miRNA: A Novel Link Between Rice Ragged Stunt Virus and *Oryza sativa*

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Abstract Rice ragged stunt disease caused leads to severe loss of rice yield. Recently, rice ragged stunt virus (RRSV) were found to be increasingly common in rice-growing regions of China and Vietnam. RRSV may cause problem by interacting with microRNAs (miRNAs) of host cells and the mechanism is not clear yet. In this study we identified 11 miRNAs in response to RRSV infection and predicted their possible targets to viral RNA segments (S1–S10) through the bioinformatics analysis. Interestingly, we found that Osa-miR-168b might bind to both the CDS region and 3'UTR of S5 and S8 and target eEF-1A to inhibit the activity of host cells to facilitate RRSV replication. These results suggest that miRNAs may be a potential target for developing rice against RRSV infection.

Keywords Rice ragged stunt virus · miRNA · *Oryza sativa*

Rice ragged stunt disease caused by rice ragged stunt virus (RRSV) is a serious rice disease in most rice-growing regions, such as South East Asia and southern China. RRSV is classified into the genus *Oryzavirus* in the family *Reoviridae*, which was first discovered in 1976–1977 in Indonesia and the Philippines [1, 2]. RRSV genome is divided into 10 double stranded RNA segments (S1–S10) with size ranging from 1.2 to 3.9 kb [1]. And these RNA segments encode 8 structural proteins and 3 non-structural proteins [1].

MicroRNAs (miRNAs) are small RNA species ranging in size from 20 to 25 nt which are found in animals, plants and some viruses, and play a variety of roles in many cellular processes [3]. The miRNA-mediated RNA interference through sequence complementarity to the 3'-untranslated regions (UTRs) or coding sequences (CDS) of target mRNAs can lead to inhibition of mRNA translation [4].

During the interaction between virus and host, the expression profiles of miRNAs have changed and these miRNAs may play potential important roles in regulating viral infection and replication [5, 6]. To identified the altered miRNAs during the course of rice virus disease, we took advantage of Gene Expression Omnibus (GEO) repository at the National Center for Biotechnology Information (NCBI; <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE11025>). In response to rice stripe virus (RSV) infection, a variety of miRNA families had changed in two japonica rice genotypes (KT95-418 and WuYun3). In both genotypes of japonica rice infected with RSV, Osa-miR-812 family was downregulated, but Osa-miR-808, Osa-miR-818, Osa-miR-1884 and Osa-miR-2123 families were upregulated (Fig. 1). In the KT95-418 genotype, the expression of Osa-miR-809, Osa-miR-399 and Osa-miR-446 families was lower but the expression of Osa-miR-172 and Osa-miR-811

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Fig. 1 The expressions of miRNAs in two japonica rice in response to RSV infection. 9-day old 20 rice genotypes WuYun3 (a) and KT95-418 (b) seedlings were exposed to approximately 100 viruliferous small brown planthoppers (*Laodelphax striatellus* Fall) for

2 days to be inoculated by RSV. Then the rice genome array were used to analyze and compare their transcriptional profiles. The data was from (GEO) repository

Table 1 The information of miRNAs involve in RSV infection

Family	Mature miRNA	Mature sequence
Osa-miR-156	Osa-miR-156a/b/c/d/e/f1/f2/g/h/i/j/m/n	UGACAGAAGAGAGUGAGCAC
	Osa-miR-156k	UGACAGAAGAGAGAGAGCACA
	Osa-miR-156l	CGACAGAAGAGAGUGAGCAUA
	Osa-miR-156o	UGACAGAAGAGAGUGAGCAU
	Osa-miR-156p	UGACAGAAGAGAGUGAGCUC
	Osa-miR-156q	UGACAGAACAGAGUGAGCAC
	Osa-miR-156r	UGACAGAAGAGAGAGAGCAC
	Osa-miR-156s	UGACAGAAGAGAGAGAGCAC
Osa-miR-168	Osa-miR-168a/m	UCGCUUGGUGCAGAUCCGGAC
	Osa-miR-168a*	CCCGCCUUGCACCAAGUGAAU
	Osa-miR-168a-3p	GAUCCCGCCUUGCACCAAGUGAAU
	Osa-miR-168b	AGGCUUGGUGCAGCUCGGGAA
Osa-miR-172	Osa-miR-172a/d	AGAAUCUUGAUGAUGCUGCAU
	Osa-miR-172b	GGAAUCUUGAUGAUGCUGCAU
	Osa-miR-172c	UGAAUCUUGAUGAUGCUGCAC
	Osa-miR-172m/n/o/p	AGAAUCCUGAUGAUGCUGCAG
Osa-miR-399	Osa-miR-399a/b/c	UGCCAAAGGAGAAUUGCCCUG
	Osa-miR-399d	UGCCAAAGGAGAGUUGCCCUG
	Osa-miR-399e/f/g	UGCCAAAGGAGAAUUGCCCAG
	Osa-miR-399h	UGCCAAAGGAGACUUGCCCAG
	Osa-miR-399i	UGCCAAAGGAGAGCUGCCCUG
Osa-miR-446	Osa-miR-446	CAUCAUAUGAAUAUGGGAAAUGG
Osa-miR-808	Osa-miR-808	AUGAAUGUGGGAAAUGUAGAA
Osa-miR-809	Osa-miR-809a/b/c/d/e/f/g/h	UGAAUGUGAGAAAUGUAGAAU
Osa-miR-811	Osa-miR-811a/b/c	ACCGUUAAGUAGAGAAAUGGACGU
Osa-miR-812	Osa-miR-812a/b/c/d/e	GACGGACGGUUAACGUUGGAC
	Osa-miR-812f	ACGGAUGAUUAAAGUUGGACACGG
	Osa-miR-812g/h/i/j	AAGACGGAUGAUUAAAGUUGGACA
Osa-miR-818	Osa-miR-818a/b/c/d/e	AAUCCCUUAUAUAUGGGACGG
Osa-miR-1884	Osa-miR-1884a	UGUGACGCCGUUGACUUUUCAU
	Osa-miR-1884b	AAUGUAUGACGUGUUGACUUUA
Osa-miR-2123	Osa-miR-2123a/b/c	UAAAAAGUCAACGGUGUCAAC

families was higher (Fig. 1a). In the WuYun3 genotype, the expression of Osa-miR-168 and Osa-miR-156 families was downregulation (Fig. 1b). These observations

suggest that altered expression profiles of miRNAs are a potential mechanism of the host cell against viral infection in rice.

Table 2 miRNAs predicted to target rice ragged stunt virus gene genome

Targetgene	miRNA	Position	P value
S1	Osa-miR-168a/m	176	2.69E–1
	Osa-miR-168*	3348	2.04E–1
	Osa-miR-172b	247	7.73E–2
S2	Osa-miR-156p	1800	4.89E–2
	Osa-miR-168a/m	1094	2.73E–1
	Osa-miR-168m	1094	2.73E–1
	Osa-miR-168b	2489	3.33E–1
	Osa-miR-168b	2894	3.11E–1
	Osa-miR-168b	3692	7.85E–2
	Osa-miR-172c	400	1.07E–1
	Osa-miR-172c	539	2.57E–1
	Osa-miR-172c	1080	2.73E–1
	Osa-miR-172m/n/o/p	3593	3.7E–1
	Osa-miR-399d/j	3252	3.51E–1
	Osa-miR-399d/j	547	2.57E–1
	Osa-miR-808	785	2.31E–1
S3	Osa-miR-812f	3689	7.85E–2
	Osa-miR-156l	1660	3.47E–1
	Osa-miR-156p	1661	3.47E–1
	Osa-miR-168a/m	2594	3.42E–1
	Osa-miR-168b	2089	9.30E–2
	Osa-miR-172c	3298	3.65E–1
	Osa-miR-812f	182	5.99E–2
	Osa-miR-812f	329	4.49E–2
S4	Osa-miR-812j/h/i/j	931	4.49E–2
	Osa-miR-168b	1323	2.34E–01
	Osa-miR-168b	1409	4.19E–02
	Osa-miR-168b	1461	3.84E–01
	Osa-miR-811a/b/c	2087	2.81E–01
	Osa-miR-812a/b/c/d/e	1996	9.74E–02
	Osa-miR-812a/b/c/d/e	2168	2.27E–01
	Osa-miR-812f	1984	9.74E–02
	Osa-miR-812g/h/i/j	1986	9.74E–02
	Osa-miR-812g/h/i/j	2164	2.27E–01
S5	Osa-miR-168a-3p	605	2.45E–1
	Osa-miR-168b	612	2.45E–1
	Osa-miR-168b	2576	1.21E–1
	Osa-miR-399e/f/g/h	1228	1.77E–1
	Osa-miR-399i	1227	1.77E–1
	Osa-miR-812a/b/c/d/e	1808	1.77E–1
	Osa-miR-812f	1804	1.77E–1
	Osa-miR-1884a	1065	2.37E–1
S6	Osa-miR-168b	148	3.18E–1
	Osa-miR-168b	741	3.36E–1
	Osa-miR-168b	795	1.41E–1
	Osa-miR-172b	541	1.08E–1

Table 2 continued

Targetgene	miRNA	Position	P value
S7	Osa-miR-168a/m	462	5.61E–2
	Osa-miR-168b	848	1.08E–1
	Osa-miR-172c	738	1.71E–1
	Osa-miR-1884b	1388	3.12E–2
S8	Osa-miR-168b	1769	2.59E–1
	Osa-miR-168b	1866	2.48E–1
	Osa-miR-172c	1036	1.58E–1
	Osa-miR-812g/h/i/j	819	2.44E–1
	Osa-miR-1884b	1245	8.31E–2

Asterisk represents an miRNA expressed at low level relative to the miRNA in the opposite arm of a hairpin

So far, it is unknown how miRNAs of host cells affect RRSV. Given the similarity between RSV and RRSV, we use these dysregulated miRNAs in Fig. 1 to investigate potential interaction between RRSV and miRNA. First, we sought to identify the direct target genes and obtained all mature sequence of these miRNA families from plant microRNA database PMRD [7] (<http://bioinformatics.cau.edu.cn/PMRD/>; Table 1). Second, we found that RRSV genome dsRNA S1, S2, S3, S4, S5, S6, S7 and S8 may serve as potential target genes of these miRNAs (Table 2) by surveying the online algorithms RNA22 (version 2.0; <https://cm.jefferson.edu/rna22/Interactive/>). And every segment could be bound by more than two miRNAs (Fig. 2). Interestingly, all of these segments could be regulated by Osa-miR-168 family (Fig. 2). Surprisingly, Osa-miR-168b might bind to the CDS region and 3'UTR of S5 and S8 (Fig. 2e, h) and only bind to the CDS region of S1, S2, S3, S4, S6, S7 (Fig. 2). Other miRNAs might only bind to the CDS region of their target genes (Fig. 2). Some researches showed that miRNAs inhibited viral genome not only by binding to 5'UTR and 3'UTR but also by binding to CDS region [8–10]. These observations suggest that these putative miRNAs of host cells (Fig. 2), especial Osa-miR-168b, may inhibit the replication of RRSV in rice. In addition, these predicted miRNAs and their targets by another software RNAhybrid are accorded with the predictions by RNA22 (Figure S1).

Viruses have many strategies to control the cellular translation machinery to perpetuate viral survival [11]. In many cases, viral proteins and host proteins could interact directly [5, 6]. Songbai et al. [12] found that a rice protein homologous to eukaryotic translation elongation factor 1A (eEF-1A, designated OseEF-1A) could interact with the Pns6 of RRSV. The interaction between OseEF-1A and Pns6 may inhibit the translation of host mRNAs but not viral mRNAs. RRSV may potentially influence the expression of eEF-1A through host miRNAs. RNA22

Fig. 2 The binding sites of multiple miRNAs in the RRSV genome dsRNA. RRSV genome dsRNA S1 (a), S2 (b), S3 (c), S4 (d), S5 (e), S6 (f), S7 (g) and S8 (h) may serve as potential target genes of these miRNAs by RNA22 analysis

Fig. 2 continued

Fig. 3 eEF-1A bears miR-168b target sites in its CDS region. According to analysis by RNA22, three binding sites of Osa-miR-168b were found in OseEF-1A mRNA

analysis predicted that eEF-1A carried 3 miR-168b target sites in its CDS region (Fig. 3). Thus we proposed that RRSV might benefit from the inhibition of host cellular mRNA by miRNAs. Further in vivo studies are needed to clarify the role.

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