Supplementary Materials: Hairpin RNA Targeting Multiple Viral Genes Confers Strong Resistance to Rice Black-Streaked Dwarf Virus

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Table S1. Disease assay of transgenic rice in the field RBSDV nursery (2012).

Repeat 1					
Rice Materials		**Disease Response of the Tested Plants**			
		N [a]	***S*** [b]	***R*** [c]	**Incidence Rate (%)**
T_1 transgenic lines	#1	39	4 [d]	35	10.26
	#2	35	0	35	0.00
	#3	36	1 [d]	35	2.78
	#4	37	0	37	0.00
	#5	28	0	28	0.00
	#6	38	10 [d]	28	26.32
	#7	34	6 [d]	28	17.65
	#8	37	2 [d]	35	5.41
Wild-type		34	10	24	29.41
Huaidao 5 [e]		32	7	25	21.88
Repeat 2					
Rice Materials		**Disease Response of the Tested Plants**			
		N [a]	***S*** [b]	***R*** [c]	**Incidence Rate (%)**
T_1 transgenic lines	#1	38	2 [d]	36	5.26
	#2	34	0	34	0.00
	#3	35	0	35	0.00
	#4	39	2 [d]	37	5.13
	#5	37	4 [d]	33	10.81
	#6	36	7 [d]	29	19.44
	#7	37	5 [d]	32	13.51
	#8	39	6 [d]	33	15.38
Wild-type		37	4	33	10.81
Huaidao 5 [e]		36	4	32	11.11

[a] Total number of rice plants examined; [b] typical disease symptoms were observed at 30 days post-inoculation; [c] no symptoms were observed during the whole growth period; [d] the intron of *AtFAD2* was not detected in these plants by PCR; [e] Huaidao 5 is a susceptible *Japonica* variety in Jiangsu Province of China used as the negative control.

Table S2. Disease assay of transgenic rice in the field RBSDV nursery (2013).

Repeat 1					
Rice Materials		**Disease Response of the Tested Plants**			
		N [a]	***S*** [b]	***R*** [c]	**Incidence Rate (%)**
T_2 transgenic lines	#1	34	0	34	0.00
	#2	37	0	37	0.00
	#3	39	1 [d]	38	2.56
	#4	40	1 [d]	39	2.50
	#5	38	4 [d]	34	10.53
	#7	38	3 [d]	35	17.5
	#8	36	9 [d]	27	25.00
Wild-type		34	6	28	17.65
Huaidao 5 [e]		39	5	34	12.82
Repeat 2					
Rice Materials		**Disease Response of the Tested Plants**			
		N [a]	***S*** [b]	***R*** [c]	**Incidence Rate (%)**
T_2 transgenic lines	#1	34	2 [d]	32	5.88
	#2	38	0	38	0.00
	#3	35	0	35	0.00
	#4	37	0	37	0.00
	#5	37	3 [d]	34	8.11
	#7	40	7 [d]	33	17.5
	#8	39	3 [d]	36	7.92
Wild-type		38	5	33	13.16
Huaidao 5 [e]		36	8	28	22.22

[a] Total number of rice plants examined; [b] typical disease symptoms were observed at 30 days post-inoculation; [c] no symptoms were observed during the whole growth period; [d] the intron of *AtFAD2* was not detected in these plants by PCR; [e] Huaidao 5 is a susceptible *Japonica* variety in Jiangsu Province of China used as the negative control.

Table S3. Disease assay of transgenic rice in the field RBSDV nursery (2014).

Rice Materials		**Disease Response of the Tested Plants**			
		N [a]	***S*** [b]	***R*** [c]	**Incidence Rate (%)**
T_3 transgenic lines	#1	86	4 [d]	82	4.65
	#2	103	0	103	0
	#3	64	9 [d]	55	14.06
	#4	135	4 [d]	131	2.96
	#5	50	1 [d]	49	2.00
	#6	160	0	160	0
	#7	74	2 [d]	72	2.70
	#8	149	0	149	0
Wild-type		111	46	65	41.44

[a] Total number of rice plants examined; [b] typical disease symptoms were observed at 30 days post-inoculation; [c] no symptoms were observed during the whole growth period; [d] the intron of *AtFAD2* was not detected in these plants by PCR.

Table S4. Primers used in this study.

Primer	Sequence * (5′–3′)	Genes	Accession Numbers
	Primers for the RNAi vector construct		
S1-For	GGTGGAACGAAAGTTCAGTAGATC	*S1*	AJ294757
S1-Rev	GGTGCTTCAGGCAAAAAGTTGTCAGAATTTGGACTACACTTGGACGAA		
S2-For	CTGACAACTTTTTGCCTGAAGCACCAGAGCGACAAGAAGAATCGAAA	*S2*	AJ409145
S2-Rev	TGGGATCAGACGAAAATATTGGACGCAAAAGTAGTTGTGTAAGCGGG		
S6-For	CGTCCAATATTTTCGTCTGATCCCACTCGAATCATCCGTCACTTCTGAGT	*S6*	AJ409148
S6-Rev	TGCGTTTGTTGACCATTACCATGAAGGACAAAACCTTTCCAATTATCGAG		
S10-For	TTCATGGTAATGGTCAACAAACGCAGGAAACATTACTTTGAAGCCC	*S10*	AF459813
S10-Rev	CCACCATAATGTGTAACATCCGTA		
iS1-For	GATGGATCCGGTGGAACGAAAGTTCAGTAGATC (*Bam*HI)		
iS10-Rev	GTAGCGGCCGCCCACCATAATGTGTAACATCCGTA (*Not*I)		
iS10-For	GATCTCGAGCCACCATAATGTGTAACATCCGTA (*Xho*I)		
iS1-Rev	GATGAATTCGGTGGAACGAAAGTTCAGTAGATC (*Eco*RI)		
	Primers for gene expression		
FAD-For	GAATTTCTCCGCTCACGA	*AtFAD2*	AJ271841
FAD-Rev	ATTTCCACCAACCCACCA		
S1-exp-For	CCTATGTTAACTTTCGCCAT	*S1*	AJ294757
S1-exp-Rev	TTCAATTACTCCATCACGTT		
S2-exp-For	CAGATTATCCGTTTATTAGCTC	*S2*	AJ409145
S2-exp-Rev	TTTCTTGTCCATCAAACGGTA		
S6-exp-For	TGCACACTTATCAATCACGTT	*S6*	AJ409148
S6-exp-Rev	GATACCTGTAGTTGACCGTTC		
S10-exp-For	TGCCACTAATACTTCAACCAC	*S10*	AF459813
S10-exp-Rev	GCACAACACTGAACTAGTCG		
S9-For	ATGGCAGACCAAGAGCGGAG	*S9*	NC_003731
S9-Rev	GGCATTTCAACTATTTTCTTCTC		
ACT-For	AGCCACACTGTCCCCATCTA	*Actin 1*	NM_001057621
ACT-Rev	AGCAAGGTCGAGACGAAGGA		

* Restriction sites/recombination sites are underlined.

Table S5. Sequence information of the four RBSDV target genes (*S1*, *S2*, *S6* and *S10*) used in his study.

Target Gene	Length (bp)	ORF Position	Protein Size (kDa)	Functions and Properties	RT-PCR Locus	Reference
S1	4501	36–4427	1464 (168.8)	RNA-dependent RNA polymerase	38–252	[9]
S2	3812	46–3723	1226 (141.5)	Core protein	117–415	[9]
S6	2645	82–2457	792 (89.9)	Silencing suppressor	202–478	[9]
S10	1801	22–1695	558 (63.3)	Coat protein	472–728	[11]

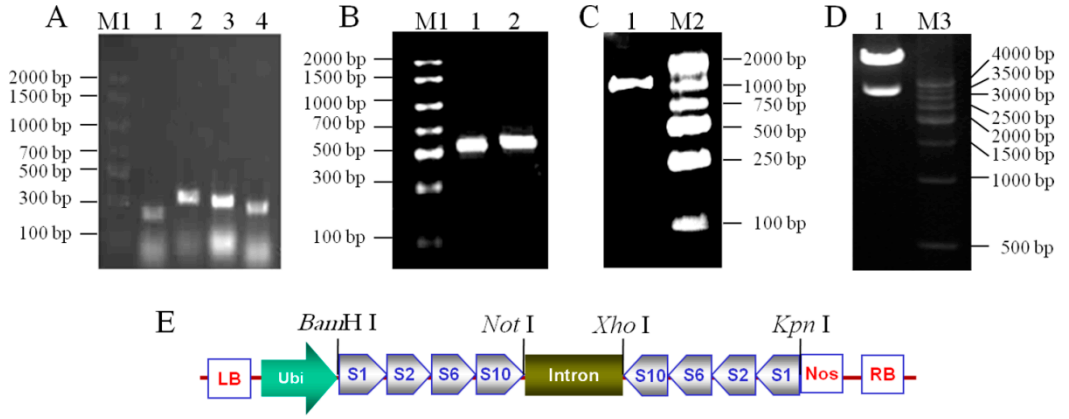

Figure S1. Construction of the hpRNA transformation vector. (**A**) Amplification of the individual gene fragments from the four target genes of RBSDV. Lanes 1–4 are the fragments of the *S1*, *S2*, *S6* and *S10* gene, respectively; (**B**) Concatenation of two segments. Lanes 1 and 2 show the concatenated intermediates of the *S1* + *S2* and the *S6* + *S10* fragments, respectively; (**C**) Concatenation of four segments. Lane 1 is the full-length fusion fragment; (**D**) Verification of the hpRNA vector. Lane 1 is the *Bam*HI/*Kpn*I double-digestion product containing the full-length fusion fragment and the vector backbone; (**E**) The schematic diagram shows the composition and organization of the hpRNA construct. LB and RB, left border and right border of the T-DNA, respectively. Ubi, maize ubiquitin promoter. Intron, the intron from *AtFAD2* serving as the spacer separating the inverted repeats of the viral sequence. Nos, the nopaline synthase terminator. M1, DNA Marker IV; M2, DNA Marker DL 2000; M3, 500-bp DNA Ladder.

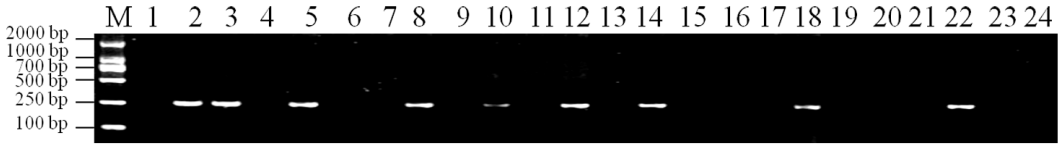

Figure S2. Identification of positive transgenic rice plants using PCR specific for the intron of *AtFAD2*. Lanes 1–21, the amplification results of 21 different transgenic rice plants; Lane 22, positive control; Lane 23, untransformed plant (negative control); Lane 24, water (negative control); M, DNA Marker DL 2000.

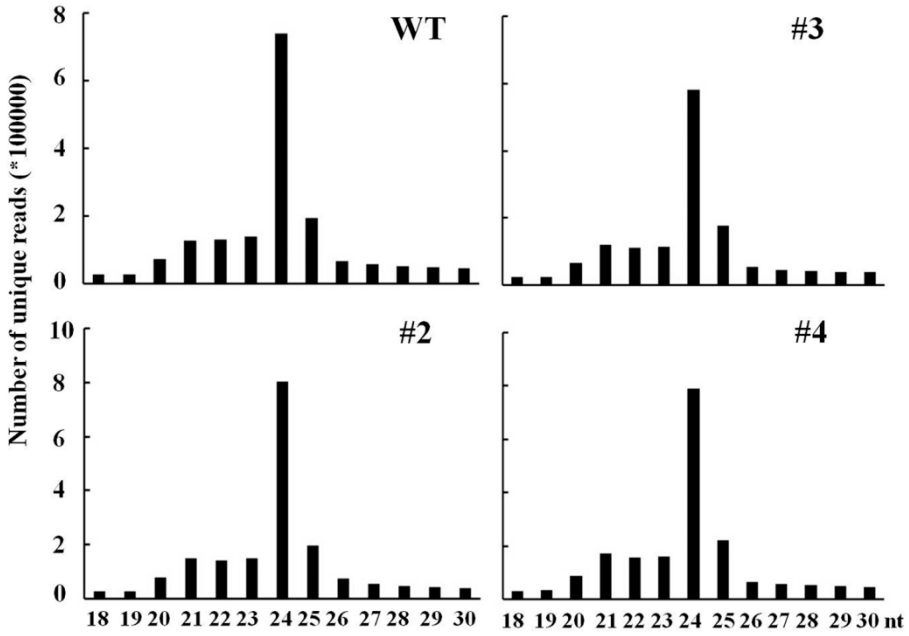

Figure S3. Size distribution of unique small RNAs from the three transgenic rice lines. WT, wild-type Kitaake; #2, #3 and #4, three independent transgenic rice lines resistant to RBSDV.