OsMATE6 gene putatively involved in host defense response toward susceptibility against Rhizoctonia solani in rice

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ABSTRACT
Sheath blight caused by Rhizoctonia solani AG1-IA is the second most serious disease of rice worldwide. Elucidating the role of multi-drug and toxic compound extrusion (MATE) gene family in host-pathogens interactions may uncover a new possible way to comprehend the mechanism of sheath blight resistance in rice. We foremost explored the role of OsMATE genes against R. solani resistance through comparative transcriptomics in PR114 (susceptible) and ShB-8 (moderately resistant) at 24 and 48 hpi (hours post-inoculation) of R. solani infection, respectively. Six OsMATE genes were differentially expressed and further validated through qRT-PCR. OsMATE6 gene was identified as a potential candidate for sheath blight susceptibility as it was significantly up-regulated in PR114. OsMATE6 is conserved within the wild relatives and might be translocated from Oryza nivara during the domestication of rice. Further studies are focused to verify its role by overexpression and protein interactions to understand the molecular mechanism of sheath blight resistance.

Introduction
Rice (Oryza sativa L.) is one of the most important cereal crops, which serves as a staple food for 90% of the human population globally (Khush 2005). With the ever-increasing population worldwide, its production has to be increased to ensure food security. This necessitates developing rice varieties that have higher yield potential and resilience to biotic and abiotic stresses. Due to climatic changes during the last 5–6 years, sheath blight of rice caused by a soil-borne, facultative, necrotrophic fungus Rhizoctonia solani kuhn AG1-IA has evolved as a second major disease of rice (Molla et al. 2020) and led to yield losses as high as 50% under prevalent conditions (Richa et al. 2016). The pathogen is genetically dynamic and has been grouped into 14 different anastomosis groups (AG) based on morphological characteristics and hyphal anastomosis interactions between them (Carling et al. 1999). R. solani AG isolates were further classified into three groups, namely 1A, 1B and 1C based on size and DNA sequence-based homology (Carling et al. 1999). Sheath blight-infesting isolates were allocated to AG1-IA that infect rice worldwide, which is remarked by morphological structures (Borthakur and Addy 1988), infection patterns (Marshall and Rush 1980), and ribosomal DNA internal transcribed spacer (rDNA-ITS) sequences (Guillemaut et al. 2003). Due to its broad host range, lack of complete resistance and survival under unfavorable conditions by forming dormant sclerotia has posed difficulties in controlling the pathogen (Singh et al. 2019).

Despite its economic importance, the molecular mechanism of resistance to R. solani AG1-IA is still unknown in rice (Molla et al. 2020). The resistance to sheath blight is complex and is regulated by multiple genes (Zuo et al. 2000). Although several studies have identified QTLs for ShB resistance distributed over all 12 chromosomes of rice using different marker types and mapping populations (Jia et al. 2012; Zuo et al. 2013; Zuo et al. 2014a; Zeng et al. 2015). Two major QTLs, qShB9-2 (Liu et al. 2009) and qSBR11-1 (Chan-namallikarjuna et al. 2010), have been verified in other studies (Liu et al. 2013; Zuo et al. 2013; Zuo et al. 2014b; Yadav et al. 2015). Also, a few QTLs have been introgressed and pyramided in other rice lines for the enhancement of ShB resistance (Singh et al. 2012; Wang et al. 2012). Wang et al. (2021) identified two disease resistance proteins, RPM1 (OsRSR1) and protein kinase domain-containing protein (OsRLCK5), using GWAS analysis in rice against R. solani and further validated through overexpression and knockdown assays.

There is an interplay of molecular events between pathogen and host as a defense mechanism. In the previous studies, it has appeared that fungus can manipulate host defense response by expression of essential pathogenicity factors such as secondary metabolites, carbohydrate-active enzymes, secreted proteins, and effectors (Anderson et al. 2016). It has also been observed that isolates that produce melanin and high amounts of cell wall degrading enzymes such as pectolytic and cellulases have played a major role in the enhancement of disease and are expressed at different stages of infection (Zheng et al. 2013). There are counteracting events that take place in the host plant in response to R. solani. The involvement of different signaling molecules...
such as jasmonic acid (JA), lipoxygenase (LOX), ethylene, and salicylic acid (SA) in resistance to *R. solani* has been demonstrated in different studies (Zhang et al. 2017; Karmarkar et al. 2019). There are few reports on deciphering the role of secondary messengers in signaling molecules and metabolic alterations in rice-*R. solani* interactions (Ghosh et al. 2017; Karmarkar et al. 2019). Among several other plant defense mechanisms such as lectins (Singh et al. 2021) and R genes (Molla et al. 2020), transporter proteins have been reported to be involved in the transport of metabolites and maintain the growth and development of the host plant (Magalhaes et al. 2007). Multidrug and toxic compound extrusion (MATE) proteins are secondary transporters to translocate substrates across the membrane (Takanashi et al. 2014).

**MATE** gene families have been well characterized in various plant species and have diverse roles like efflux of alkaloids in *N. bentahmiana* (Shitan et al. 2014), flavonoids in *A. thaliana* (Ghosh et al. 2017), and aluminum detoxification in sorghum, wheat, and rice (Magalhaes et al. 2007; Ryan et al. 2009; Yokosho et al. 2011). Mackon et al. (2021) depicted putatively *OsMATE34*(Os08g0562800) as a transporter of anthocyanin in black rice caryopsis, which has an important role in plant growth and development. Contrary to its positive roles, a few others reported MATE as susceptibility genes since heterologous expression of these led to increased susceptibility to disease in *Arabidopsis* and *Citrus sinensis* (Tiwari et al. 2014; Juliao et al. 2020). Available literature posed a question on the role of the MATE gene in the resistance/susceptibility of rice and prompted us to investigate its role in the rice-*R. solani* pathosystem at the molecular level. A precise understanding of fungal virulence factors vis-à-vis host defense mechanism could help design effective strategies to combat this disease (Gururani et al. 2012). In this direction, certain secondary metabolites produced by the fungus during infection stages have been identified. To understand the fate of these metabolites in the plant cells it is foremost important to identify genes that have a potential role in their sequestration or extrusion of out-of-cell. In the present study, two diverse genotypes of rice, PR114 (susceptible) and ShB-8 (moderate resistant), were challenged with *R. solani* to correlate the role of MATE genes at different hpi (hours post-inoculation) using RNA-seq analysis and further role of putative candidate MATE gene was *in-silico* characterized through promoter analysis and protein interactome studies. The results of the present study have laid a foundation for the functional genomics aspect that can be further targeted with overexpression or knockdown of candidate MATE gene to understand the regulation mechanism of host–pathogen interactions that could help design effective strategies to combat this disease.

## Material and methods

### Data retrieval

The nucleotide and amino-acid sequences were retrieved from Rice Genome Annotation Project (http://rice.plantbiology.msu.edu) and Ensembl plant database (https://plants.ensembl.org/index.html) respectively. To identify MATE gene family members in rice, we used fifty-seven *Arabidopsis* AtMATE proteins (Table S1) as queries for the Basic Local Alignment Search Tool for Protein (BLASTP) against the rice database. Additionally, we downloaded the Pfam entry PF01554 for the MATE domain conformation using the Pfam database and the rice proteomes by HMMER 3.0 software, with an E-value cutoff of $10^{-5}$. We designated the 52 OsMATE transporters as *OsMATE1−OsMATES2* based on their physical locations in the rice genome (Table S2).

### Evolutionary relationship of MATE genes

The amino acid sequences of the functionally annotated MATE proteins from *Arabidopsis* thaliana (At1), Medicago truncatula (Mt), Nicotiana tabacum (Nt), Brassica oleracea (Bo), Brassica rapa (Br), Eucalyptus camaldulensis (Ec), Populus trichocarpa (Pt), Ricinus communis (Rc), Vitis vinifera (Vv), Glycine max (Gm), Hordeum vulgare (Hv), Oryza sativa (Os), Sorghum bicolor (Sb), Secale cereal (Sc), Vigna umbellate (Vu), Triticum aestivum (Ta), and Zea mays (Zm) were retrieved from published articles (Table S3). All these protein sequences were used for multiple sequence alignment using the MUSCLE program of MEGA X (v10.0.2) (https://www.megasoftware.net) software. The phylogenetic tree for evolutionary relationships was constructed by the neighbor-joining method with 1000 bootstrap values.

### Plant materials and inoculation with R. solani

Two genotypes of *Oryza sativa*, viz., PR114 (susceptible) and ShB-8, (moderately resistant) were used in the current study based on their response to sheath blight infection. Plants were grown in earthen pots containing sterile soil mixed in a greenhouse and maintained at a temperature of 28°C±4°C. Five days before inoculations, plants were shifted to Conviron growth chamber at 28°C±4°C day/night temperature, 16/8 h of photoperiod, and 80% relative humidity. Fifty-day-old (tillering stage) plants of PR114 and ShB-8 were mocked and single sclerotia of *R. solani* AG1-IA pathogen was inoculated at the base of the flag leaf using the detached tiller method. The inoculated plants were sprayed with water to maintain moisture for their growth and development. Plants were immediately covered with a transparent polythene bag with a hole to maintain their humidity level (Lore et al. 2013). Infected samples were collected at a time interval of 0 h (control), 24, and 48 hpi (hour post-inoculation) and were further preceded for RNA isolation. The sheath blight disease development was also evaluated under field conditions in three replicates. Maize sand meal medium with sclerotia and mycelia of *R. solani* were placed in the whorl of 50-day-old rice plants after transplanting. After 15 days of inoculations, the percent relative lesion height (lesion height/ plant height * 100) was estimated, and disease reaction was recorded on a scale of 0–9 (SES, IRRI).

### RNA-Seq analysis

The raw reads of transcriptome data were retrieved from NCBI Sequence Read Archive (SRA) with BioProject accession number PRJNA692031 (www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA692031; Kumar et al. 2021a, 2021b). The BioProject comprised RNA-seq data of different time intervals at 0 h, 24, and 48 hpi of moderately resistant genotype, ShB-8 and susceptible genotype, PR114. The Fragments per Kilobase of transcript per Million mapped reads (FPKM) showed transcripts abundance and log2 fold values were employed for hierarchical
clustering of differentially expressed OsMATE genes. The heat map of differentially expressed OsMATE genes was generated with MeV v4.4.1 software (http://mev.tm4.org).

**Table 1. List of primers sequences of six differentially expressed OsMATE genes for the qRT-PCR analysis.**

| Locus/gene name | Forward primer (5'-3') | Reverse primer (5'-3') |
|-----------------|------------------------|------------------------|
| LOC_Os02g42380/OsMATE6 | GTCGGCATGTCCTGCGTGA | ACGCGACGACGTGTTCTT |
| LOC_Os04g30490/OsMATE18 | TGCCCCATCTGTCGCTCG | CGCAGAAGTGATAGCTCCA |
| LOC_Os05g48040/OsMATE20 | GCATGCGTGCGTTCGGT | TCTGGCAGGTTCGCGGAA |
| LOC_Os07g31884/OsMATE27 | GCCGGCTCTGTCGTACGCACT | TCAAACTACGGCTGAGC |
| LOC_Os010g20470/OsMATE42 | TTGTGGACGCGCGAACCCTC | GATGGATCGCGCGAGAGA |
| LOC_Os010g37920/OsMATE43 | CATCGTGTGTCCGACCTTCA | GTAGAACGCGCGCGAGGTA |

**Transcript profiling through qRT-PCR**

The coding sequence of genes was retrieved from Rice Genome Annotation Project (http://rice.plantbiology. msu.edu/). The primers for differentially expressed genes were designed by GenScript primers designing software (https://www.genscript.com/tools/pcr-primers-designer) (Table 1). Total RNA was extracted from infected sheath tissues from PR114 and ShB-8 at 0, 24, and 48 hpi (hour post-inoculation) using the Qiagen RNeasy Mini kit as per the manufacturer’s instructions. The RNA concentration and integrity were checked by a Thermo scientific spectrophotometer (USA) and by 1.2% denaturing agarose gel, respectively. The complementary DNA (cDNA) was synthesized by a Verso cDNA synthesis kit [Thermo fisher scientific (USA)] by taking 1 μg of RNA from pooled biological samples in three technical replicates. SYBR Green-based Quantitative Real-time PCR (qRT-PCR) was performed in a Roche light cycler. β-tubulin and actin were used as an internal reference control. The PCR reaction was performed in 15 μl containing 1 μl cDNA template (50 ng), 0.5 μl (10 mM) of each specific forward and reverse primer, 6.5 μl of 2 × SYBR Premix Ex Taq (TaKaRa, Bio Inc.), and 6.5 μl double distilled water. The PCR profile was set up as initial denaturation at 95°C for 3 min followed by 40 cycles of denaturation at 95°C for 10 s, primer annealing at 64°C for 30 s, extension at 72°C for 30, and 30 s at 55°C for the melting curve analysis. The qRT-PCR analysis was performed in three technical replicates and data were analyzed through the ΔΔct method, as described by Livak and Schmittgen (2001).

**Characterization of candidate OsMATE6 gene**

The evolutionary relationship of candidate genes was studied using genomic information of various plants, including flowering and non-flowering families (rice, maize, Arabidopsis) and using ortholog and paralog information from Ensembl Plants database (http://plants.ensembl.org). To explore cis- regulating elements, genomic sequences of 1 kb upstream from TSS (transcriptional start site) of OsMATE6 gene were retrieved from the whole genome assembly of ShB-8 and PR114, which were further processed through New PLACE (https://www dna.afric.go.jp/PLACE/?action=newplace) for analyzing DNA-binding motifs. TMHMM 2.0 (http://www.cbs.dtu.dk) was used to predict helices (inside, outside, and transmembrane) to understand the protein topology of candidate genes by the Hidden Markov model. The candidate protein sequence was further processed in the STRING (https://string-db.org) database to identify an interactive protein–protein interaction network.

**Results**

**Functional divergence of MATE genes**

A phylogenetic tree was constructed using full-length protein sequences of 57 AtMATEs, 52 OsMATEs, and well-characterized 38 MATEs proteins from other studies (Table S3). These 147 MATE proteins were grouped into four major clades, namely I, II, III, and IV which consisted of 22, 56, 33, and 31 MATE genes, respectively (Figure 1). The functions of OsMATE proteins could be anticipated from the known MATE transporters according to their phylogenetic relationships. The clade I constituted ten rice MATEs, previously reported well-known MATE proteins, such as AtDS1, which negatively regulates plant disease resistance and also accumulates salicylic acid in Arabidopsis. OsMATE6 and OsMATE43 belong to clade I. Similarly, AtZFP14 and ELS1 also belonged to clade I, which was recognized for organ initiation, iron homeostasis, and hypocotyl cell elongation. Clade II included 21 OsMATEs along with MATEs from Arabidopsis, Medicago, Vigna, and Brassica. The AtFFT (clade II) was previously reported for flavonoid transporter and AtTT12 for anthocyanin transportation, and flavonoid transport in vacuoles, and also acts as an H+ antiporter. The Clade III consisted of 13 rice and 18 Arabidopsis MATEs along with previously reported AtDTX for xenobiotic efflux and NiJAT-I for nicotine efflux. Clade IV consisted of six OsMATEs along with Arabidopsis, Medicago, Glycine max, Brassica, Zea mays, Secale cereale, and Triticum aestivum. AtALF5 is responsible for xenobiotics efflux, while AtFRD3 is for aluminium and iron translocation and AtMATE for aluminum detoxification. Clade IV is comprised of previously reported OsMATE genes, such as OsFRDL1, known to play a role in iron translocation, and OsFRDL4 and OsFRDL2 for aluminum detoxification. Clade IV majorly consisted of all MATEs that belong to different plant species that indicate it as the major diverse group. One of the OsMATE genes, OsMATE27 (LOC_Os07g31884) formed a separate lineage involved in salt stress tolerance in rice.

**Impact of R. solani infection**

The disease development after R. solani infection on rice genotypes (PR114 and ShB-8) was started after 24 hpi and was observed at 48 hpi, as mycelium increases at a fast rate at this time interval. With the gradual increase in time, disease symptoms like water-soaked lesions with grey markings and irregular grey patches were observed, which eventually increases with time and leads to leaf necrosis of the plant (Figure 2(a and b)). PR114 being susceptible showed higher
lesion height than ShB-8. The average relative lesion height (RLH) of PR114 was 32.81 ± 0.60 with a disease score of 7.6. Whereas RLH was 21.38 ± 1.33 in ShB-8 with a disease score of 4.3, which was significantly less than PR114 which led to moderate resistant reaction (Table 2).

**Identification of differentially expressed genes**

The RNA-seq assembled data of differential fold values were calculated using FPKM data from PRJNA692031. Out of 52 OsMATE genes reported in rice, only six genes were expressed upon R. solani infection. The reads of other OsMATEs were not obtained in the transcriptomic data. The set of six differentially expressed MATE genes viz., LOC_Os02g45380.1 (OsMATE6), LOC_Os04g30490 (OsMATE18), LOC_Os05g48040 (OsMATE20), LOC_Os07g31884 (OsMATE27), LOC_Os10g20470 (OsMATE42), and LOC_Os10g37920 (OsMATE43) were identified based on their log2 fold value change localized on chromosomes 2, 4, 5, 7, 10 and 10, respectively (Table 3; Figure 3). Their molecular weight and isoelectric points ranged from 50.9 KD to 61.0 KD and from 7.35 to 9.38, respectively, whereas amino acids varied from 484 to 567 indicating genetic variations within the rice MATE family (Table 3). OsMATE6 gene showed 2-fold changes at 24 hpi, and ≥4.89 folds at 48 hpi in PR114. However, −4.72 folds at 24 hpi and ≥−2.69 folds at 48 hpi were observed in ShB-8. Similarly, OsMATE43 was upregulated 3.4 folds at 24 hpi and 4.88 folds at 48 hpi in PR114, whereas the expression pattern was non-significant (less than 2 folds) at 24 and 48 hpi in ShB-8. Non-significant differences in the expression level of OsMATE18 and OsMATE27 were observed between PR114 and ShB-8, while OsMATE20 and OsMATE42 genes showed significant down-regulation in ShB-8 but >1 fold change was perceived in PR114 compared to control.

![Figure 1. Phylogenetic analysis of Oryza sativa and Arabidopsis thaliana and other functionally annotated MATE genes into four different sub-groups. The red circle represents MATE genes from Oryza sativa.](image-url)
Validation of OsMATE expression profiling

The qRT-PCR-based expression footprints of differentially expressed OsMATE genes were analyzed under R. solani stress at 24 and 48 hpi, for 0 hr (control). The qRT-PCR results were in agreement with the RNAseq data. In susceptible PR114 genotype, OsMATE6 and OsMATE43 represented ≥2-fold values change, whereas OsMATE18, OsMATE20, OsMATE27, and OsMATE42 represented ≤1-fold values at 24 hpi (Table S4). At 48 hpi, most of the MATE genes showed upregulation of ≥2 fold. However, OsMATE6 and OsMATE43 showed a maximum of a 4-fold change indicating their crucial role under infection conditions (Figure 4). In contrast, the moderately resistant ShB-8 genotype depicted significantly decreased transcript abundance for all the six genes at 24 hpi. Similarly, down-regulation of MATE genes was observed at 48 hpi except for OsMATE18 which represented ≥2-fold change values compared to control (Figure 4). As per the results of transcriptome and qRT-PCR experiments, it could be concluded that OsMATE6 might have a role in sheath blight susceptibility by acting as a negative regulator channel for sheath blight resistance.

Synteny of OsMATE6 gene

The candidate OsMATE6 gene was explored for its origin through evolutionary tree patterns across the species of land plants, including flowering and non-flowering plants (orthologs and paralogs), using the Ensembl plant database. The candidate OsMATE6 gene (BGIOSGA008799) showed closer homology with O. nivara and with O. barthii, O. glumipatula, and O. meridonalis (Figure 5). However, its paralogs are also present in Commelinids and Petrosaviidae, suggesting that this gene is highly diverse and might be evolved due to neo-functionalization and cross-species transfer from O. nivara.

Characterization of cis-acting regulatory elements in the OsMATE6 promoter region

To elucidate the MATE transcriptional regulation in sheath blight resistance/susceptibility, the OsMATE6 promoter region was retrieved from the whole genome assembly of PR114 and ShB-8. Stress and hormone-responsive genes of regulators functions were depicted. A total of 219 cis-regulating elements were identified in the targeted region mostly related to abiotic and biotic stress tolerance. Abiotic and biotic stress-related cis-regulatory elements were identified, such as oxygen deficiency response gene (CURECORECR), stress response transcription factor (WRKY710S), defense-related genes (MYB1LEPR), bZIP transcription factors such as DPBF-1 and DPBF-2 (DPBF COREDCDC3), MYB-binding sites (MYBCOREATCYVB1, MYBATRD22), and for polyadenylation signal (POLASIG3). The detailed information on cis-regulatory elements of OsMATE6 promoter is given in Table S5.

Protein topology and interactome studies

The transmembrane helix prediction plugin 2.0 software was used to predict the topology of OsMATE6 protein by the N-best algorithm. Analysis of results suggests that the posterior probability of protein helix was to be inside, outside, and in transmembrane, which was summed over all the paths, and a plot was generated (Figure 6). A total of 549 amino acids were present in OsMATE6 protein, and 250 amino acids were lying in the transmembrane. The expected numbers of amino acids in the transmembrane were >18, which indicates that OsMATE6 is a transmembrane protein.

Table 2. Parameters measured for the evaluation of sheath blight disease score in PR114 and ShB-8.

| Genotype/trait | Plant height | Lesion height | Relative lesion height | Disease score | Reaction       |
|----------------|--------------|---------------|------------------------|---------------|----------------|
| PR114          | 103.3 ± 2.86 | 34 ± 4.3      | 32.81 ± 0.60           | 7.6 ± 1.15    | Susceptible    |
| ShB-8          | 127.3 ± 11.89| 27.3 ± 3.7    | 21.38 ± 1.33           | 4.3 ± 1.15    | Moderately resistant |

Values are presented as mean ± standard error (SE) (replicates, n = 3).
The protein–protein interaction map of OsMATE6 with other proteins was generated by STRING 11.0 software (Search Tool for the Retrieval of Interacting Genes/Proteins). Ten edges represented association networks that were seen to be associated with different transporter proteins and jointly contributed to shared functions (Figure 7). The protein interaction suggested that OsMATE6 (OsJ_15781) is a membrane-bound protein, belongs to the multi-antimicrobial extrusion (MATE) family. It is involved in drug/sodium antiporters. OsJ_28160 refers to LOC_Os08g0545000 and is a putatively uncharacterized protein. Os03T0859500-01 (LOC_Os03g0859500) and OsJ_28532 interacting proteins are ABC transporter family proteins. The further interacting partners are OS08T0545700-01, OS04T0636600-02, OS04T0643100-01, OS11T0177400-01, and S06T0581000-01 (putative nitrate transporter NTL1). Similarly, OsJ_01678 and OS04T0501000-00 proteins are putative 60S ribosomal protein L23A and uncharacterized, respectively, belong to the universal ribosomal protein uL23 family. The interactome analysis depicts OsMATE6 might have a role in ions and metals transportation by the ABC transporter.

**Discussion**

Sheath blight of rice is a serious problem across the globe that causes various consequential plant health issues, which can be addressed by identifying QTLs and candidate resistance genes (transcription factors and transporters) (Tiwari et al. 2014; Molla et al. 2020). Limited genetic variation for sheath blight resistance is present in rice germplasm. Even a few of the wild species of rice were moderately resistant to sheath blight. This indicates the dynamic nature of the pathogen and the complex genetic resistance mechanism. Despite the identification of various QTLs and candidate genes against sheath blight, study related to host–pathogen interactions and their impact on metabolites actuations need to be explored more especially through MATE transporters. In general, MATE transporters are believed to extrude compounds by a rocker-switcher mechanism, in which the transporter exists in two conformations: straight or bent depending on the protonation state of the acidic residues (Law et al. 2008). These conformations have a differential affinity for substrate- and ion-binding and can be inter-converted by the rearrangement of helices (Tanaka et al. 2013). In-silico analysis of the MATE gene family has been reported in Arabidopsis (Wang et al. 2016), tomato (Santos et al. 2017), cotton (Xu et al. 2019), soybean (Liu et al. 2016), potato (Huang et al. 2021), Brassica (Qiao et al. 2020), rice (Tiwari et al. 2014), and maize (Zhu et al. 2016) documented their role in plant growth and development and also under abiotic and biotic stresses. However, to our best knowledge, the genome-wide identification of the MATE gene family in rice and its potential role during R. solani infection is still unexplored.

In the present study, the comparative transcriptomic analysis revealed that six MATE genes were up-regulated in PR114 (susceptible) compared to ShB-8 (moderately resistant), which depicts that these transporters may be responsible for enhancing disease susceptibility or down-regulating resistance mechanism in plants. In the present

### Table 3. Genome coordinates of six differentially expressed OsMATEs.

| Name      | MSU locus Id | Clade | Chromosome location | CDS Coordinates (5′-3′) | Nucleotide length (bp) | Protein length (aa) | Molecular weight (KDa) | Isoelectric point (pI) |
|-----------|--------------|-------|---------------------|-------------------------|------------------------|---------------------|------------------------|------------------------|
| OsMATE6   | LOC_Os02g45380.1 | I     | 2                   | 27591163–27593013       | 1650                   | 550                 | 57.83                  | 7.34                   |
| OsMATE18  | LOC_Os04g30490 | II    | 4                   | 18212826–18216756       | 1452                   | 484                 | 50.96                  | 7.58                   |
| OsMATE20  | LOC_Os05g48040 | II    | 5                   | 27541216–27546073       | 1503                   | 501                 | 53.48                  | 7.37                   |
| OsMATE27  | LOC_Os07g31884 | U     | 7                   | 18962323–18965322       | 1701                   | 567                 | 61.66                  | 9.38                   |
| OsMATE42  | LOC_Os10g20470 | II    | 10                  | 10304395–10305755       | 1461                   | 487                 | 51.44                  | 8.3                    |
| OsMATE43  | LOC_Os10g37920 | I     | 10                  | 20307371–20308990       | 1620                   | 540                 | 56.03                  | 7.35                   |
study, OsMATE6 (LOC_Os02g45380.1) was significantly up-regulated during 48 hpi in PR114 but significantly down-regulated in ShB-8 at both 24 and 48 hpi compared to other MATE genes. The GO analysis predicted that the candidate MATE gene, OsMATE6, has a direct role in the regulation of ATPase-coupled transmembrane transport.

**Figure 4.** qRT-PCR analysis of six differentially expressed MATE genes in PR114 and ShB-8 at different time intervals of *R. solani* infection compared to control.

**Figure 5.** Evolution of OsMATE6 gene across plant species. On the left side, gene collinearity is represented with branch length and nodes of speciation. On the right, structural feature of the MATE gene in terms of homology across species.
orthologous in Arabidopsis (LOC101489496) were highly up-regulated during salinity P. expansum infection. It has been also reported that its V. inaequalis genes were down-regulated after family in apples has a diverse function in responses to bio-
Zhang et al. (2021) also predicted that the pathogenicity during the occurrence of rice blast fungus. (2013) reported that the that could function as an antiporter. Fernandez et al. (2014) revealed that over-expression of OsMATE2 gene in heterologous host Arabidopsis showed increased susceptibility to Pseudomonas syringae and changed the morphology of transgenic defining its role in the development and growth of plants. OsMATE42 is predicted as a chloroplast transmembrane arsenic detoxification protein with xenobiotic activity in rice (Norton et al. 2008).

Among differentially expressed six genes, only one gene, OsMATE6, showed a significantly higher relative gene expression in the susceptible cultivar PR114 and comparative down-regulation in moderately resistant cultivar ShB-8 upon R. solani colonization. Therefore, the OsMATE6 gene could be considered as a putative candidate gene for sheath blight susceptibility at 48 hpi when R. solani shows maximum growth of mycelium (Singh et al. 2016) and might be acting as a negative regulator for resistance. Furthermore, evolutionary characterization of the candidate OsMATE6 gene revealed that it might have originated from its wild progenitor O. nivara as it showed close homology with O. nivara (Yamanaka et al. 2003). This divergence may support the hypothesis that most of the genes have been transferred and are being utilized in rice breeding programs (Aggarwal et al. 2019).

It is well reported that cis-regulating elements also play a significant role in gene regulation (Kumar et al. 2021a, 2021b). A total of 15 cis-regulatory elements that are related to biotic stress were present in OsMATE6, suggesting these elements may play a putative role in the elicitor-induced initiation of transcription through various W box-related elements (WRKY710S, WBoxNCTCHN48, and WBoxNTERF3) and pathogen-responsive core sequence of MYB1LEPR and GCCC (Tiwari et al. 2014). These cis-regulatory elements, therefore, also act as strong regulators for the candidate OsMATE6 gene. The high number of cis-elements detected in the target gene speculates its versatile role in response to various stresses. Protein modeling suggests that OsMATE6 protein has typically 549 amino acid residues encompassing 11 transmembrane domains (TMD). Previous reports suggest that MATE proteins are membrane-bound and are important for translocation activity across membranes (Tiwari et al. 2014; Das et al. 2018). This rearrangement is facilitated through the introduction of a kink by a conserved proline residue. The binding of cations, preferably H+/Na+, changes an outward-facing conformation to an inward one, thereby either facilitating or inhibiting the binding of a compound, respectively (Lu et al. 2013).
MATEs are also known for the regulation of secondary metabolites as these compounds can be toxic to cells due to their high reactivity and protein-denaturing properties and play important roles in defense against pathogens (Freeman and Beattie 2008). The plants can protect their cellular machinery against the toxic effects of these secondary metabolites either to store them in compartments after primary processing, such as conjugation or oxidation or to expel them from the cell (Freeman and Beattie 2008). Based on this context, we have hypothetically proposed a model for the mode of action of the OsMATE6 gene for sheath blight susceptibility (Figure 8). It is well known that during pathogen attacks multi-layered defense systems are activated in plant cells. In the present study, it could be hypothesized that infection-induced stress conditions build up higher amounts of either secondary metabolites or reactive oxygen species (ROS) in PR114, and for effluxing these toxic compounds OsMATE6 gene was highly up-regulated, and energy was consumed by cells to counteract pathogen but over the time of disease development, OsMATE6 might have exhausted and not be able to efflux the large number of toxins that increased the toxicity of the cell and finally resulted in the onset of activation of R-genes. In a previous study also, it has been postulated that OsBAHD-AT genes showed higher expression in sheath blight susceptible rice genotype PR114. Also, higher hypomethylated regions of OsBAHD-AT genes are related to their higher expression in susceptible rice genotype, indicating epigenetic regulation of OsBAHD-AT genes in response to R. solani (Kumar et al. 2021a, 2021b). Therefore, it could be hypothesized that the synergistic effect of the enhanced activity of OsMATE6 and OsBAHD-AT significantly increased susceptibility in PR114 against R. solani.

**Conclusion**

The study of plant responses at different time intervals of infection in ShB-8 and PR114 is of major concern for conceptualizing the role of MATE genes in resistance/susceptibility to R. solani. Transcript profiling of MATE genes upon R. solani infection identified OsMATE6 as the potential candidate gene for sheath blight susceptibility due to its high relative transcript abundance at 48 h with more fold changes in susceptible PR114 genotype compared to moderately resistant ShB-8 genotype. It acts as a transmembrane secondary transporter protein that couples the translocation of substrates with an electrochemical gradient of cations (such as H+ or Na+ ions) across the membrane and is conserved among plant families. This study has provided a basis to understand the OsMATE6 relationship with host–pathogen interactions toward the susceptibility for sheath blight in rice that can be further validated by functional genomics.
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Conceived and designed the study: AK, JSL, YV; Computational Bioinformatics: PK, RK, RB; Gene modeling: RK, RS; Performed the experiments: RK, PK PP; Analyzed the data: RK, PK, GK; Original draft: RK, PK, GK; Finalized the manuscript: YV, KN, PK, RS. Funding: AK; All authors approved the final version.

Disclosure statement

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Data availability statement

All supporting data can be found within the manuscript and its additional files.

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