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The roles of rice microRNAs in rice-*Magnaporthe oryzae* interaction

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Abstract

MicroRNAs (miRNAs) are a class of small (20–24 nucleotides (nt) long) non-coding RNAs. One mature miRNA can be transcribed from one or more gene loci known as miRNA genes (*MIRs*). The transcript of a *MIR* forms a stem-loop structure that is processed into a 20–24-nt miRNA-5p/−3p duplex by RNase III family endoribonucleases such as Dicer-like1 (DCL1). In turn, the overhang ends of the duplex are methylated by HUA ENHANCER 1 (HEN1), generating stabilized mature miRNAs. The mature miRNAs are loaded onto ARGONAUTE (AGO) proteins, forming a miRNA-induced gene silencing complex (miRISC). Then, the miRISC binds to target sites with sequences complementary to the miRNAs, leading to either cleavage or translational inhibition of the target mRNAs, or methylation of the target sequences, resulting in post-transcriptional and transcriptional gene silencing, respectively. In the past decade, more than 700 miRNAs have been identified in rice, a subset of which have been found to be responsive to the rice blast fungus, *Magnaporthe oryzae*, or its elicitors. Moreover, members of 10 miRNA families have been found to positively or negatively regulate rice defense against *M. oryzae*, namely miR160, miR164, miR166, miR167, miR169, miR319, miR396, miR398, miR444 and miR7695. This review summarizes the identification and functional characterization of the miRNAs, which respond to *M. oryzae* or its elicitors and describes the current understanding of the complicated but well-organized network in the context of rice-*M. oryzae* interaction.

Keywords: Dicer-like 1, MicroRNA, miR164, miR167, miR169, miR319, miR396, miR398, miR7695

Background

Rice is a staple food for half of the world population and thus is one of the most important grains to secure global food supply. Rice blast is one of the most devastating diseases of rice, and hence threatens food security. Exploitation of blast-resistant cultivars is rated to be the most appropriate strategy to control this disease. Rice mounts an innate immune system consisting of two perception layers to defend itself against invasion by the blast fungus, *Magnaporthe oryzae*. The first layer of immunity, known as pathogen-associated molecular pattern- (PAMP-) triggered immunity (PTI), is activated following recognition of the fungal pattern molecule chitin, by four lysin motif- (LysM-) containing protein (LYP) receptors, i.e., chitin elicitor binding protein (CEBiP), chitin elicitor receptor kinase (CERK1), LYP4 and LYP6 (Shimizu et al. 2010; Liu et al. 2012). Virulent rice blast strains employ effectors, such as secreted LysM protein1 (Slp1), or avirulence genes, such as *AVR-Pizt*, to subvert PTI, leading to effector-triggered susceptibility (Mentlak et al. 2012; Park et al. 2016; Wang et al. 2017). In turn, rice resistance (*R*) genes recognize avirulence effectors to activate the second layer of immunity, i.e., effector-triggered immunity (ETI), to contain the *M. oryzae* infection (Bialas et al. 2018). To date, more than 30 *R* genes, most of which encode nucleotide-binding site leucine-rich repeat (NLR) family receptors, have been functionally characterized from rice, and nine associated cognate avirulence effectors from *M. oryzae* have been identified (Wang et al. 2017; Xie et al. 2019). Some of these *R* genes have been widely exploited in rice production and have made major contributions to the control of rice blast. However, activation of immunity often results in yield penalties in rice, known as fitness costs. Some recent reports have demonstrated that both PTI and ETI can be deliberately regulated by immunity-

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associated regulators that could reduce or abolish the fitness cost (Deng et al. 2017; Wang et al. 2018b; Chandran et al. 2019). MicroRNAs (miRNAs) are among such regulators capable of fine-tuning growth, development and immunity.

miRNAs are a class of small non-coding RNAs transcribed from miRNA genes (*MIRs*). To date, more than 500 *MIR* genes have been identified in the rice genome, generating more than 700 mature miRNAs (Tang and Chu 2017). *MIR* genes are transcribed into primary miRNAs (pri-miRNAs) that are spliced to form stem-loop precursor miRNAs (pre-miRNAs). The pre-miRNAs are processed into miRNA-5p/miRNA-3p (previously miRNA/miRNA*) duplex by Dicer-like1s (DCL1s) or DCL3 (Rogers and Chen 2013). While DCL3 produces mainly 24-nt miRNAs, DCL1s produce 21-nt miRNAs (Liu et al. 2005; Wu et al. 2010; Wei et al. 2014). The overhang ends of the miRNA-5p/miRNA-3p duplex are methylated by HUA ENHANCER 1 (HEN1) to stabilize the duplex (Abe et al. 2010). Then, the 21-nt and 24-nt miRNAs are loaded to ARGONAUTE 1 s (AGO1s) and AGO4, respectively, forming miRNA-induced gene silencing complex (miRISC) (Seo et al. 2013). Whereas the miRNA-AGO1 miRISC mediates sequence-complementary transcript cleavage or translational inhibition, the miRNA-AGO4 miRISC mediates target site methylation (Wu et al. 2009; Wu et al. 2010). Thus, miRNAs negatively regulate the expression of their target genes at either the transcriptional or post-transcriptional level (Fig. 1).

Evidence from Arabidopsis has demonstrated the fine-tuning roles of miRNAs in both PTI and ETI. The conserved flagellin peptide flg22, a PAMP molecule, induces the expression of a subset of miRNAs, including miR160 and miR393 (Li et al. 2010a). miR393 is the first miRNA to be identified to act in both PTI and ETI against *Pseudomonas syringe* pv. *tomato* (*Pst*) DC3000 via targeting of the auxin receptor genes *TIR1*, *AFB2* and *AFB3* to block auxin signaling. On the one hand, the expression of miR393 is induced by flg22 to promote basal defense against bacterial strains (Navarro et al. 2006). The expression of miR393 can be suppressed by AvrPto and AvrPtoB, two bacterial effectors that can subvert PTI (Navarro et al. 2008). On the other hand, both miR393-5p and miR393-3p can be up-regulated during

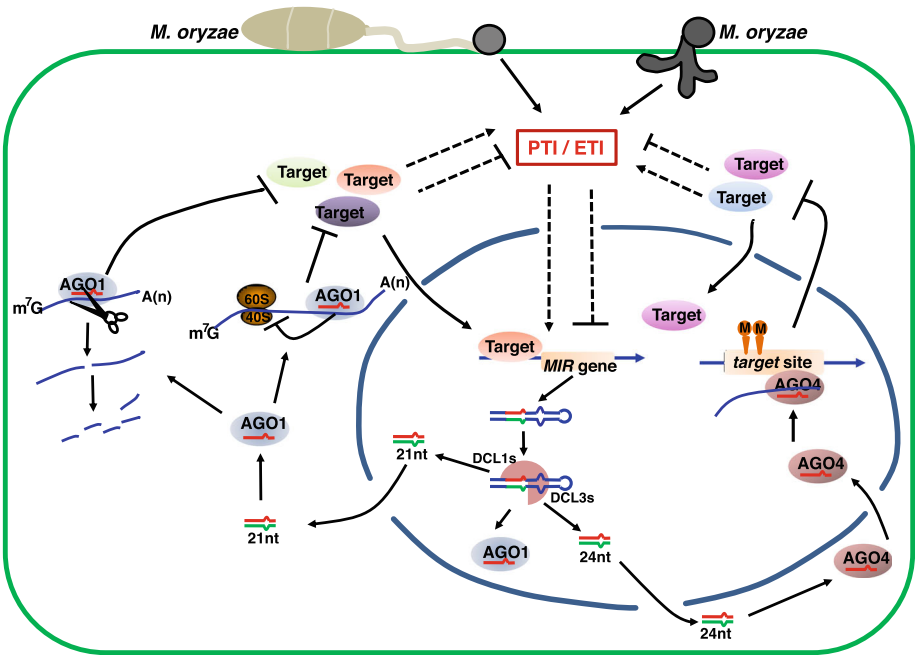

Fig. 1 Hypothetical microRNA-mediated feedback regulation of rice immunity against *Magnaporthe oryzae*. Infection by *M. oryzae* activates pathogen-associated molecular pattern- (PAMP-) triggered immunity (PTI) and/ or effector-triggered immunity (ETI). During PTI/ETI, a subset of miRNA genes (*MIRs*) can be induced or suppressed, depending on their roles as positive or negative regulators, respectively. The *MIRs* are transcribed and processed by the RNase III family endoribonucleases such as Dicer-like1s (DCL1s) and DCL3s. While DCL3s mainly produce 24-nt (nucleotide) miRNAs, DCL1s produce 21-nt miRNAs. After modification by a series of regulators, the mature single strand 21-nt and 24-nt miRNAs are loaded into ARGONAUTE1 (AGO1) and ARGONAUTE 4 (AGO4), respectively, forming miRNA-induced gene silence complex (miRISCs). The miRISC could then hypothetically mount feedback regulation on PTI/ETI at several layers. First, the 24-nt-AGO4 miRISC re-enters the nucleus to mediate methylation of the DNA sequences complementary to the 24-nt miRNAs, resulting in transcriptional gene silencing of target genes that act in the regulation of PTI/ETI. Second, the 21-nt-AGO1 miRISC mediates the cleavage or translational inhibition of the target mRNAs, leading to post-transcriptional gene silencing of target genes that act in the regulation of PTI/ETI. Third, some target genes of miRNAs are transcription factors that can regulate the transcription of *MIR* genes and/or genes acting in the regulation of PTI/ETI, forming feedback regulatory networks

ETI as a result of the recognition of the avirulent effector AvrRpt2 by the R receptor RPS2 (Zhang et al. 2011). Whereas miR393-5p is loaded onto AGO1, miR393-3p is loaded onto AGO2 and mediates suppression of a SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) gene, *MEMB12*, to promote immunity against this bacterial pathogen (Zhang et al. 2011). Therefore, a miRNA may be involved in both PTI and ETI, and thus be involved in host-pathogen "arms race". In addition, several miRNAs directly target resistance genes encoding NLR family proteins that mediate ETI, such as miR2109 in *Medicago* (Zhai et al. 2011), miR482 and miR2118 in tomato (Shivaprasad et al. 2012) and miR472 in Arabidopsis (Boccara et al. 2014). miR472 targets *NLR*s, such as *RPS5* that recognizes the effector AvrPphB to activate ETI against *P. syringae* in Arabidopsis (Boccara et al. 2014). In rice, miRNAs are well-documented in fine-tuning many biological processes to control many important agronomic traits, such as tiller development, flowering time, panicle establishment, grain formation and yield production, as well as responses to biotic and abiotic stresses (Wu et al. 2015; Bakhshi et al. 2016; Tang and Chu 2017; Wu et al. 2017; Tripathi et al. 2018; Yao et al. 2019). Increasing numbers of reports have shown that miRNAs also play important roles in rice-blast fungus interactions. Because detailed reviews on the biogenesis and functions of miRNAs have been published (Xie et al. 2015; Baldrich and San 2016; Huang et al. 2016b; Tang and Chu 2017; Song et al. 2019), we focus in the current review on rice miRNAs that are responsive to *M. oryzae* or its elicitors, and summarize our understanding of their roles in rice-*M. oryzae* interaction.

Core components of the miRNA signaling pathway involved in rice-*M. oryzae* interaction

DCLs and AGOs are two key groups of core component proteins in the miRNA signaling pathway, encoded separately by distinct groups of genes. The rice genome contains eight *DCL* and 19 *AGO* genes (Kapoor et al. 2008). Evidence is emerging that both *DCL*s and *AGO*s play important regulatory roles in rice immunity against *M. oryzae*. For example, *AGO4* has been demonstrated to regulate the expression of the blast-susceptibility NLR receptor gene *PigmS*, which, in turn, balances the trade-off between yield formation and activation of immunity against *M. oryzae* mediated by the broad-spectrum blast resistance gene *PigmR* (Deng et al. 2017), indicating that *AGO4* positively regulates *PigmR*-mediated ETI. However, the roles of the other AGOs in rice immunity against *M. oryzae* remain to be elusive. Among the eight *DCL*s, *OsDCL1a*, *OsDCL1b*, *OsDCL2a*/*b*, *OsDCL3a* and *OsDCL4* have been shown to be responsive to *M. oryzae* (Zhang et al. 2015; Salvador-Guirao et al. 2019), implying an involvement in rice-*M. oryzae* interaction.

To date, of the eight rice *DCL* genes, only *OsDCL1a* has been deeply investigated, being found to negatively regulate PTI against *M. oryzae*. Knock-down of *OsDCL1a* led to enhanced resistance to the blast disease (Zhang et al. 2015). This resistant phenotype could be due to constitutive activation of defense-related genes, such as 13 *Pathogenesis-related* genes (*PR*s) and the PTI-related genes *OsKS4* and *OsNAC4*, as well as higher and faster production of H_2O_2 in the knock-down mutant than in the control (Zhang et al. 2015). By contrast, up-regulation of *OsDCL1a* led to increased susceptibility to both *M. oryzae* and the bakanae disease fungus *Fusarium fujikuroi* in an activation-tagged mutant (Salvador-Guirao et al. 2019). Moreover, such activation mutation also resulted in reprogramming of both the transcriptome and the miRNAome, leading to suppression of defense-related genes, phytoalexin biosynthesis and ROS detoxification (Salvador-Guirao et al. 2019). These reports indicate that the functions of *DCL1* in rice differ from those in Arabidopsis, in which *DCL1* is required for PTI against bacterial pathogens (Navarro et al. 2008).

Consistent with the function of *OsDCL1* in the biogenesis of miRNAs, the up-regulation of this gene remarkably influenced the expression of more than 90 miRNAs from 61 miRNA families, including up-regulation and down-regulation of a subset of miRNAs, which, in turn, resulted in altered expression of 216 genes in the categories of "biotic stress," "signaling," and "metabolism" (Salvador-Guirao et al. 2019).

Therefore, *OsDCL1*s seem to act as a key node in the miRNA signaling pathway, mediating crosstalk between the miRNA network and rice-*M. oryzae* interaction (Fig. 1).

Identification of rice miRNAs responsive to *M. oryzae* or its elicitors

To investigate the roles of miRNAs in rice-*M. oryzae* interaction, it is vital to first identify those miRNAs that are responsive to *M. oryzae* or its elicitors. A number of research groups have independently identified such miRNAs, as a result of using one of three methods described below. In the first approach, rice tissues were treated with *M. oryzae* mycelial extracts containing the fungal elicitors. Samples from mock- and elicitor-treated rice tissues were collected for miRNA analysis. Candidate miRNAs were those showing altered accumulation between elicitor- and mock-treated samples. Using this method, some conserved miRNAs/miRNA families and novel miRNAs were found to be responsive to the blast fungal elicitors, suggesting their possible involvement in rice-*M. oryzae* interaction (Baldrich et al. 2015). These miRNAs have been shown to target a wide range of

genes involved in a number of pathways, via degradomics analysis (Baldrich et al. 2015). In the second strategy, rice seedlings were inoculated with a virulent *M. oryzae* strain, following which samples were collected for miRNA analysis. Candidate miRNAs were those showing altered abundance following inoculation with *M. oryzae*. Using this method, the expression levels of a number of miRNAs were found to be significantly altered in the rice accession Nipponbare (NPB) or its mutant following infection with *M. oryzae* (Zhang et al. 2015; Wang et al. 2018a; Zhang et al. 2018). In the third approach, rice seedlings from one susceptible and one resistant accession were inoculated with a *M. oryzae* strain, with samples subsequently collected for miRNA analysis. Candidate miRNAs were those showing altered abundance between the susceptible and resistant accessions in response to *M. oryzae* infection. Using this method, a subset of miRNAs was found to be differentially expressed in the susceptible and resistant accessions, suggesting their possible roles in rice-*M. oryzae* interaction (Li et al. 2014; Li et al. 2016b; Dong et al. 2018).

To date, more than 70 miRNAs from 61 *MIR* families have been identified to be responsive to *M. oryzae* or its elicitors (Table 1). These miRNAs have been predicted to regulate various signaling pathways by targeting a wide range of genes, including those involved in the biogenesis of small RNAs, auxin signaling, salicylic acid (SA)/jasmonic acid (JA)/ethylene (ET) signaling, and ROS signaling, as well as genes containing upstream open reading frames (Baldrich et al. 2015; Wang et al. 2018a; Zhang et al. 2018; Li et al. 2019a). Intriguingly, candidate miRNAs responsive to *M. oryzae* included members from the miR162 and miR168 families (Campo et al. 2013; Li et al. 2014). It is known that miR162 regulates *DCL1* and miR168 controls *AGO1* (Wu et al. 2015; Zhang et al. 2015), suggesting that the miRNA signaling pathway plays important roles in rice immunity against *M. oryzae*. The identification of these miRNAs opens up a door to explore the fine-tuning mechanisms underlying rice-*M. oryzae* interaction.

The microRNAs that play positive roles in rice immunity against *M. oryzae*

To date, members of four miRNA families have been reported to act as positive regulators of rice immunity against *M. oryzae*, namely miR160, miR166, miR398 and miR7695.

The miR160 family is a highly conserved miRNA family, consisting of six loci in the rice genome and being capable of generating three mature isoforms (Table 1). The abundance of each of the three mature isoforms was altered in response to infection by *M. oryzae* or treatment with its elicitors (Campo et al. 2013; Li et al. 2014). The mature miR160a/b/c/d isoform seems to be transcribed mainly from *OsMIR160a* and *OsMIR160b* and accumulates to greater concentrations than miR160e and miR160f in leaves (Li et al. 2014; Huang et al. 2016a). miR160 targets five *Auxin Response Factor*s (*ARF*s) transcription factor genes, namely *ARF8* (*LOC_Os02g41800*), *ARF10* (*LOC_Os04g43910*), *ARF13* (*LOC_Os04g59430*), *ARF18* (*LOC_Os06g47150*) and *ARF22* (*LOC_Os10g33940*) (Wu et al. 2009; Li et al. 2010b). Transgenic lines overexpressing miR160a showed reduced susceptibility to *M. oryzae*, a phenotype which was associated with suppression of the expression of target genes such as *ARF8*, *ARF10* and *ARF13*, indicating that miR160 positively regulates rice blast disease resistance via *ARF*s that may involve auxin signaling; this finding, is also, consistent with the opinion that auxin signaling antagonizes immunity. However, the mechanism by which suppression of *ARF*s leads to increased resistance remains to be elucidated.

The miR166 family has 13 members in the rice genome, forming six mature isoforms (Table 1). The function of miR166 has been reported in plant development, targeting transcription factor genes of the class III homeodomain-leucine zipper family, such as *PHABULOSA* and *PHABOLUTA*, to specify the fate of the shoot apical meristem (Zhu et al. 2011; Li et al. 2019b). Its function in plant immunity has been reported only in rice. The difference in expression between resistant and susceptible accessions or in response to *M. oryzae* or its elicitors varied between individual miR166 members. For example, miR166m was down-regulated following treatment with *M. oryzae* elicitors (Campo et al. 2013). The expression of miR166k−/l-3p was constitutively higher in the susceptible accession Lijiangxin Tuan Hegu (LTH) than in the resistant accession IRBLkm-Ts, but was up-regulated in both accessions in response to infection by *M. oryzae* (Li et al. 2014). In the intermediate susceptible/resistant accession NPB, expression of both miR166k-3p and miR166j-3b was up-regulated following *M. oryzae* infection (Zhang et al. 2018). In a knockdown *dcl1* mutant, expression of miR166j-5 was down-regulated, whereas, expression of miR166a/b/c/d-3p/f was up-regulated following *M. oryzae* infection (Zhang et al. 2015). The expression patterns suggest that the functions may differ among different members of the miR166 family. However, in-depth functional investigation has been carried out only with respect to the miR166k-166 h polycistron, that co-expresses miR166k and miR166h (Baldrich et al. 2016). Up-regulation of miR166k and miR166h from an activation mutant *miR166k-166 h-Ac* led to increased resistance to rice blast disease, a phenotype which was associated with activation of the ET-signaling pathway and high marked up-regulation of defense-related genes, although

Table 1 miRNAs responsive to *Magnaporthe oryzae* or its elicitors[a]

miRNA	Loci	Mature length (nt)	Mature isoforms	Target gene	Literature
miR156	13	20, 21	3	*SPL13* (*LOC_Os07g32170*); *SPL18* (*LOC_Os09g32944*); *SPL19* (*LOC_Os11g30370*); *SPL14* (*LOC_Os08g39890*); *SPL3* (*LOC_Os02g04680*); *SPL4*(*LOC_Os02g07780*)	Campo et al. 2013; Li et al. 2014; Baldrich et al. 2015; Zhang et al. 2015; Li et al. 2016b; Wang et al. 2018a; Zhang et al. 2018
miR159	6	21	6	*GAmyb* (*LOC_Os01g59660, LOC_Os06g40330*); *Zinc finger* (*LOC_Os10g05230*)	Li et al. 2014; Xu et al. 2014; Li et al. 2016b
miR160	6	21	3	*ARF8* (*LOC_Os02g41800*); *ARF10* (*LOC_Os04g43910*); *ARF13* (*LOC_Os04g59430*); *ARF18* (*LOC_Os06g47150*); *ARF22* (*LOC_Os10g33940*)	Campo et al. 2013; Li et al. 2014; Xu et al. 2014; Baldrich et al. 2015; Li et al. 2016b
miR162	2	21	2	*DCL1a* (*LOC_Os03g02970*)	Li et al. 2014; Zhang et al. 2015; Li et al. 2016b; Wang et al. 2018a; Zhang et al. 2018
miR164	6	21	4	*MTN4* (*LOC_Os06g46270*); *MTN6* (*LOC_Os08g10080*); *MTN5* (*LOC_Os06g23650*); *MTN3* (*LOC_Os12g41680*); RGHIA; *Salicylic acid-induced protein 19* (*LOC_Os12g41680*)	Campo et al. 2013; Li et al. 2014; Xu et al. 2014; Li et al. 2016b; Wang et al. 2018a
miR166	13	21	6	*START domain-containing protein* (*LOC_Os03g01890*); *Homeobox-leucine zipper protein* (*LOC_Os03g43930, LOC_Os10g33960, LOC_Os12g41860*); *Vacuolar protein 8* (*LOC_Os06g01304*)	Campo et al. 2013; Li et al. 2014; Xu et al. 2014; Zhang et al. 2015; Li et al. 2016b; Zhang et al. 2018
miR167	10	21	2	*ARF6* (*LOC_Os02g06910*); *ARF12* (*LOC_Os04g57610*); *ARF17* (*LOC_Os06g46410*); *ARF25* (*LOC_Os12g41950*); *Retinol dehydrogenase 14* (*LOC_Os06g03830*); *Zinc finger protein 207* (*LOC_Os09g38790*)	Liu et al. 2012; Campo et al. 2013; Li et al. 2014; Xu et al. 2014; Zhang et al. 2015; Li et al. 2016b; Wang et al. 2018a
miR168	2	21, 24	3	*AGO1a* (*LOC_Os02g45070*); *AGO1b* (*LOC_Os04g47870*); *AGO1c* (*LOC_Os02g58490*); *AGO1d* (*LOC_Os06g51310*)	Campo et al. 2013; Li et al. 2014; Zhang et al. 2015; Li et al. 2016b
miR169	17	21, 22	9	*NF-YC1* (*LOC_Os02g07450*); *NF-YC2* (*LOC_Os03g14669*); *NF-YC3* (*LOC_Os04g58680*); *NF-YC4* (*LOC_Os06g45640*); *NF-YA11* (*LOC_Os02g53620*); *NF-YA10* (*LOC_Os12g42400*)	Campo et al. 2013; Li et al. 2014; Zhang et al. 2015; Li et al. 2016b; Wang et al. 2018a; Zhang et al. 2018
miR171	9	21	10	*SCARECROW gene regulator* (*LOC_Os06g01620, LOC_Os04g44360, LOC_Os04g46860*); *AP2 domain containing TF (ERF#043-ERF#073-ERF#090)*	Campo et al. 2013; Li et al. 2014; Xu et al. 2014; Baldrich et al. 2015; Li et al. 2016b
miR172	4	20, 21	4	*Retrotransposon protein* (*LOC_Os05g43220*); *SSH1* (*LOC_Os07g13170*); *ARF9* (*LOC_Os04g36054*); *AP2-like factor* (*LOC_Os03g60430, LOC_Os04g55560, LOC_Os05g03040, LOC_Os07g13170*); *Putative zinc-finger motif* (*LOC_Os09g21770*)	Li et al. 2014; Xu et al. 2014; Baldrich et al. 2015; Li et al. 2016b; Wang et al. 2018a
miR319	2	20, 21	1	*TCP21* (*LOC_Os12g07480*); *myb proto-oncogene protein* (*LOC_Os01g59660*)	Xu et al. 2014; Li et al. 2016b; Zhang et al. 2018
miR390	1	20, 21	2	*STRUBBELIG-RECEPTOR FAMILY 6* (*LOC_Os03g51040*); *Wall-associated receptor kinase-like 10* (*LOC_Os04g30060*); *Leucine-rich repeat family protein* (*LOC_Os04g45170*)	Campo et al. 2013; Li et al. 2016b
miR393	2	21, 22	2	*TIR1* (*LOC_Os05g05800*); *AFB2* (*LOC_Os04g32460*)	Campo et al. 2013; Li et al. 2014; Baldrich et al. 2015; Li et al. 2016b
miR394	1	20	1	*FBX32* (*LOC_Os01g69940*); *RNA polymerase sigma factor rpoD* (*LOC_Os05g51150*)	Campo et al. 2013; Li et al. 2016b; Wang et al. 2018a
miR395	25	21, 22	10	*Cytochrome b5-like Heme/steroid binding domain containing protein* (*LOC_Os10g35870*); *Bifunctional 3-phosphoadenosine 5-phosphosulfate synthetase* (*LOC_Os03g53230*); *Protein kinase domain containing protein* (*LOC_Os05g44290*)	Xu et al. 2014; Baldrich et al. 2015; Li et al. 2016b
miR396	8	21, 22	5	*GRF6* (*LOC_Os03g51970*); *GRF7* (*LOC_Os12g29980*); *GRF8* (*LOC_Os11g35030*); *GRF9* (*LOC_Os03g47140*); *GRF2* (*LOC_Os06g10310*); *GRF6* (*LOC_Os03g51970*); *Deaminase* (*LOC_Os06g29430*)	Campo et al. 2013; Li et al. 2014; Zhang et al. 2015; Li et al. 2016b
miR398	2	21	2	*CSD1* (*LOC_Os03g22810*), *CSD2* (*LOC_Os07g46990*), *CSD3* (*LOC_Os03g11960*), *CSD4* (*LOC_Os08g44770*)	Campo et al. 2013; Li et al. 2014; Li et al. 2016b; Wang et al. 2018a
miR435	1	20	1	*COBRA-like protein precursor* (*LOC_Os07g41320*) (predicted)	Li et al. 2014; Baldrich et al. 2015; Li et al. 2016b; Zhang et al. 2018
miR439	10	21	1	*Chl9* (*LOC_Os03g36540*); *Cyclin-dependent kinase G-1* (*LOC_Os02g39010*)	Li et al. 2014; Xu et al. 2014; Li et al. 2016b

Table 1 miRNAs responsive to *Magnaporthe oryzae* or its elicitors[a] *(Continued)*

miRNA	Loci	Mature length (nt)	Mature isoforms	Target gene	Literature
miR444	6	21	6	*MADS23* (*LOC_Os08g33488*); *MADS27* (*LOC_Os02g36924*); *MADS57* (*LOC_Os02g49840*)	Campo et al. 2013; Li et al. 2014; Li et al. 2016b; Zhang et al. 2018
miR528	2	21	2	*Laccase* (*LOC_Os01g62600*); *Copper ion binding protein* (*LOC_Os01g03620, LOC_Os01g03640*)	Campo et al. 2013; Li et al. 2014; Li et al. 2016b
miR529	2	20, 21	2	*SBP-box gene family member* (*LOC_Os02g07780*)	Campo et al. 2013; Baldrich et al. 2015; Li et al. 2016b
miR530	1	20	1	*Transcriptional regulator* (*LOC_Os05g34720*); *Expression protein* (*LOC_Os01g52920*); *Cyclin-T1–2*; *LORICRIN*	Li et al. 2014; Baldrich et al. 2015; Li et al. 2016b
miR535	1	21	2	*Expressed protein* (*LOC_Os02g09080, LOC_Os03g14880*); *Squamosa promoter-binding-like protein 11* (*LOC_Os06g45310*)	Li et al. 2014; Xu et al. 2014; Li et al. 2016b
miR810	2	21	3	*FBX160* (*LOC_Os05g07950*); *Retrotransposon protein* (*LOC_Os02g38610*) (predicted)	Baldrich et al. 2015; Li et al. 2016b
miR812	22	21, 22, 24	15	*Calcium/calmodulin dependent protein kinase* (*LOC_Os03g22050*)	Li et al. 2014; Li et al. 2016b
miR818	6	21, 22	3	*RDR2* (*LOC_Os04g39160*)	Baldrich et al. 2015; Li et al. 2016b
miR820	3	21	1	*DRM2* (*LOC_Os03g02010*); *Cellulose synthase like C12* (*LOC_OS11g13650*)	Campo et al. 2013; Li et al. 2016b
miR827	2	21	2	*SPX-MFS1* (*LOC_Os04g48390*); *SPX-MFS2* (*LOC_Os02g45520*); *WAK receptor-like protein kinase* (*LOC_Os02g56370*)	Campo et al. 2013; Li et al. 2014; Li et al. 2016b
miR1319	2	24	2	*LTPL6* (*LOC_Os10g05720*)	Baldrich et al. 2015; Li et al. 2016b
miR1320	1	21	1	*Ethylene-responsive transcription factor* (*LOC_Os10g41330*); *ANTHOCYANIDIN 3-O-glucosyltransferase*	Li et al. 2014; Wang et al. 2018a
miR1423	2	21, 24	2	*Expressed protein* (*LOC_Os11g28540*); *Heat shock protein DnaJ* (*LOC_Os06g09560*)	Baldrich et al. 2015; Li et al. 2016b
miR1425	1	21	1	*Rf4* (*LOC_Os10g35240*); *Rf6* (*LOC_Os10g35436*); *Protein kinase* (*LOC_Os01g49614*)	Campo et al. 2013; Li et al. 2014; Baldrich et al. 2015; Li et al. 2016b
miR1427	1	21	1	*YGL8* (*LOC_Os01g17170*)	Xu et al. 2014; Baldrich et al. 2015; Li et al. 2016b
miR1430	1	21	1	*ASYMMETRIC LEAVES 2* (*LOC_Os05g34450*); *myb/SANT domain protein* (*LOC_Os03g13790*)	Campo et al. 2013; Li et al. 2016b
miR1433	1	21	1	*NAD dependent epimerase/dehydratase family protein* (*LOC_Os09g15420*), *Retrotransposon protein* (*LOC_Os04g17640*) (predicted)	Li et al. 2014; Li et al. 2016b
miR1437	2	21, 22, 24	3	*Ubiquitin carboxyl-terminal hydrolase domain containing protein* (*LOC_Os06g0184300*); *FAD2–1*(*LOC_Os02g48560*)	Xu et al. 2014; Baldrich et al. 2015; Li et al. 2016b
miR1846	5	20, 21, 22	3	*Expression protein* (*LOC_Os08g10350*); *Anther-specific proline-rich protein* (*LOC_Os02g18870*); Zinc-finger, C3H4 type domain containing TF	Baldrich et al. 2015; Li et al. 2016b
miR1847	2	21, 24	2	*AGO4b* (*LOC_Os04g06770*)	Baldrich et al. 2015; Li et al. 2016b
miR1850	3	21, 22	3	*Pectinesterase inhibitor domain* (*LOC_Os08g04650*); *RDR2*; *NB-ARC*	Campo et al. 2013; Li et al. 2016b
miR1854	1	21, 22	2	*Glucose-6-phosphate 1-dehydrogenase* (*LOC_Os03g20300*) (predicted)	Baldrich et al. 2015; Li et al. 2016b
miR1858	2	21	1	*CSLC9-cellulose synthase-like family C* (*LOC_Os03g56060*) (predicted)	Baldrich et al. 2015; Li et al. 2016b
miR1861	15	22	8	*Starch binding domain containing protein* (*LOC_Os01g63810*); *ATP binding protein* (*LOC_Os05g51790*)	Li et al. 2014; Baldrich et al. 2015; Li et al. 2016b
miR1862	7	20, 24	4	*SART-1 family protein* (*LOC_Os02g30730*)	Zhang et al. 2015; Li et al. 2016b
miR1863	3	23, 24	4	*Predicted expression protein* (*LOC_Os05g13804*)	Li et al. 2014; Li et al. 2016b; Wang et al. 2018a
miR1865	1	24	2	*Aspartate aminotransferase* (*LOC_Os02g14110*)	Campo et al. 2013; Li et al. 2016b
miR1867	1	24	1	*tRNA methyltransferase* (*LOC_Os10g30550*)	Li et al. 2014; Li et al. 2016b
miR1871	1	24	1	*Ribosomal protein L7Ae* (*LOC_Os09g32976*)	Li et al. 2014; Li et al. 2016b

Table 1 miRNAs responsive to *Magnaporthe oryzae* or its elicitors[a] *(Continued)*

miRNA	Loci	Mature length (nt)	Mature isoforms	Target gene	Literature
miR1874	1	24	2	*Expression protein* (*LOC_Os02g20950*)	Li et al. 2014; Baldrich et al. 2015; Li et al. 2016b
miR1876	1	24	1	*Esterase/lipase/thioesterase* (*LOC_Os02g31200*)	Campo et al. 2013; Li et al. 2016b
miR1879	1	24	1	*Catalase isozyme B* (*LOC_Os06g51150*)	Campo et al. 2013; Li et al. 2016b
miR1882	8	24	2	*ABC transporter, ATP-binding protein* (*LOC_Os11g39020*) (predicted)	Zhang et al. 2015; Li et al. 2016b
miR2871	2	21	1	*Glycosyltransferase family 43 protein* (*LOC_Os10g13810*); Zinc finger, C3HC4 type, domain containing TF	Xu et al. 2014; Baldrich et al. 2015
miR2873	3	21, 24	3	*TKL_IRAK_CrRLK1L_1.4* (*LOC_Os01g06280*) (predicted)	Li et al. 2014; Li et al. 2016b
miR2878	1	24	2	*Plastocyanin-like domain containing protein* (*LOC_Os08g37670*)	Li et al. 2014; Li et al. 2016b
miR5150	1	24	2	*Receptor-like protein kinase 2 precursor* (*LOC_Os01g70260*)	Li et al. 2016b; Zhang et al. 2018
miR5153	1	24	1	*NHX1* (*LOC_Os07g47100*); *Chloroplast ribonuclease III domain protein* (*LOC_Os01g59510*)	Li et al. 2016b; Wang et al. 2018a
miR5794	1	21	1	*Rapid ALkalinization Factor14* (*RALF14, LOC_Os11g26880*); *RALF4* (*LOC_Os12g35670*)	Zhang et al. 2015; Li et al. 2016b
miR7695	1	21, 24	5	*OsNramp6* (*LOC_Os01g31870*)	Campo et al. 2013

[a]This list only includes those miRNAs that are functionally characterized or reported by two or more papers

miR166k-5p suppresses the expression of two *OsEIN2* genes (Salvador-Guirao et al. 2018). This finding was consistent with an involvement of ET signaling in rice immunity against *M. oryzae* (Yang et al. 2017), although it was unclear how suppression of *OsEIN2*s led to activation of the ET-signaling pathway.

The miR398 family has two members in rice genome, generating two mature isoforms (Table 1). This family is highly conserved in both monocotyledons and dicotyledons, including rice, wheat, barley, switchgrass, Arabidopsis, *Brassica rapa* and *Populus trichocarpa*. miR398 targets four members of the 15 superoxidase dismutase (SOD) gene family in rice, i.e., *CSD1*, *CSD2*, *SODX* and *CCSD* (Li et al. 2019a). Overexpression of miR398b led to increased resistance to the rice blast disease, in association with the down-regulation of all four target genes, up-regulation of defense-related genes and increased accumulation of H_2O_2 (Li et al. 2014). By contrast, blocking miR398b by overexpressing a target gene mimic resulted in increased susceptibility, which was associated with up-regulation of all four target genes, delayed induction of defense-related genes and reduced H_2O_2 accumulation (Li et al. 2019a). Detailed analysis indicated that *CSD1*, *CSD2* and *SODX* negatively regulated H_2O_2 accumulation, whereas *CCSD* positively regulated it; the loss-of-function mutants *csd1*, *csd2* and *sodx* exhibited increased susceptibility. *CSD1*, *CSD2* and *SODX* appear to determine the production of H_2O_2 by coordinately regulating the expression of the other *SOD* family members and hence the total SOD enzyme activity. In contrast, *CCSD* is probably required for CSD enzyme activity because the *ccsd* mutant exhibited little CSD activity (Li et al. 2019a). Therefore, miR398 fine-tunes ROS signaling through these four *SOD* members to regulate rice immunity against *M. oryzae*. Nevertheless, the function of miR398 in rice immunity obviously differs from those in Arabidopsis and barley, where miR398 negatively regulates immunity to the bacterial pathogen *Pst* DC3000 and the powdery mildew pathogen *Blumeria graminis* f. sp. *hordei*, respectively (Li et al. 2010a; Xu et al. 2014). Therefore, the detailed functions of miR398 need to be further investigated in different phytopathological systems.

miR7695 was the first miRNA to be identified as being responsive to the elicitors of *M. oryzae*, and is expressed in leaves throughout the rice vegetative growth stage (Campo et al. 2013). Its abundance is reduced in the *dcl4*, but not in *dcl1* mutant, indicating that *MIR7695* is a recently evolved *MIR* locus, the primary transcript of which is processed by *DCL4*, producing multiple miRNAs (Campo et al. 2013). Indeed, five mature miR7695s were detected by northern blotting analysis (Campo et al. 2013). Mature miR7695.3-3p targets one transcript of *NRAMP6* (*Natural resistance-associated macrophage protein 6*), a locus that generates eight transcripts as a result of alternative splicing (Campo et al. 2013). miR7695 seems to occur specifically in rice, particularly in the *japonica* subspecies and in some *indica* accessions. Its expression is higher in blast-resistant accessions than in blast-susceptible ones, and is further up-regulated in response to infection by *M. oryzae* (Quoc et al. 2019). Overexpression of its precursor leads to increased resistance to *M. oryzae*. Therefore, miR7695 plays positive roles in rice immunity against *M. oryzae*. Consistent with this role of miR7695, loss-of-function mutants of its target gene *NRAMP6* exhibited reduced

plant growth but increased resistance to *M. oryzae* (Peris-Peris et al. 2017). *NRAMP6* encodes a metal transporter that has iron and manganese transport activity. Therefore, it is presumed that *NRAMP6* acts via metal homeostasis, linking immunity to *M. oryzae* to rice plant growth, a process which is fine-tuned by miR7695.

Taken together, some miRNAs played positive roles in regulating rice immunity against *M. oryzae* via regulating a number of signaling pathways, such as auxin signaling, ethylene signaling, ROS signaling and metal homeostasis.

The microRNAs that facilitate infection by *M. oryzae* in rice

Currently, members of six miRNA families have been reported to facilitate the infection by *M. oryzae*, namely miR164, miR167, miR169, miR319, miR396 and miR444.

The miR164 family is a highly conserved miRNA family containing six loci, which generate four mature isoforms, targeting six genes encoding NAC (NAM, ATAF1/2 and CUC2) transcription factors in rice (Fang et al. 2014). The miR164-NAC regulatory module functions in ET-signaling to control leaf senescence in Arabidopsis and drought tolerance in rice (Li et al. 2013; Fang et al. 2014). Recently, evidence has emerged of its roles in rice-*M. oryzae* interactions. The abundance of miR164a/b/f was altered in response to infection by *M. oryzae* or treatment with the blast fungus elicitors (Campo et al. 2013; Li et al. 2014; Wang et al. 2018a). Overexpressing miR164a led to increased susceptibility to *M. oryzae*, failing to up-regulate the expression of defense-related genes and H_2O_2 production following infection by *M. oryzae* (Wang et al. 2018a). Consistently, mutation in one target gene, *OsNAC60*, resulted in increased susceptibility to *M. oryzae*. In contrast, transient overexpression of *OsNAC60* activated cell death that was associated with H_2O_2 production in *Nicotiana benthamiana* (Wang et al. 2018a). Therefore, miR164 negatively regulated rice immunity to *M. oryzae* via at least one of its targeted *NAC* transcription factor genes coupled to ET signaling. However, it is unclear whether the other target genes were involved in miR164-mediated regulation of rice-*M. oryzae* interaction.

In rice, the miR167 family has 10 members, forming two mature isoforms that target four *Auxin Responsive Factor*s and three other genes, including one encoding a NLR family protein (Zhao et al. 2019). Over-expressing miR167d resulted in increased susceptibility to *M. oryzae* in a resistant accession genetic background, a phenomenon that was associated with failure to induce defense-related genes and consequent reduced H_2O_2 production (Zhao et al. 2019). In contrast, blocking miR167d by expressing a target mimic led to increased resistance to *M. oryzae*, associated with high expression of defense-related genes and increased H_2O_2 production. The miR167d-mediated regulation of immunity seemed to be associated with neither *ARF25* (*LOC_Os12g41950*) nor the gene encoding the NLR protein (*LOC_Os07g29820*), because knocking-out *ARF25* or overexpressing or knocking-out of *LOC_Os07g29820* did not generate any obvious change in the rice blast disease phenotype (Zhao et al. 2019). In contrast, knocking-out of *ARF12* led to increased susceptibility to *M. oryzae* (Zhao et al. 2019), implying that miR167d mediates its functions via *ARF12*. In addition, the miR167d-mediated regulation of rice-*M. oryzae* interaction may be associated with both IAA- and JA-signaling pathways, because JA concentration decreased in transgenic lines overexpressing miR167d, but increased in those overexpressing the target mimicry (Zhao et al. 2019). Therefore, it would be interesting to investigate the context of the connection between *ARF12* and IAA/JA signaling in terms of rice immunity against *M. oryzae*.

miR169 is a relatively large miRNA family containing 17 members, which generate nine mature isoforms in rice, targeting eight of the 11 *nuclear transcription factor Y subunit A* (NF-YA) genes (Wu et al. 2009; Li et al. 2010b). NF-YA, together with NF-YB and NF-YC, forms a heterotrimer to control the expression of downstream genes. The different isoforms of miR169 show considerable variation in expression patterns and are differentially responsive to *M. oryzae* or its elicitors (Campo et al. 2013; Li et al. 2014; Li et al. 2017). However, they may play negative roles in rice immunity against *M. oryzae*, because the total miR169 abundance was significantly up-regulated in the susceptible rice accession in response to *M. oryzae*, but varied only slightly in the resistant accession in response to infection by *M. oryzae* (Li et al. 2017). Consistent with this observation, overexpression of miR169a resulted in increased susceptibility to *M. oryzae,* which was associated with compromised defense responses, such as reduced induction of defense-related genes and decreased H_2O_2 production (Li et al. 2017). In contrast, blocking miR169 via expressing a target mimicry of miR169a led to increased resistance to *M. oryzae*, which may be attributed to up-regulation of some or all of the eight target *NF-YA*s (Li et al. 2017). However, it remains to be determined whether (or which of) the target *NF-YA*s positively regulates rice immunity against *M. oryzae* and which signal transduction pathway is involved.

The miR319 family includes two members, which form one mature miRNA targeting *teosinte branched/cycloidea/proliferation cell factors21* (*TCP21*) and the positive regulator of gibberellin (GA) signaling, *GAMYB*, in rice (Zhang et al. 2018). The expression of miR319 was up-regulated, whereas that of its target genes was down-regulated in response to *M. oryzae* infection in a susceptible accession (Zhang et al. 2018). Similarly, a miR319-resistant *TCP21*

mutant exhibited resistance to *M. oryzae*, whereas knock-down of *TCP21* led to increased susceptibility to *M. oryzae*. The resistant phenotypes were associated with up-regulation of expression of genes involved in JA biosynthesis/signaling, marked up-regulation of defense-related genes and increased accumulation of H_2O_2. In contrast, the susceptible phenotypes were associated with down-regulation of genes involved in JA biosynthesis/signaling and a failure of H_2O_2 production (Zhang et al. 2018). Therefore, miR319 probably regulates rice immunity against *M. oryzae* by *TCP21*-mediated regulation of JA biosynthesis and signaling.

The miR396 family is a highly conserved and abundant miRNA family. It consists of eight members in the rice genome, forming five mature isoforms, which target 11 *Growth Regulating Factor* (*GRF*) transcription factor genes (Duan et al. 2015; Gao et al. 2015; Li et al. 2016a). The miR396-GRF module has been reported to fine-tune inflorescence development controlling panicle and individual grain size (Tang and Chu 2017). Over-expressing miR396a, miR396c, miR396d or miR396h led to increased susceptibility to *M. oryzae*, which was associated with reduced H_2O_2 production and a failure to induce defense-related genes (Chandran et al. 2019). In contrast, blocking miR396 by expressing a target mimicry of miR396d or a short tandem target mimicry (STTM) of miR396d-396e led to increased resistance to *M. oryzae*, which was associated with marked up-regulation of defense-related genes and increased H_2O_2 production. Consistent with these being the target genes of miR396, overexpression of *GRF6*, *GRF7*, *GRF8* or *GRF9* resulted in increased resistance to *M. oryzae*, whereas, knock-down of *GRF7* via RNA interference (RNAi) led to increased susceptibility (Chandran et al. 2019). Thus, miR396 facilitates the infection by *M. oryzae* via at least four *GRF* genes. Because blocking miR396 led to improvements in both rice yield and immunity against *M. oryzae*, the miR396-GRF module is invaluable in breeding programs aiming to improve both of yield and disease resistance simultaneously.

The miR444 family is a monocot-specific miRNA family with six gene loci in the rice genome, each generating 1–3 mature miRNAs, leading to six mature isoforms (Table 1). miR444 targets four MADS-box transcription factor genes and four other genes (Wu et al. 2009; Li et al. 2010b), and plays roles in root and tiller development, as well as in response to *Rice stripe virus* (RSV) (Yan et al. 2014; Wang et al. 2016). Overexpression of miR444s resulted in increased resistance to RSV that was associated with up-regulation of *OsRDR1* expression via suppression of the miR444-targeted *MADS* genes (Wang et al. 2016), indicating that miR444a plays a positive role in resistance to the virus. On the other hand, miR444b seemed to play negative roles with respect to rice immunity against *M. oryzae*. In response to *M. oryzae* infection, the abundance of miR444b.2 was increased in a blast-susceptible rice accession, but was decreased in a blast-resistant accession (Xiao et al. 2017). In contrast, expression of the target genes of miR444b.2, including *MADS27b* and *MADS57*, changed only slightly in the susceptible accession but increased significantly in the resistant accession, indicating that miR444b facilitates the infection by *M. oryzae* via impact on these target genes. In addition, overexpression of miR444b resulted in increased susceptibility to *M. oryzae*; whereas blocking miR444b.2 via overexpression of a target mimicry of miR444b.2 resulted in increased rice blast disease resistance (Xiao et al. 2017). However, the identity of the target genes, which contribute to miR444b-mediated facilitation of infection by *M. oryzae*, has still to be confirmed.

Taken together, the signaling pathways associated with rice defense against the blast disease were also regulated by the miRNAs that facilitate infection of *M. oryzae*, including auxin signaling, ET signaling, JA signaling and some yet-to-be identified signaling pathways, which may also regulate the trade-off between plant growth and immunity.

Challenges facing functional investigations into the roles of miRNAs in rice-*M. oryzae* interaction

It is quite challenging to investigate the roles of miRNAs in rice-*M. oryzae* interaction due to the complicated miRNA regulatory nature. Usually, one mature miRNA can be transcribed from one or more *MIR* gene loci, whereas one gene locus may differ from another in terms of response to *M. oryzae*. One mature miRNA may suppress the expression of one or several target genes and such suppression may vary among different target genes. Moreover, one target gene can be regulated by multiple miRNAs. However, the most challenging problem is to identify the authentic target genes *in planta* that contribute to the miRNA-mediated phenotypes, because functional analysis of the target genes is essential for an investigation of a miRNA-mediated regulatory mechanism. In this section, we briefly summarize methods reported to achieve the identification of target genes.

The strategy for target gene identification is based on how the miRNA regulates the expression of its target genes. Most plant miRNAs regulate target gene expression via mRNA cleavage. In such a situation, the target genes can be relatively and easily predicted and experimentally validated via degradome sequencing and/or RNA ligase-mediated 5′-rapid amplification of cDNA ends (RLM-5′-RACE) (German et al. 2008). Because miRNA-mediated mRNA cleavage generates 3′ decay

products that have a 3′ poly(A) tail and a 5′ monophosphate end, the 5′ monophosphate ends can be ligated to an adaptor sequence by an RNA ligase. The ligated products can then be reverse-transcribed into cDNA and subjected to high-throughput sequencing or 5′-RACE analysis (Wu et al. 2009). However, this strategy is invalid for those target genes that are translationally inhibited by miRNAs or where target site-methylation is mediated by miRNAs. In such cases, the target genes can first be predicted based on sequence complementarity, using online programs such as psRNATarget (http://plantgrn.noble.org/psRNATarget/) or psRobot (http://omicslab.genetics.ac.cn/psRobot/) (Wu et al. 2012; Dai et al. 2018). The predicted target genes can be confirmed via a reporter assay, in which the reporter gene is constructed by fusing the predicted target site to a fluorescence protein such as the Yellow Fluorescence Protein (YFP). Then, the reporter gene is transiently expressed or co-expressed with the miRNA. If the predicted target site is authentic, the YFP signal should be weaker or the YFP protein concentration lower as a result of co-expression with miRNA than that from expression of the reporter alone (Li et al. 2017). The reporter assay approach should be valid for targets regulated by miRNA-mediated cleavage and translational inhibition, but may not be valid for identification of target genes regulated by miRNA-mediated methylation. In the latter case, the predicted target sites could be confirmed by examining the DNA methylation status via bisulfite sequencing analysis (Wu et al. 2010). After testing each of the above strategies, it may still be impossible to confirm the predicted target genes. Under such circumstances, a combination of the above strategies may be appropriate, with multiple omics analyses, by comparing the transgenic line overexpressing the miRNA with the transgenic line overexpressing its target mimicry with the authentic target genes being among the predicted target genes showing opposite expression patterns in the two transgenic lines. Finally, even if we identified the authentic target genes, we may still face the problem that the target genes identified did not contribute to the miRNA-mediated phenotypes. Therefore, the process can be very challenging. Fortunately, most of the blast fungus-responsive miRNAs have known target genes, the regulatory roles of which have been confirmed in a variety of biological processes, such as growth and development (Table 1) (Tang and Chu 2017).

Conclusions

At present, our understanding of the miRNA-mediated regulation of rice-*M. oryzae* interaction is quite limited and fragmentary. In the context of the rice-*M. oryzae* interaction, the activation of either PTI or ETI, or both, could lead to either activation or silencing of certain subsets of *MIR* genes, which, in turn, could recruit networks of downstream genes that may achieve feedback regulation of PTI and/or ETI (Fig. 1). On the one hand, the key component genes of the miRNA signaling pathway, such as *DCL*s and *AGO*s, are regulated during PTI and/or ETI, which, in turn, mediate the engagement of the miRNA network in the regulation of rice-*M. oryzae* interaction. Evidence supporting this conclusion comes from *DCL1* and *AGO4*, in that *DCL1* negatively and *AGO4* positively regulates rice immunity against *M. oryzae* (Zhang et al. 2015; Deng et al. 2017; Salvador-Guirao et al. 2019). On the other hand, a subset of *MIR* genes may be down- or up-regulated during PTI and/or ETI, depending on their roles as negative or positive regulators of PTI and/or ETI, whereas the positive regulators would be up-regulated during PTI and/or ETI, the negative regulators would be down-regulated. Evidence supporting this conclusion comes from functionally characterized miRNAs, including miR160, miR164, miR166, miR167, miR169, miR319, miR396, miR398, miR444 and miR7695 (Campo et al. 2013; Li et al. 2014; Li et al. 2017; Xiao et al. 2017; Wang et al. 2018a; Zhang et al. 2018; Chandran et al. 2019; Quoc et al. 2019; Zhao et al. 2019). However, we still do not know how PTI and/or ETI is coupled with the miRNA signaling pathway or with each of the blast fungus-responsive miRNAs.

Some miRNAs may be involved in either PTI or ETI, or both, according to their response to *M. oryzae* or its elicitors. For example, miR398b may act in PTI, because its abundance was up-regulated in both the susceptible and resistant accessions following *M. oryzae* infection, with overexpression of miR398b leading to increased induction of the PTI marker genes *OsKS4* and *OsNAC4* in response to PAMP treatment (Li et al. 2014). However, current data cannot exclude the involvement of miR398 in ETI. Nevertheless, the miRNA-mediated regulatory nature bestows miRNA with the potential to simultaneously regulate multiple genes. For example, miR169 targets eight of the *NF-YA* family transcription factor genes and miR396 targets eleven of the *GRF* genes. In such cases, blocking or overexpressing a single miRNA can achieve up- or down-regulation of multiple genes, facilitating gene manipulation when these target genes have functional redundancy. For example, in tomato, blocking miR482 and miR2118 via STTM resulted in increased resistance to at least two different pathogens (Canto-Pastor et al. 2019). Therefore, it would be worthwhile investigating the roles of miRNAs in rice-*M. oryzae* interaction and applying them to breeding programs to improve agronomic traits such as disease resistance.

Taken together, progress is clearly being made with respect to unveiling the roles of miRNAs in conferring rice immunity against *M. oryzae*. The future focus should be

on dissecting the functions of key components in the appropriate miRNA signaling pathway and of each blast fungus-responsive miRNA in the rice-*M. oryzae* interactions. It is highly anticipated that, through *MIR* genes manipulation we can improve important agronomic traits with lower fitness costs.

Abbreviations

AGO: Argonaute; ARF: Auxin response factor; CCSD: Copper chaperone for superoxide dismutase; CEBiP: Chitin elicitor binding protein; CERK1: Chitin elicitor receptor kinase; CSD: Cu/Zn-superoxidase dismutase; DCL: Dicer-like; ETI: Effector-triggered immunity; GRF: Growth regulating factor; HEN1: Hua enhancer 1; LTH: Lijiangxin Tuan Hegu; LYP: Lysin motif–containing protein; miRISC: miRNA-induced silencing complex; NF-YA: Nuclear transcription factor Y subunit A; NLR: Nucleotide-binding site leucine-rich repeat; NRAMP 6: Natural resistance-associated macrophage protein 6; PAMP: Pathogen-associated molecular pattern; PTI: PAMP-triggered immunity; ROS: Reactive oxygen species; SOD: Superoxide dismutase

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W-MW, YL and JMJJ wrote the manuscript. YL and W-MW drew the Fig. QF, Z-XZ and JMJJ collected data for the table. MIK and JF edited the manuscript. All authors read and approved the final manuscript.

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Competing interests

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References

Abe M, Yoshikawa T, Nosaka M, Sakakibara H, Sato Y, Nagato Y, et al. *WAVY LEAF1*, an ortholog of Arabidopsis *HEN1*, regulates shoot development by maintaining microRNA and trans-acting small interfering RNA accumulation in rice. Plant Physiol. 2010;154:1335–46.

Bakhshi B, Mohseni Fard E, Nikpay N, Ebrahimi MA, Bihamta MR, Mardi M, et al. MicroRNA signatures of drought signaling in rice root. PLoS One. 2016;11:e0156814.

Baldrich P, Campo S, Wu MT, Liu TT, Hsing YI, San SB. MicroRNA-mediated regulation of gene expression in the response of rice plants to fungal elicitors. RNA Biol. 2015;12:847–63.

Baldrich P, Hsing Y, San SB. Genome-wide analysis of polycistronic microRNAs in cultivated and wild rice. Genome Biol Evol. 2016;8:1104–14.

Baldrich P, San SB. MicroRNAs in rice innate immunity. Rice. 2016;9:6.

Bialas A, Zess EK, De la Concepcion JC, Franceschetti M, Pennington HG, Yoshida K, et al. Lessons in effector and NLR biology of plant-microbe systems. Mol Plant-Microbe Interact. 2018;31:34–45.

Boccara M, Sarazin A, Thiebeauld O, Jay F, Voinnet O, Navarro L, et al. The Arabidopsis miR472-RDR6 silencing pathway modulates PAMP- and effector-triggered immunity through the post-transcriptional control of disease resistance genes. PLoS Pathog. 2014;10:e1003883.

Campo S, Peris-Peris C, Sire C, Moreno AB, Donaire L, Zytnicki M, et al. Identification of a novel microRNA (miRNA) from rice that targets an alternatively spliced transcript of the *Nramp6* (*Natural resistance-associated macrophage protein 6*) gene involved in pathogen resistance. New Phytol. 2013;199:212–27.

Canto-Pastor A, Santos B, Valli A, Summers W, Schornack S, Baulcombe D. Enhanced resistance to bacterial and oomycete pathogens by short tandem target mimic RNAs in tomato. Proc Natl Acad Sci U S A. 2019;116:2755–60.

Chandran V, Wang H, Gao F, Cao X-L, Chen Y-P, Li G-B, et al. miR396-OsGRFs module balances growth and rice blast disease-resistance. Front. Plant Sci. 2019;9:1999.

Dai X, Zhuang Z, Zhao P. psRNATarget: a plant small RNA target analysis server (2017 release). Nucleic Acids Res. 2018;46:W49–54.

Deng Y, Zhai K, Xie Z, Yang D, Zhu X, Liu J, et al. Epigenetic regulation of antagonistic receptors confers rice blast resistance with yield balance. Science. 2017;355:962–5.

Dong S, Zhang J, Sun D, Liu H, Yang Q, Wang H, et al. Identification of *Magnaporthe oryzae*-elicited rice novel miRNAs and their targets by miRNA and degradome sequencing. Eur J Plant Pathol. 2018;151:629–47.

Duan P, Ni S, Wang J, Zhang B, Xu R, Wang Y, et al. Regulation of *OsGRF4* by OsmiR396 controls grain size and yield in rice. Nat Plants. 2015;2:15203.

Fang Y, Xie K, Xiong L. Conserved miR164-targeted NAC genes negatively regulate drought resistance in rice. J Exp Bot. 2014;65:2119–35.

Gao F, Wang K, Liu Y, Chen Y, Chen P, Shi Z, et al. Blocking miR396 increases rice yield by shaping inflorescence architecture. Nat Plants. 2015;2:15196.

German MA, Pillay M, Jeong D-H, Hetawal A, Luo S, Janardhanan P, et al. Global identification of microRNA-target RNA pairs by parallel analysis of RNA ends. Nat Biotechnol. 2008;26:941–6.

Huang J, Li Z, Zhao D. Deregulation of the *Os*miR160 target gene *OsARF18* causes growth and developmental defects with an alteration of auxin signaling in rice. Sci Rep. 2016a;6:29938.

Huang J, Yang M, Zhang X. The function of small RNAs in plant biotic stress response. J Integr Plant Biol. 2016b;58:312–27.

Kapoor M, Arora R, Lama T, Nijhawan A, Khurana JP, Tyagi AK, et al. Genome-wide identification, organization and phylogenetic analysis of dicer-like, Argonaute and RNA-dependent RNA Polymerase gene families and their expression analysis during reproductive development and stress in rice. BMC Genomics. 2008;9:451.

Li S, Gao F, Xie K, Zeng X, Cao Y, Zeng J, et al. The OsmiR396c-OsGRF4-OsGIF1 regulatory module determines grain size and yield in rice. Plant Biotechnol J. 2016a;14:2134–46.

Li X, Lian H, Zhao Q, He Y. MicroRNA166 monitors *SPOROCYTELESS/NOZZLE (SPL/NZZ)* for building of the anther internal boundary. Plant Physiol. 2019b;181:208–20.

Li Y, Cao X-L, Zhu Y, Yang X-M, Zhang K-N, Xiao Z-Y, et al. Osa-miR398b boosts H_2O_2 production and rice blast disease-resistance via multiple superoxide dismutases. New Phytol. 2019a;222:1507–22.

Li Y, Lu YG, Shi Y, Wu L, Xu YJ, Huang F, et al. Multiple rice microRNAs are involved in immunity against the blast fungus *Magnaporthe oryzae*. Plant Physiol. 2014;164:1077–92.

Li Y, Zhang Q, Zhang J, Wu L, Qi Y, Zhou JM. Identification of microRNAs involved in pathogen-associated molecular pattern-triggered plant innate immunity. Plant Physiol. 2010a;152:2222–31.

Li Y, Zhao SL, Li JL, Hu XH, Wang H, Cao XL, et al. Osa-miR169 negatively regulates rice immunity against the blast fungus *Magnaporthe oryzae*. Front Plant Sci. 2017;8:2.

Li YF, Zheng Y, Addo-Quaye C, Zhang L, Saini A, Jagadeeswaran G, et al. Transcriptome-wide identification of microRNA targets in rice. Plant J. 2010b;62:742–59.

Li Z, Peng J, Wen X, Guo H. Ethylene-insensitive3 is a senescence-associated gene that accelerates age-dependent leaf senescence by directly repressing miR164 transcription in Arabidopsis. Plant Cell. 2013;25:3311–28.

Li ZY, Xia J, Chen Z, Yu Y, Li QF, Zhang YC, et al. Large-scale rewiring of innate immunity circuitry and microRNA regulation during initial rice blast infection. Sci Rep. 2016b;6:25493.

Liu B, Li JF, Ao Y, Qu J, Li Z, Su J, et al. Lysin motif-containing proteins LYP4 and LYP6 play dual roles in peptidoglycan and chitin perception in rice innate immunity. Plant Cell. 2012;24:3406–19.
Liu B, Li P, Li X, Liu C, Cao S, Chu C, et al. Loss of function of *OsDCL1* affects microRNA accumulation and causes developmental defects in rice. Plant Physiol. 2005;139:296–305.
Mentlak TA, Kombrink A, Shinya T, Ryder LS, Otomo I, Saitoh H, et al. Effector-mediated suppression of chitin-triggered immunity by *Magnaporthe oryzae* is necessary for rice blast disease. Plant Cell. 2012;24:322–35.
Navarro L, Dunoyer P, Jay F, Arnold B, Dharmasiri N, Estelle M, et al. A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. Science. 2006;312:436–9.
Navarro L, Jay F, Nomura K, He SY, Voinnet O. Suppression of the microRNA pathway by bacterial effector proteins. Science. 2008;321:964–7.
Park C, Shirsekar G, Bellizzi M, Chen S, Songkumarn P, Xie X, et al. The E3 ligase APIP10 connects the effector AvrPiz-t to the NLR receptor Piz-t in rice. PLoS Pathog. 2016;12:e1005529.
Peris-Peris C, Serra-Cardona A, Sánchez-Sanuy F, Campo S, Ariño J, Segundo BS. Two NRAMP6 isoforms function as iron and manganese transporters and contribute to disease resistance in rice. Mol Plant-Microbe Interact. 2017;30:385–98.
Quoc NB, Phuong NDN, Trang HTT, Phi NB, Chau NNB. Expression of *osa-miR7695* against the blast fungus *Magnaporthe oryzae* in Vietnamese rice cultivars. Eur J Plant Pathol. 2019;155:307–17.
Rogers K, Chen X. Biogenesis, turnover, and mode of action of plant microRNAs. Plant Cell. 2013;25:2383–99.
Salvador-Guirao R, Baldrich P, Tomiyama S, Hsing Y, Okada K, Segundo BS. *OsDCL1a* activation impairs phytoalexin biosynthesis and compromises disease resistance in rice. Ann Bot. 2019;123:79–93.
Salvador-Guirao R, Hsing YI, San SB. The polycistronic miR166k-166h positively regulates rice immunity via post-transcriptional control of *EIN2*. Front Plant Sci. 2018;9:337.
Seo JK, Wu J, Lii Y, Li Y, Jin H. Contribution of small RNA pathway components in plant immunity. Mol Plant-Microbe Interact. 2013;26:617–25.
Shimizu T, Nakano T, Takamizawa D, Desaki Y, Ishii-Minami N, Nishizawa Y, et al. Two LysM receptor molecules, CEBiP and OsCERK1, cooperatively regulate chitin elicitor signaling in rice. Plant J. 2010;64:204–14.
Shivaprasad PV, Chen HM, Patel K, Bond DM, Santos BA, Baulcombe DC. A microRNA superfamily regulates nucleotide binding site-leucine-rich repeats and other mRNAs. Plant Cell. 2012;24:859–74.
Song X, Li Y, Cao X, Qi Y. MicroRNAs and their regulatory roles in plant-environment interactions. Annu Rev Plant Biol. 2019;70:489–525.
Tang J, Chu C. MicroRNAs in crop improvement: fine-tuners for complex traits. Nat Plants. 2017;3:17077.
Tripathi A, Chacon O, Singla-Pareek SL, Sopory SK, Sanan-Mishra N. Mapping the microRNA expression profiles in glyoxalase over-expressing salinity tolerant rice. Curr Genomics. 2018;19:21–35.
Wang B-H, Ebbole DJ, Wang Z-H. The arms race between *Magnaporthe oryzae* and rice: diversity and interaction of *Avr* and *R* genes. J Integr Agric. 2017;16:2746–60.
Wang H, Jiao X, Kong X, Hamera S, Wu Y, Chen X, et al. A signaling cascade from miR444 to RDR1 in rice antiviral RNA silencing pathway. Plant Physiol. 2016;170:2365–77.
Wang J, Zhou L, Shi H, Chern M, Yu H, Yi H, et al. A single transcription factor promotes both yield and immunity in rice. Science. 2018b;361:1026–8.
Wang Z, Xia Y, Lin S, Wang Y, Guo B, Song X, et al. Osa-miR164a targets *OsNAC60* and negatively regulates rice immunity against the blast fungus *Magnaporthe oryzae*. Plant J. 2018a;95:584–97.
Wei L, Gu L, Song X, Cui X, Lu Z, Zhou M, et al. Dicer-like 3 produces transposable element-associated 24-nt siRNAs that control agricultural traits in rice. Proc Natl Acad Sci U S A. 2014;111:3877–82.
Wu H, Ma Y, Chen T, Wang M, Wang X. PsRobot: a web-based plant small RNA meta-analysis toolbox. Nucleic Acids Res. 2012;40:W22–8.
Wu J, Yang R, Yang Z, Yao S, Zhao S, Wang Y, et al. ROS accumulation and antiviral defence control by microRNA528 in rice. Nat Plants. 2017;3:16203.
Wu J, Yang Z, Wang Y, Zheng L, Ye R, Ji Y, et al. Viral-inducible Argonaute18 confers broad-spectrum virus resistance in rice by sequestering a host microRNA. eLife. 2015;4:e05733.
Wu L, Zhang Q, Zhou H, Ni F, Wu X, Qi Y. Rice microRNA effector complexes and targets. Plant Cell. 2009;21:3421–35.
Wu L, Zhou H, Zhang Q, Zhang J, Ni F, Liu C, et al. DNA methylation mediated by a microRNA pathway. Mol Cell. 2010;38:465–75.
Xiao Z-Y, Wang Q-X, Wang H, Li J-L, Zhao S-L, Fan J, et al. MiR444b.2 regulates resistance to *Magnaporthe oryzae* and tillering in rice. Acta Phytopathola Sin. 2017;47:511–22 (in Chinese). http://zwblxb.magtech.com.cn/CN/10.13926/j.cnki.apps.000019.
Xie M, Zhang S, Yu B. MicroRNA biogenesis, degradation and activity in plants. Cell Mol Life Sci. 2015;72:87–99.
Xie Z, Yan B, Shou J, Tang J, Wang X, Zhai K, et al. A nucleotide-binding site-leucine-rich repeat receptor pair confers broad-spectrum disease resistance through physical association in rice. Philos Trans R Soc B. 2019;374:20180308.
Xu W, Meng Y, Wise RP. *Mla*- and *Rom1*-mediated control of microRNA398 and chloroplast copper/zinc superoxide dismutase regulates cell death in response to the barley powdery mildew fungus. New Phytol. 2014;201:1396–412.
Yao S, Yang Z, Yang R, Huang Y, Guo G, Kong X, et al. Transcriptional regulation of miR528 by OsSPL9 orchestrates antiviral response in rice. Mol Plant. 2019;12:1114–22.
Yan Y, Wang H, Hamera S, Chen X, Fang R. MiR444a has multiple functions in the rice nitrate-signaling pathway. Plant J. 2014;78:44–55.
Yang C, Li W, Cao J, Meng F, Yu Y, Huang J, et al. Activation of ethylene signaling pathways enhances disease resistance by regulating ROS and phytoalexin production in rice. Plant J. 2017;89:338–53.
Zhai J, Jeong D-H, De Paoli E, Park S, Rosen BD, Li Y, et al. MicroRNAs as master regulators of the plant *NB-LRR* defense gene family via the production of phased, *trans*-acting siRNAs. Genes Dev. 2011;25:2540–53.
Zhang D, Liu M, Tang M, Dong B, Wu D, Zhang Z, et al. Repression of microRNA biogenesis by silencing of *OsDCL1* activates the basal resistance to *Magnaporthe oryzae* in rice. Plant Sci. 2015;237:24–32.
Zhang X, Bao Y, Shan D, Wang Z, Song X, Wang J, et al. *Magnaporthe oryzae* induces the expression of a microRNA to suppress the immune response in rice. Plant Physiol. 2018;177:352–68.
Zhang X, Zhao H, Gao S, Wang W, Katiyar-Agarwal S, Huang H, et al. *Arabidopsis* Argonaute 2 regulates innate immunity via miRNA393(*)-mediated silencing of a Golgi-localized SNARE gene, *MEMB12*. Mol Cell. 2011;42:356–66.
Zhao ZX, Feng Q, Cao XL, Zhu Y, Wang H, Chandran V, et al. *Osa-miR167d* facilitates infection of *Magnaporthe oryzae* in rice. J Integr Plant Biol. 2019. https://doi.org/10.1111/jipb.12816.
Zhu H, Hu F, Wang R, Zhou X, Sze SH, Liou LW, et al. *Arabidopsis* Argonaute10 specifically sequesters miR166/165 to regulate shoot apical meristem development. Cell. 2011;145:242–56.