Zhao *et al.* 2011). In tobacco, MATE proteins can transport alkaloids into vacuoles through a similar mechanism (vacuolar flavonoid sequestration; Morita *et al.* 2009, Shoji *et al.* 2009).

Rice is one of the most important crops around the world and a model plant in studies involving the molecular genetics of monocotyledons. Its yields are severely affected by abiotic stresses, such as drought, low temperature, salt damage, heavy metals, and pathogens every year. Many plants, including rice, form various mechanisms of stress tolerance to ensure their normal growth. One of these mechanisms is detoxification, and an example of this mechanism is the excretion of toxic compounds from cells. In this process, MATE proteins play vital roles. In the rice genome, at least 50 MATE genes are available, but only a small number have been analyzed (Yokosho *et al.* 2009, 2011, 2016b, 2016a). Considering the effects of the reported MATE transporters on plant growth and stress responses, we believe that many unknown MATE transporter members will participate in plant development and stress resistance.

This study aimed to identify the MATE protein gene structure, chromosome localization, subcellular localization, and expression patterns of various subfamily genes in different tissues and organs in rice through bioinformatics tools and to understand the information and function of MATEs. This can study provides useful information on the research of MATE transporters in rice.

Materials and methods

Identification of putative *MATE* genes in rice and sequence structure analysis: The nucleotide and protein sequences of rice (*Oryza sativa* L. cv. Nipponbare) were retrieved from *Phytozome v. 11.0* database (<http://phytozome.jgi.doe.gov/pz/portal.html>). Many putative *MATE* sequences (E-value was $\leq 1e-7$) were filtered on the basis of the presence of the conserved *MATE* domain (Pfam: PF01554) by using *Pfam* (<http://pfam.sanger.ac.uk/>), and 55 rice MATE proteins with the MATE domain were identified. The *MATE* transporter genes were denominated systematically on the basis of their physical locations on the chromosomes of rice. The molecular mass (Mr) and theoretical isoelectric point (pI) of rice MATE proteins were computed using the *ExPASy Compute pI/Mw* tool (http://www.expasy.ch/tools/pi_tool.html). The numbers of transmembrane helices and subcellular localizations of the MATE proteins were predicted by *TMHMM* server (www.cbs.dtu.dk/services/TMHMM2.0/) and *WoLF PSORT* (<http://www.genscript.com/wolf-psort.html>), respectively.

All of the assumed *MATE* genes were located on the 12

rice chromosomes by using *MapInspect* software (http://www.plantbreeding.wur.nl/uk/software_mapinspect.html). The information of gene linkage was described by analyzing the relative location relationship among the different family members.

Phylogenetic and gene structure analysis: The full protein sequences of 55 rice MATE were aligned using *ClustalW* (<http://clustalw.ddbj.nig.ac.jp/top-e.html>) with the 30 previously reported MATE from other plant species (Table 1 Suppl.). Then, an unrooted phylogenetic tree was constructed by using the maximum likelihood (ML) algorithm with 1000 bootstraps in *MEGA7.0*. The result of the ML tree was viewed and edited in *Evolview* (He *et al.* 2016). The gene structure map and motifs in MATE proteins were drawn and statistically identified by the online tools of *GSDS* (<http://gsds.cbi.pku.edu.cn/>) and *MEME* (<http://meme-suite.org/>) with default settings, respectively (Hu *et al.* 2015). The maximum number of motif was set at 12.

Extraction of microarray data and cluster analysis: The microarray data of the expression of all *MATE* transporter genes, including those at 27 different growth stages and tissue organs, were found and obtained from the *Rice Oligonucleotide Array Database* (<http://www.ricearray.org/>). For multiple probes corresponding to a single gene, the average of the expression values was applied. A heat map for the expression of MATEs was constructed using *HeatI* (v.1.0) software. Hierarchical clustering analysis was applied to examine these gene expression values.

Expression analysis of selected *MATE* genes by reverse transcription quantitative PCR: Seeds from *Oryza sativa* L. var. *japonica* cv. Nipponbare were surface disinfected with 0.1 % (m/v) HgCl_2 for 10 min, washed with distilled water, and placed in darkness at 30 °C for 3 d. The germinating seeds were selected and grown in a beaker with the Yoshida culture solution (Sun *et al.* 2012). For different treatments, 14-d-old rice seedlings were exposed to 25 μM CdCl_2 , 25 μM CuCl_2 , 50 μM AlCl_3 , and 50 μM FeSO_4 for 3 h. The root tips were excised and subjected to RNA isolation. Root samples were also obtained from the 14-d-old seedlings, which were exposed to a 25 μM Cu for 0, 3, 6, 12, and 48 h.

Total RNA was extracted from all of the samples by using *Trizol* reagent in accordance with the manufacturer's protocol (*TaKaRa*, Dalian, China). First-strand cDNAs were synthesized by *Prime Script* reverse transcriptase. The amount of transcripts of *OsMATE1*, *OsMATE2*, *OsMATE21*, and *Actin 1* (internal control) were determined by using *SYBR Green I* with the following primers: *sMATE1*, 5'-CAGCTCATCTCCACCGTCA-3' (forward) and 5'-GCGATTGGTACACTCACAACA-3' (reverse); *OsMATE2*, 5'-CGGTGAGCCTGACGAAC TAC-3' (forward) and 5'-AGGTGTGCACTCCCAGAGTA-3' (reverse); *OsMATE21*, 5'-TC AGCCTCCTCGTT GGAATG-3' (forward) and 5'-ATCACGGGGTCTGAC CTAT-3' (reverse); *Actin 1*, 5'-CATGCTATCCCTCGTCT CGACCT-3' (forward) and 5'-CGCACTTCATGATGGA

GTTGTAT-3' (reverse). Expression data were normalized to the expression of *Actin 1* and relative to the untreated control by the $\Delta\Delta C_t$ method.

Results and discussion

Fifty-five MATE genes were identified through the genome-wide analysis of *Oryza sativa*, and the necessary information of all the genes was obtained (Table 2 Suppl.). On the basis of positions on rice chromosomes, we named these genes *OsMATE1* - *sMATE55*. The length and number of the transmembrane domains of the rice MATE transporters were in the range of 110 - 1197 amino acids and 4 - 13 were transmembranes (TMs), respectively. Specifically, the *OsMATE23* contains no TMs domain. However, the MATE proteins in *Arabidopsis* consist of 400 - 700 amino acids, and most of them possess 12 TMs domains (Li *et al.* 2002), indicating that the rice MATE family members vary. The predicted Mr of the rice MATE proteins ranged from 11.49 to 116.4 kDa and pI from 4.27 to 9.76. The predicted subcellular localization of MATEs was diverse, and it included plasma membrane (PM), cytosol, chloroplast, endoplasmic reticulum, and vacuole. Of these MATEs, 58.18 % (32 out of 55 MATE proteins) were localized in PM, 12.73 % (7 out of 55) were detected in the cytosol, and 10.91 % (6 out of 55), 1.82 % (1 out of 55), and 16.36 % (9 out of 55) were found in the chloroplast, endoplasmic reticulum, and vacuole, respectively. The rice MATE proteins probably play roles in the endomembrane system and the cytosol. They might be associated with differences in rice living environment and MATE protein function, which might result in changes in MATE subcellular localization.

The *MATEs* were widely located on chromosomes 1 to 12 but they were not evenly distributed (Fig. 1).

The number of *OsMATE* genes per chromosome ranged from 1 to 10. The few *OsMATE* genes were located on chromosomes 1, 2, 4, 5, 7, 8, 9, and 11, and numerous *OsMATE* genes were found on chromosomes 3, 6, 10, and 12. The density of genes on chromosomes varied from 1 to 18 %. The largest number of *MATE* genes was observed on chromosome 3, whereas the least number of *MATE* genes was detected on chromosome 5 (Fig. 1). One *MATE* gene was located at an average of 2.8 Mb region. The most densely distributed regions were the intermediate regions of chromosomes 3, 6, and 10; the lower arms and apex of chromosome 8, 9 and 11, 12 separately. The MATE gene was also distributed near the centromeres of chromosomes 1, 3, 6, and 10. Some genes existed in the form of gene clusters (Fig. 1), including six genetic clusters, suggesting that they might originate from the same fragment replication and tandem duplication during evolution. Gene duplication is the original driving force in gene evolution, and the reproducibility of the whole genome provides an unprocessed genetic material for the evolution of sequence and the expression of multiple replicas (Zhu *et al.* 2012). Gene duplication might play an important role in the formation of the functional diversity of the *MATE* gene family. Duplication events in *ZmMATE1* might be associated with transposable elements (Pereira *et al.* 2019). The same phenomena might be valid for some *OsMATEs* in rice.

Phylogenetic analysis indicated that all of the MATEs in rice could be classified into the C1, C2, C3, and C4 clades (Fig. 2). The phylogenetic relationships suggested that the function of MATE proteins in rice could be identified on the basis of the known function of MATE transporters in other plants.

Clade C1 consisted of subgroup C1-1 and C1-2 subgroups (Fig. 2). The C1-1 subgroup contained 11 rice MATE transporters, and C1-2 included 3 rice MATE

Fig. 1 Localization of multidrug and toxic compound extrusion family genes on rice chromosomes. The number of chromosomes is indicated above each bar, and the scale on the left is in megabases (Mb). The size of chromosomes is indicated by its relative length by using the information from *Phytozome*. Tandemly duplicated genes are shown in red boxes.

transporters and the known functional proteins AtDTX1 from *Arabidopsis* and NtJAT1 from tobacco (Morita *et al.* 2009, Li *et al.* 2002). AtDTX1 is localized in the plasma membrane and implicated in the transport of toxic compounds and some heavy metals, such as Cd²⁺ (Li *et al.* 2002). Nt-JAT1 participates in nicotine translocation, unloads alkaloids from aerial parts, and become deposited in vacuoles (Morita *et al.* 2009). Among them, *OsMATE21* (*OsMATE2*) is related to the development and stress and disease resistance in rice (Tiwari *et al.* 2014). Therefore, clade C1 may be involved in the development and efflux of various compounds.

Clade C2 consists of 10 rice *MATE* transporters and 2 previously reported *MATE* transporters, namely, AtADS1/ABS3 and AtZF14/BCD1/ABS4 (Fig. 2) (Burko *et al.* 2011, Sun *et al.* 2011, Seo *et al.* 2012). The AtADS1/ABS3 negatively regulates plant disease resistance (Sun *et al.* 2011), and AtZF14/BCD1/ABS4 mediates ion balance and plant development (Burko *et al.* 2011, Seo *et al.* 2012). Both transporters can regulate hypocotyl cell elongation (Wang *et al.* 2015). In summary, the functions of *MATE* transporters in this group are diverse, and further studies are needed to confirm this finding.

Clade C3 had two subgroups (Fig. 2). The C3-1 subgroup contained three *MATE* transporters, including the known *MATE* protein AtALF5/AtDTX19 in which mutation results in the defective formation of lateral roots and the increased sensitivity of roots to various compounds (Diener *et al.* 2001); thus, this subgroup likely provides plant resistance to toxins. The C3-2 subgroup

had 8 rice *MATE* proteins and 13 previously reported *MATE* proteins. *OsMATE5* and salicylic acid transporter AtEDS5, which endows plant disease resistance, form an individual branch. The majority of the rest remaining functionally characterized members in C3-2 are citrate transporters, such as SbMATE (Magalhaes *et al.* 2007), HvACCT1 (Furukawa *et al.* 2007), AtMATE (Liu *et al.* 2009), TaMATE1B (Ryan *et al.* 2009), ScFRDL2 (Yokosho *et al.* 2010), OsFRDL4 (*OsMATE4*) (Yokosho *et al.* 2011), OsFRDL2 (*OsMATE40*) (Yokosho *et al.* 2016a), ZmMATE1 (Maron *et al.* 2010), BoMATE (Wu *et al.* 2014), EcMATE1 (Sawaji *et al.* 2013), AtFRD3 (Roschztardt *et al.* 2011), GmFRD3b (Rogers *et al.* 2009), ScFRDL1 (Yokosho *et al.* 2010), and OsFRDL1 (*OsMATE10*) (Yokosho *et al.* 2009), which endow plant with aluminum resistance and maintain the iron metabolism balance in root. GmMATE75 also enhances the tolerance to aluminum in soybean (Liu *et al.* 2016). Therefore, we hypothesize that the function of clade C3 members may be related to the mediation of citrate efflux and iron translocation and induce rice adaptation to acidic soils.

Clade C4 contained subgroup C4-1, C4-2, C4-3, and C4-4 subgroups (Fig. 2). The C4-1 subgroup includes nine rice *MATE* transporters, and one of them, namely, *OsMATE9* (*OsMATE1*), is involved in the development of rice, especially fertility, and disease and stress tolerance (Tiwari *et al.* 2014). The C4-2 subgroup comprises six rice *MATE* proteins and five characterized transporters of various glycosides. Several transporters are involved

Fig. 2 Aphylogenetic tree of a rice multidrug and toxic compound extrusion (*MATE*) family. The phylogenetic tree was constructed by *MEGA 6.0* using the maximum likelihood method. Bootstrap values in percentage (1 000 replicates) are indicated on the *nodes*. Different subfamilies are highlighted using different colors (C1 in blue, C2 in orange, C3 in yellow, and C4 in green), and subgroups are marked with *colored arcs* outside the cycle tree.

in the transport of acylated anthocyanins into vacuoles, where they accumulate in the form of glycosides, such as VvAM1 and VvAM3 from *Vitis vinifera* (Gomez *et al.* 2009, Gomez *et al.* 2011), SIMATE from *Solanum lycopersicum* (Mathews *et al.* 2003), and MtMATE2 from

Medicago truncatula (Zhao *et al.* 2011). The substrate of Arabidopsis FLOWER FLAVONOID TRANSPORTER is a flavonoid, and AtFFT acts on reproductive growth, including anther dehiscence and pollen development (Thompson *et al.* 2010). One rice MATE protein and five

Fig. 3 Gene structures of a rice multidrug and toxic compound extrusion (*MATE*) family. The structures of 55 rice *MATE* genes are plotted using green boxes (coding DNA sequences), gray lines (introns), and blue boxes (upstream/downstream sequences - untranslated regions). The scale is in kb on the bottom. The genes are listed according to the order of subfamilies C1 to C4 from the phylogenetic tree, and different subfamilies are highlighted in different colors: C1 in blue, C2 in orange, C3 in yellow, and C4 in green.

previously reported proteins form C4-3. The AtTT12 from *A. thaliana* (Debeaujon *et al.* 2001), BrTT12 from turnip (Chai *et al.* 2009) and MtMATE1 from *M. truncatula* (Zhao and Dixon 2009) transport flavonoids and epicatechin 3'-O glucoside into the vacuoles. VvMATE1 and VvMATE2 from *V. vinifera* are involved in mediating the transport of proanthocyanidins (Perez-Diaz *et al.* 2014). The OsMATE55 is clustered with these proteins and may be involved in the transport of various compounds. The C4-4 is composed of five rice MATE proteins and two known proteins. Alkaloids are transported by NtMATE1 and NtMATE2 from *Nicotiana tabacum* and stored in vacuoles (Shoji *et al.* 2009). The OsMATE in C4-4 may be involved in alkaloids transport. The rice MATEs in this clade may be related to vacuolar transport and flavonoid or alkaloid accumulation in plant as indicated by the functions of the known MATE transporters in this clade.

The online tool *GSFS* was used to explore the diagrams of an exon-intron structure with the coding sequence and genomic sequence, and this analysis was expected to provide further insights into the evolution of gene structures. Their exon-intron structures and the order

of the subgroups were plotted in the phylogenetic tree (Fig. 3). The length and number of introns and exons vary in different branches of *MATE*. Most genes in clade C1 have approximately seven introns except *OsMATE42* and *OsMATE43* that have only four introns and no untranslated region. Most genes in clade C2 have no introns except *OsMATE17*, *OsMATE54*, and *OsMATE11*, which contain only one intron. In clade C3, *OsMATE23* is special and does not contain intron. The number of introns in the remaining genes is between 5 and 13. The *MATEs* in clade C4 contain 2 - 8 introns. Within the same subfamily, the *MATE* gene structures are similar.

The protein motifs of the rice MATE family were analyzed by *MEME* (Fig. 1 Suppl.). Our results show that the sequences and types of protein motifs were similar in the MATE family except the C3-2 subgroup. The MATE proteins in the C3-2 subgroup were significantly different from other proteins which include few motifs. This remarkable difference in MATE protein structures might be due to functional diversities that occurred during the evolution.

Genes with similar structural sequence or homologous

Fig. 4 A hierarchical cluster display of expression profiles for rice multidrug and toxic compound extrusion (*MATE*) genes in different tissues and/or organs. A heat map with hierarchical clustering 49 rice *MATE* genes was constructed using the *HemI* (v1.0) software. A color key is the relative transcript abundance of the rice *MATE* genes in 27 rice tissues. The whole plant, callus, suspension cell, dry seed, germinating seed, plumule, radicle, coleoptile, seedling, shoot, leaf, stem, internode, internode pith parenchyma, root, root tip, shoot apical meristem, flag leaf, spikelet, plasmalemma, stigma, ovary, developing anther, mature anther, embryo sac, embryo, and endosperm are represented by numbers 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, and 27, respectively.

genes have different expression patterns in distinct tissues and organs. In this study, to elucidate the possible function of rice *MATE* family during plant development, we collected and analyzed the relative transcript abundance of *MATE* genes in different rice tissues from the *Rice Oligonucleotide Array Database*. A heat map with the hierarchical clustering of the 49 remaining rice *MATEs* was generated using *HemI* software because some genes lacked chip probes (Fig. 4). The results showed that *MATEs* were expressed in various tissues and organs, thereby confirming that *MATE* transporters are relatively important functional proteins, which may be involved in various processes of plant physiology and development. Hierarchical clustering analysis revealed that 49 genes could be divided into four groups (I to IV), and each group included two to three subfamilies.

Group I contained six genes. The expression characteristics in this group indicated that the genes were mainly expressed in callus, suspension cell, seeds, roots, embryo sac, embryo, and endosperm, and the expression was high (Fig. 4). Therefore, the six genes might play important roles in these tissues and organs.

Group II consisted of clade C1 (four members), clade C2 (four members), clade C3 (one member), and clade C4 (four members). All of the genes were strongly expressed in seedlings, coleoptiles, shoot apical meristem, and plasmalemma. However, only a few genes were highly expressed in mature anther (*OsMATE36*), embryo sac (*OsMATE14*, *OsMATE20*, and *OsMATE12*), embryo (*OsMATE2*), and endosperm (*OsMATE9*, *OsMATE26*, *OsMATE23*, and *OsMATE24*), and the remaining ones were almost not expressed (Fig. 4). Therefore, the members in Group II might be involved in regulating the seedling stage and the vegetative growth.

Group III contained 18 genes, which, although low expressed, were expressed throughout the plant (Fig. 4), thus implying that they are essential for plant growth and development.

Group IV had 12 genes mainly expressed in plumule, radical, coleoptiles, and whole internode. Among them, *OsMATE4*, *OsMATE28*, *OsMATE32*, and *OsMATE39* are specifically expressed in plumule (Fig. 4); they might be involved in the development of rice plumule. *OsMATE54*

and *OsMATE55* were highly expressed in stigma, spikelet, and embryo sac, indicating that the two genes participated in the reproductive development of rice.

The rice *MATE* family can be divided into four clades based on the results of phylogenetic analysis and the known function of *MATE* transporters (Fig. 2). Its members were also sorted into four groups through hierarchical clustering analysis (Fig. 4). However, the genes of each branch between two different analytical methods were different possibly because of the distinct algorithm. One is based on the genetic distances and their differences in evolutionary location to analyze evolutionary relationship and process. The other is based on similarity coefficients to collect data, classify them, and interpret the proximity and distances of kinship.

On the basis of the expression patterns in different tissues of rice and the cluster analysis of *MATE* genes, we speculate that the *MATE* gene expressions differ. The diversity and richness of rice *MATE* function further increase in the course of evolution. However, the expressions of the rice *MATE* genes follow a certain pattern and are not random indicating that functional complement and functional redundancy among rice *MATE* genes are the result of mutual coordination and interaction.

The *AtDTX1* in the C1-2 subgroup is involved in transport of some heavy metals (Li *et al.* 2002). In our study, the expression patterns of all of the three *OsMATE* genes in the C1-2 subgroup in response to metal toxicity were analyzed through reverse transcription quantitative PCR. The relative expressions of the three C1-2 *OsMATE* genes in the root tips of rice seedlings after metal stress are shown in Fig. 5A. The *OsMATE1* and *OsMATE2*, especially *OsMATE1*, had an evidently high expression after Cu treatment. A previous microarray study reported that Os01g31980 (*OsMATE1*) was upregulated (approximately 2.56-fold) by Cu treatment for 24 h in rice (Sudo *et al.* 2008). We determined whether *OsMATE1* strongly responds to Cu stress and found that the *OsMATE1* expression was upregulated by 5.09-fold after Cu treatment for 3 h (Fig. 5B), suggesting its role in the tolerance of rice to Cu stress.

Conclusions

Fig. 5 Analysis of *OsMATE* expression in rice seedlings (reverse transcription quantitative PCR). A - A relative expression of three rice C1-2 *MATE* genes in response to different metal stresses (25 μ M CdCl₂, 25 μ M CuCl₂, 50 μ M AlCl₃, and 50 μ M FeSO₄) for 3 h. B - A time-dependent expression of *OsMATE1* to 25 μ M CuCl₂. The expression of *OsActin1* was used as an internal control. Means $\pm$ SDs, $n = 3$, different letters indicate significant difference at $P < 0.05$ (the Student *t*-test).

This study maximized the use of the information about rice genome and gene expression chip to analyze the *MATE* family in rice and divided it into four major clades: C1, C2, C3, and C4. The rice *MATE* family remarkably differed in gene structure, protein domain, and expression pattern indicating that the function of *MATE* gene family members differentiated in the course of evolution. A few of *OsMATE* genes were tandem duplicated genes on some chromosomes. The *OsMATE1* from C1-2 subgroup showed a relatively high transcription in response to Cu treatment suggesting its potential role in the tolerance of rice to Cu toxicity. This study provided a valuable reference for further verification of gene function and application, and the candidate gene for metal tolerance in rice.

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