

Supplementary Information

Title: Identification and analysis of brown planthopper-responsive microRNAs in resistant and susceptible rice plants

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Supplemental Materials

Supplemental Figure S1. The preference distribution for uridine at the 5' end position of different lengths (nt) of known miRNAs.

Supplemental Figure S2. The preference distribution for uridine at the 5' end position of different lengths (nt) of novel miRNAs compared to known miRNAs of other selected plants in miRBase.

Supplemental Figure S3. The preference distribution for uridine at the 5' end position of different lengths (nt) of novel miRNAs identified by secondary structure prediction.

Supplemental Figure S4. Predicted secondary structures of the seven novel miRNAs.

Supplemental Figure S5. Original blots showing cropped areas for making Fig. 8c and 8d.

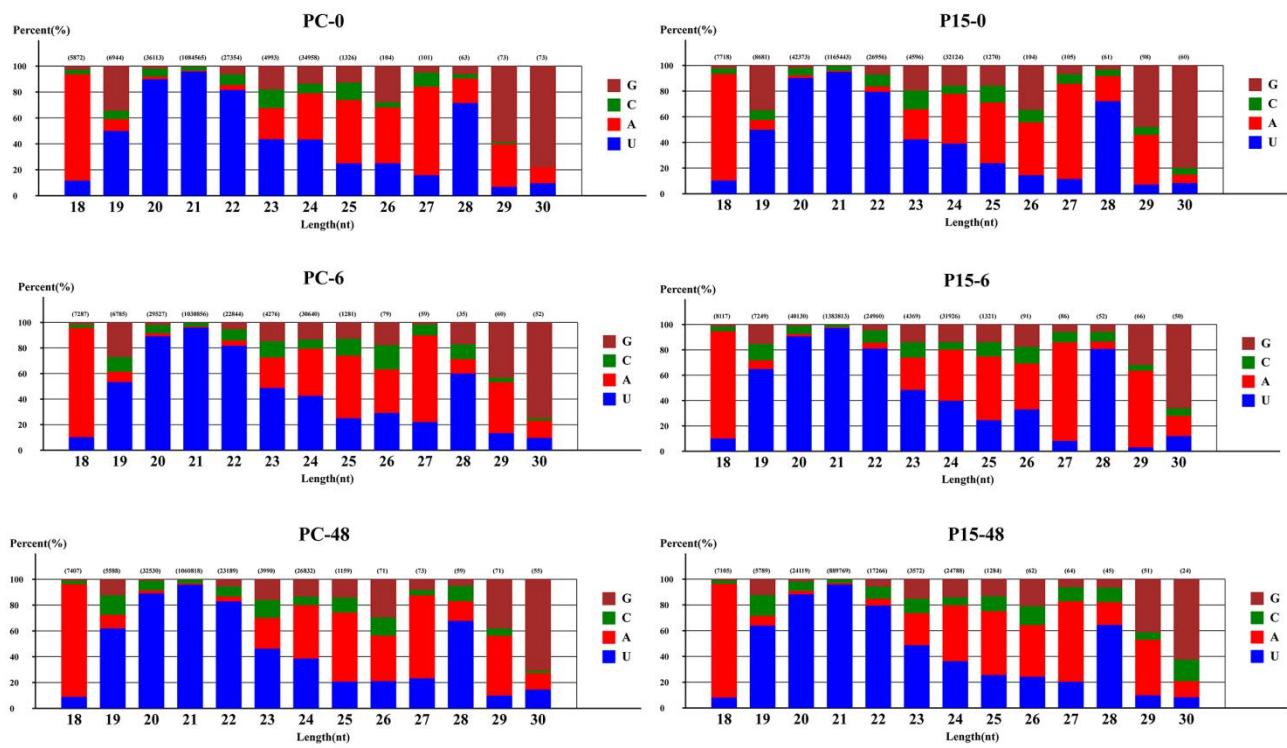
Supplemental Table S1. Novel miRNAs compared to the known miRNAs of other selected plants in miRBase and their total counts ≥ 100 .

Supplemental Table S2. Novel miRNAs identified by secondary structure prediction and their total counts ≥ 100 .

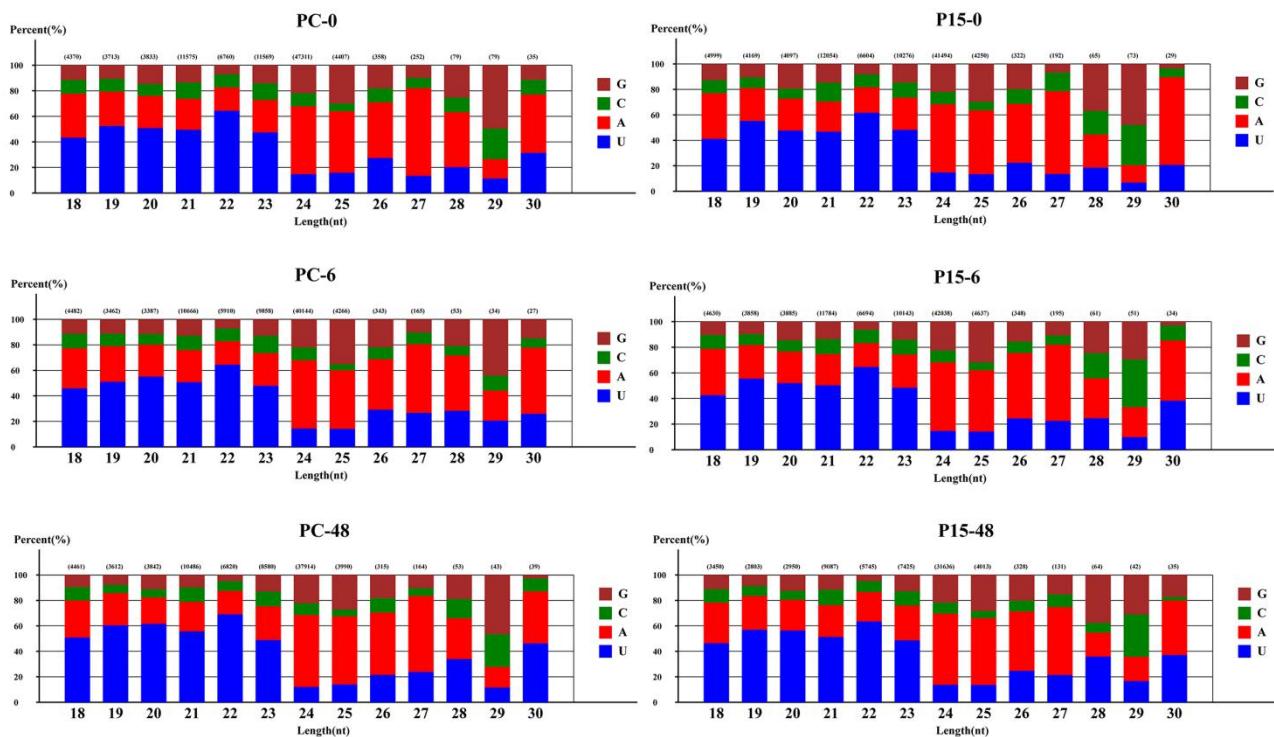
Supplemental Table S3. The target prediction of the differently expressed miRNAs in P15-0/PC-0.

Supplemental Table S4. The GO annotation of 94 resistance-related targets exhibiting the opposite expression to 46 differently expressed miRNAs.

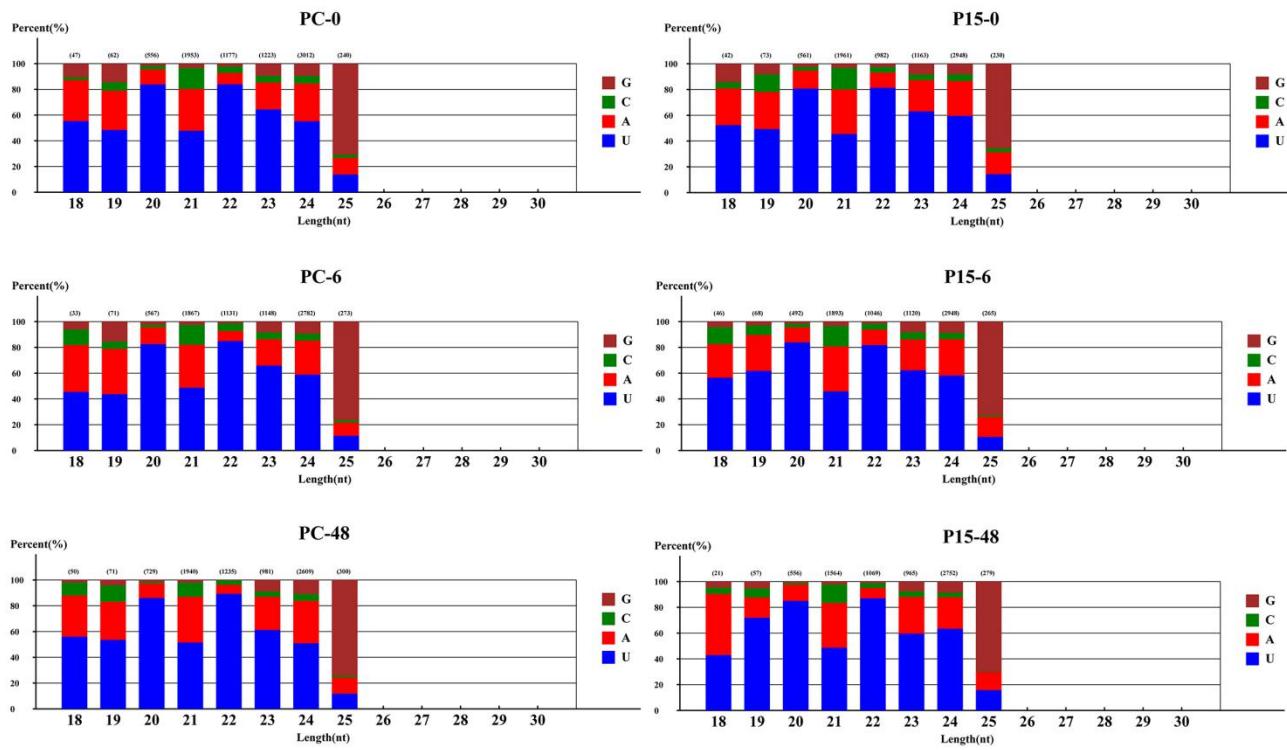
Supplemental Table S5. Primers used for qRT-PCR and transformation of rice protoplasts.



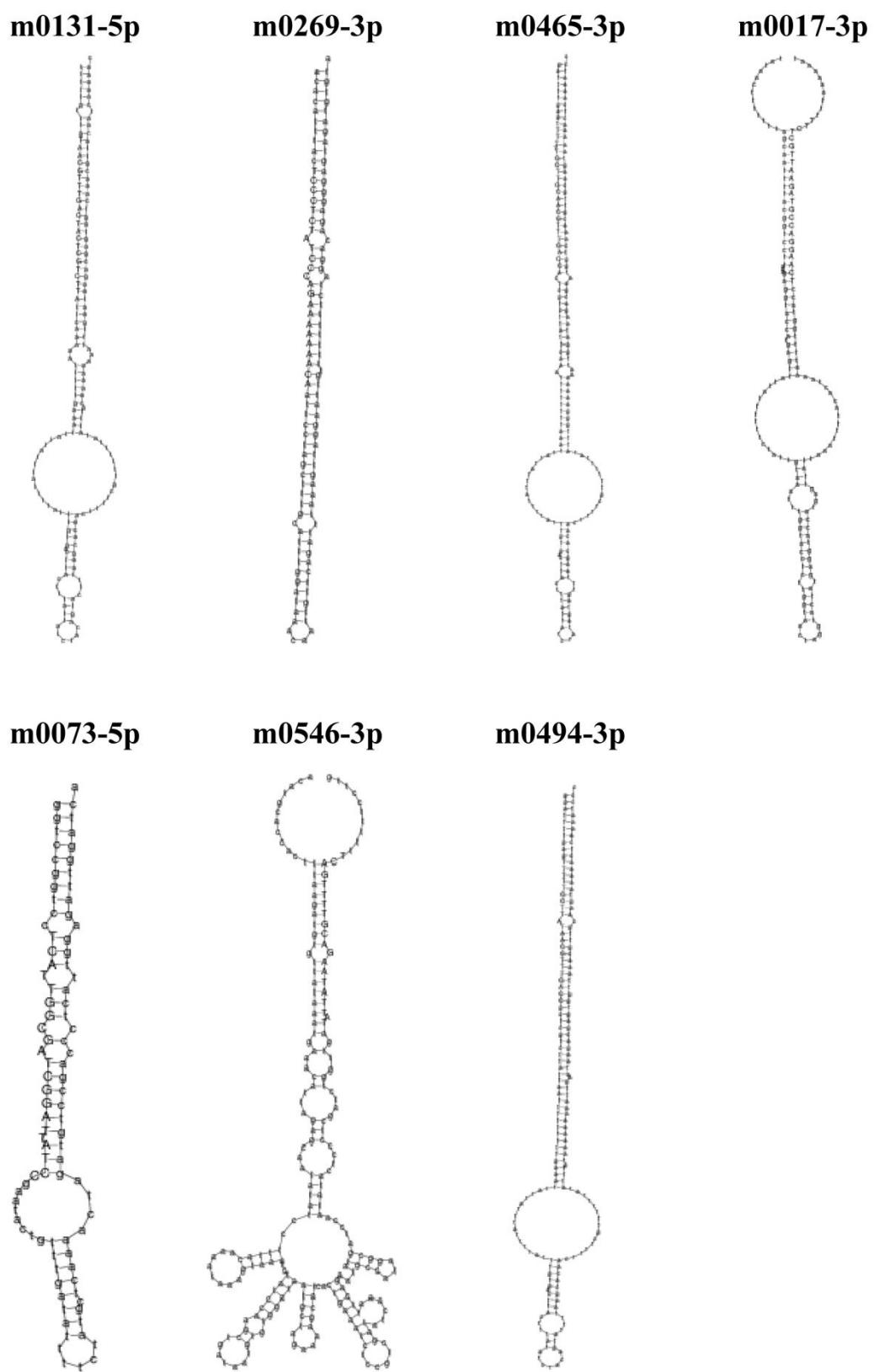
Supplemental Figure S1. The preference distribution for uridine at the 5' end position of different lengths (nt) of known miRNAs.



Supplemental Figure S2. The preference distribution for uridine at the 5' end position of different lengths (nt) of novel miRNAs compared to known miRNAs of other selected plants in miRBase.



Supplemental Figure S3. The preference distribution for uridine at the 5' end position of different lengths (nt) of novel miRNAs identified by secondary structure prediction.



Supplemental Figure S4. Predicted secondary structures of the seven novel miRNAs.

a Original blots

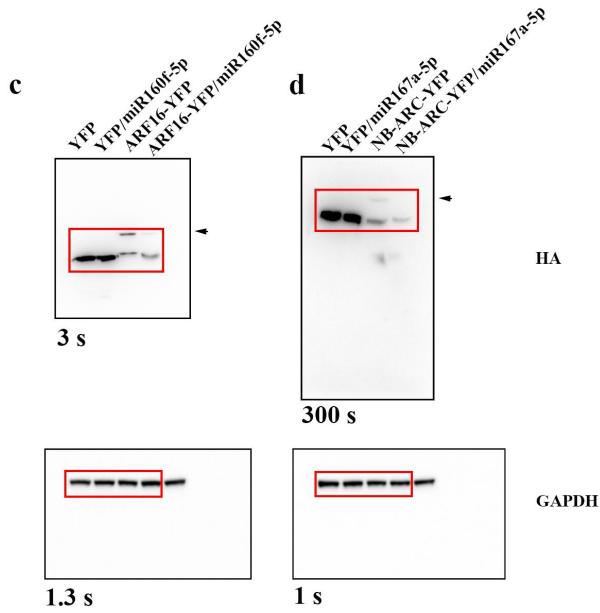
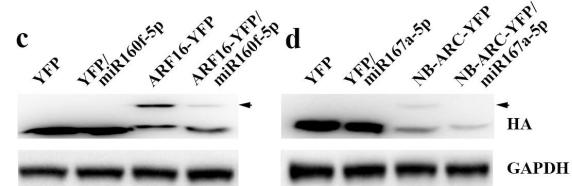
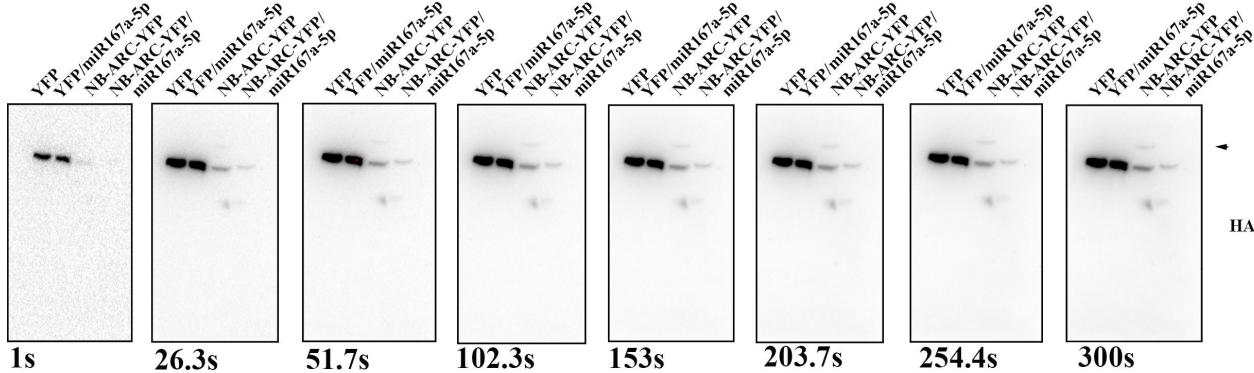


Fig. 8c, 8d



b



Supplemental Figure S5. Original blots showing cropped areas for making Fig. 8c and 8d. a. Red boxes in the original blots indicate cropped areas for the construction of Fig. 8c and 8d. The numbers below the original blots indicate the exposure time (s). **b.** The western detected by anti-HA antibody in Fig. 8d exposed at different time points (s).

MIR2880-x	24	GACGATATCCCGTTCGGACAAGAT	150	21	31	24	26	25	23	15.626	24.6279	18.7019	18.2321	15.2062	21.3216
MIR393-y	19	TCAGTCAATCCCTTGT	145	21	24	32	27	25	16	15.626	19.0668	24.9358	18.9333	15.2062	14.8324
MIR390-x	21	AGCTCAGGATAGATAGCGCCT	142	33	14	20	28	21	26	24.5552	11.1223	15.5849	19.6345	12.7732	24.1027
MIR1883-x	25	CCTGTGACGGGCCGAGAACATGGAACT	138	20	26	20	21	27	24	14.8819	20.6557	15.5849	14.7259	16.4227	22.2487
MIR2877-y	24	TTGCATCCTCTGCACATTGGGCCA	129	19	16	20	19	32	23	14.1378	12.7112	15.5849	13.3234	19.464	21.3216
MIR1120-y	23	TTGTTATACTCTGAGACGGAGGG	129	20	25	14	28	22	20	14.8819	19.8612	10.9094	19.6345	13.3815	18.5406
MIR6478-x	20	CCGACCTTAACTCAGTTGGT	129	19	18	24	30	20	18	14.1378	14.3001	18.7019	21.037	12.165	16.6865
MIR394-x	22	TTGGCATTCTGTCACCTCTCT	128	24	19	17	15	27	26	17.8583	15.0945	13.2471	10.5185	16.4227	24.1027
MIR9777-y	22	GTAGCAAATATCTGAGCACA	126	31	14	17	37	15	12	23.067	11.1223	13.2471	25.9456	9.1237	11.1243
MIR1876-y	24	ATAAATGGGTTGTGGCTGGCCC	123	17	21	12	25	25	23	12.6496	16.6834	9.3509	17.5308	15.2062	21.3216
MIR2871-y	23	TATTTAGTTCTATGGTCACTC	121	20	21	19	18	14	29	14.8819	16.6834	14.8056	12.6222	8.5155	26.8838
MIR399-y	21	TGCCAGAGGAGAACTGCACTG	120	28	15	9	31	23	14	20.8347	11.9167	7.0132	21.7382	13.9897	12.9784
MIR2871-x	21	TGACCGTAGAAACTAGCATTT	118	15	26	17	16	22	22	11.1614	20.6557	13.2471	11.2197	13.3815	20.3946
MIR5568-x	18	TTATAAGACGTTTGACT	114	14	21	23	20	22	14	10.4173	16.6834	17.9226	14.0247	13.3815	12.9784
MIR2873-y	18	TGAAGTTGAGTTGGACT	113	26	17	16	19	22	13	19.3465	13.5056	12.4679	13.3234	13.3815	12.0514
MIR1846-x	23	TCCCCCGAGCAGCGGATCTT	112	22	16	10	23	26	15	16.3701	12.7112	7.7924	16.1284	15.8145	13.9054
MIR5205-x	24	CTTATAATTGGGACGGAGGGAGT	110	21	19	20	17	19	14	15.626	15.0945	15.5849	11.921	11.5567	12.9784
MIR1423-y	24	GAGCGCCCGAGCGGTAGTTGTCTG	105	22	19	9	22	15	18	16.3701	15.0945	7.0132	15.4271	9.1237	16.6865
MIR1873-x	21	TCAACATGGTATCAGAACTGT	103	18	16	25	16	20	8	13.3937	12.7112	19.4811	11.2197	12.165	7.4162

novel-m0450-3p	23	TAGTTATTCGCCACATCTAAAAG	150	24	33	27	12	14	40	17.8583	26.2168	21.0396	8.4148	8.5155	37.0811
novel-m0447-5p	21	GTCACGGCCGGCGGAGCCTC	148	27	20	12	36	34	19	20.0906	15.889	9.3509	25.2444	20.6804	17.6135
novel-m0371-3p	22	TCAACGTTGGACACGGATTC	147	31	34	27	18	19	18	23.067	27.0113	21.0396	12.6222	11.5567	16.6865
novel-m0322-3p	24	CAGATTCTGTAGTCTAGGATGTGT	146	20	29	42	18	19	18	14.8819	23.039	32.7283	12.6222	11.5567	16.6865
novel-m0420-3p	21	TCGTCATCCCTTGTGGAGG	146	19	30	17	21	33	26	14.1378	23.8335	13.2471	14.7259	20.0722	24.1027
novel-m0360-3p	23	AAACCGGGACTAAAGATCGAGCT	145	27	28	19	33	20	18	20.0906	22.2446	14.8056	23.1407	12.165	16.6865
novel-m0392-3p	21	GTCAAACGTTGGACACGGATA	144	29	34	26	18	19	18	21.5788	27.0113	20.2603	12.6222	11.5567	16.6865
novel-m0322-5p	23	ACATCCTAGGACATGAATCTGGA	143	22	16	29	21	29	26	16.3701	12.7112	22.5981	14.7259	17.6392	24.1027
novel-m0052-5p	22	TTAATATATGACGTCGTTGACT	143	34	23	34	22	9	21	25.2993	18.2723	26.4943	15.4271	5.4742	19.4676
novel-m0328-5p	22	TAGGTCAAATTAGTAAGTAACC	140	24	22	18	22	21	33	17.8583	17.4779	14.0264	15.4271	12.7732	30.5919
novel-m0302-3p	21	TTCAGATTCTGTAGTATTATGA	136	24	22	36	16	12	26	17.8583	17.4779	28.0528	11.2197	7.299	24.1027
novel-m0496-5p	21	TTTGAGGAGGTACCATGAGGT	135	33	25	16	27	24	10	24.5552	19.8612	12.4679	18.9333	14.598	9.2703
novel-m0440-5p	20	TTTTTATTCAACGTTTGACT	131	19	13	22	33	24	20	14.1378	10.3278	17.1434	23.1407	14.598	18.5406
novel-m0174-5p	22	TTAATATATGACGTCGTTGACT	130	31	21	32	20	8	18	23.067	16.6834	24.9358	14.0247	4.866	16.6865
novel-m0533-5p	23	TAACCGGGACTAAAAATGATCTT	128	22	24	22	22	27	11	16.3701	19.0668	17.1434	15.4271	16.4227	10.1973
novel-m0266-5p	22	TTAATAGATGACGTTGTTGACT	127	28	35	26	18	13	7	20.8347	27.8057	20.2603	12.6222	7.9072	6.4892
novel-m0314-5p	22	TTTTTATTGAACTGTTGACC	124	20	22	26	24	17	15	14.8819	17.4779	20.2603	16.8296	10.3402	13.9054
novel-m0567-3p	21	AGACCAAGGAGAAATTAACTC	123	24	21	21	25	13	19	17.8583	16.6834	16.3641	17.5308	7.9072	17.6135
novel-m0458-5p	21	GTGCGCCAATGCTTTCAAGG	122	25	29	9	36	17	6	18.6024	23.039	7.0132	25.2444	10.3402	5.5622
novel-m0210-5p	22	TTCTAGTACAATGAATTGGAC	117	25	25	13	17	20	17	18.6024	19.8612	10.1302	11.921	12.165	15.7595
novel-m0051-3p	22	TTAAAACAGTTGACTTTAACCC	113	14	15	23	18	24	19	10.4173	11.9167	17.9226	12.6222	14.598	17.6135
novel-m0517-3p	22	TTAAAACAGTTGACTTTAACCC	113	14	15	24	17	24	19	10.4173	11.9167	18.7019	11.921	14.598	17.6135
novel-m0309-3p	21	AATCGGAAAAGATTGGTACC	109	0	0	0	39	34	36	0.01	0.01	0.01	27.3481	20.6804	33.373
novel-m0144-3p	22	TTGCGAGATGAGAAGTTGATT	107	14	26	26	14	16	11	10.4173	20.6557	20.2603	9.8173	9.732	10.1973
novel-m0164-3p	21	CTGCCCGCGTCGGCTCTCTCC	106	21	19	6	20	31	9	15.626	15.0945	4.6755	14.0247	18.8557	8.3433
novel-m0034-3p	21	TCATGCGCACATTATTAACG	105	14	15	21	24	19	12	10.4173	11.9167	16.3641	16.8296	11.5567	11.1243
novel-m0083-3p	22	TTAGAGTAGTTGACTTTGACT	103	24	12	19	18	16	14	17.8583	9.5334	14.8056	12.6222	9.732	12.9784
novel-m0364-5p	22	TTAATAGATGACGTTGTTGACT	102	21	26	23	16	10	6	15.626	20.6557	17.9226	11.2197	6.0825	5.5622
novel-m0252-5p	21	CGTTTGACCGTGGTCTTGCT	101	16	22	17	17	16	13	11.9055	17.4779	13.2471	11.921	9.732	12.0514
novel-m0024-5p	21	CGTTTGACCGTGGTCTTGCT	101	16	22	17	17	16	13	11.9055	17.4779	13.2471	11.921	9.732	12.0514
novel-m0062-3p	22	CAAAATTTCCATGACTTCGA	100	15	28	7	12	24	14	11.1614	22.2446	5.4547	8.4148	14.598	12.9784

									protein, putative, expressed						
osa-miR1846d-3p	6	17	4.4646	11.921	1.434409235	0.034791993	*	LOC_Os08g39220	OsWAK75 - OsWAK receptor-like protein kinase, expressed	0	0	NA	0	0	
osa-miR1846d-3p	6	17	4.4646	11.921	1.434409235	0.034791993	*	LOC_Os09g28400	alpha-amylase precursor, putative, expressed	0	0	NA	0	0	
osa-miR531a	0	23	0.01	16.1284	7.506928563	2.41E-07	**								
osa-miR531c	0	23	0.01	16.1284	7.506928563	2.41E-07	**								
osa-miR1862a	26	533	19.3465	373.7576	4.301495202	1.59E-117	**								
osa-miR1862b	26	533	19.3465	373.7576	4.301495202	1.59E-117	**								
osa-miR1862c	26	533	19.3465	373.7576	4.301495202	1.59E-117	**								
osa-miR396e-3p	12	41	8.9292	28.7506	1.71276181	0.000130031	**								

					signaling pathway				
LOC_Os04g54900.1	LOC_Os04g54900.1	Similar to H0423H10.4 protein.	1E-20	GO:0009740	gibberellic acid mediated signaling pathway	0.027333669	2.729240457	osa-miR3979-3p	P15
LOC_Os07g10620.1	Os07g0206900	Protein of unknown function DUF642 domain containing protein.	4E-100	GO:0009740	gibberellic acid mediated signaling pathway	0.027333669	2.729240457	osa-miR3979-3p	P15
LOC_Os09g02410.1	Os09g0112100	Hypothetical protein.	2E-20	GO:0009863	salicylic acid mediated signaling pathway	0.029318731	2.161558442	osa-miR399a	P15 and PC
LOC_Os09g02410.1	Os09g0112100	Hypothetical protein.	2E-20	GO:0009863	salicylic acid mediated signaling pathway	0.029318731	2.161558442	osa-miR399b	P15 and PC
LOC_Os09g02410.1	Os09g0112100	Hypothetical protein.	2E-20	GO:0009863	salicylic acid mediated signaling pathway	0.029318731	2.161558442	osa-miR399c	P15 and PC
LOC_Os12g36210.1	Os12g0548450	Similar to Proteinase inhibitor.		GO:0009607	response to biotic stimulus	0.0325429	1.514185782	osa-miR531b	P15
LOC_Os12g06210.1	Os12g0158900	Late embryogenesis abundant protein, LEA-14 domain containing protein.	4E-14	GO:0009607	response to biotic stimulus	0.0325429	1.514185782	osa-miR530-3p	P15 and PC
LOC_Os04g36040.1	Os04g0441800	TGF-beta receptor, type I/II extracellular region family protein.	0	GO:0009607	response to biotic stimulus	0.0325429	1.514185782	osa-miR6249a	P15 and PC
LOC_Os04g36040.1	Os04g0441800	TGF-beta receptor, type I/II extracellular region family protein.	0	GO:0009607	response to biotic stimulus	0.0325429	1.514185782	osa-miR6249b	P15 and PC
LOC_Os03g11010.1	Os03g0208500	Integral membrane protein.	0	GO:0009607	response to biotic stimulus	0.0325429	1.514185782	osa-miR1846e	PC
LOC_Os04g45290	Os04g0535600	Similar to Beta-fructofuranosidase 1 precursor (EC 3.2.1.26) (Sucrose 1) (Invertase 1).	1E-169	GO:0009738	abscisic acid-activated signaling pathway	0.038850955	1.833382902	osa-miR408-3p	P15
LOC_Os05g07880	Os05g0171000	Phospholipase D2 (EC 3.1.4.4).	0	GO:0009738	abscisic acid-activated signaling pathway	0.038850955	1.833382902	osa-miR6249a	P15 and PC
LOC_Os05g07880	Os05g0171000	Phospholipase D2 (EC 3.1.4.4).	0	GO:0009738	abscisic acid-activated signaling pathway	0.038850955	1.833382902	osa-miR6249b	P15 and PC
LOC_Os02g12810	Os02g0220700	Hypothetical conserved gene.	0	GO:0016310	phosphorylation	0.046977778	1.316618512	osa-miR167d-3p	P15
LOC_Os04g21110.1	LOC_Os04g21110.1	Hypothetical conserved gene.	0	GO:0016310	phosphorylation	0.046977778	1.316618512	osa-miR172d-5p	P15
LOC_Os06g05070.1	Os06g0142650	Similar to Avr9/Cf-9 rapidly elicited protein 11 (Fragment).	9E-106	GO:0016310	phosphorylation	0.046977778	1.316618512	osa-miR531b	P15
LOC_Os03g53230.1	Os03g0743900	Similar to ATP sulfurylase.	0	GO:0016310	phosphorylation	0.046977778	1.316618512	osa-miR395l	P15 and PC
LOC_Os03g53230.1	Os03g0743900	Similar to ATP sulfurylase.	0	GO:0016310	phosphorylation	0.046977778	1.316618512	osa-miR395m	P15 and PC
LOC_Os03g53230.1	Os03g0743900	Similar to ATP sulfurylase.	0	GO:0016310	phosphorylation	0.046977778	1.316618512	osa-miR395n	P15 and PC
LOC_Os03g53230.1	Os03g0743900	Similar to ATP sulfurylase.	0	GO:0016310	phosphorylation	0.046977778	1.316618512	osa-miR395p	P15 and PC
LOC_Os03g53230.1	Os03g0743900	Similar to ATP sulfurylase.	0	GO:0016310	phosphorylation	0.046977778	1.316618512	osa-miR395q	P15 and PC
LOC_Os03g53230.1	Os03g0743900	Similar to ATP sulfurylase.	0	GO:0016310	phosphorylation	0.046977778	1.316618512	osa-miR395r	P15 and PC
LOC_Os02g17910.1	Os02g0280400	Similar to casein kinase I isoform delta-like.	5E-164	GO:0016310	phosphorylation	0.046977778	1.316618512	osa-miR399a	P15 and PC
LOC_Os02g17910.1	Os02g0280400	Similar to casein kinase I isoform delta-like.	5E-164	GO:0016310	phosphorylation	0.046977778	1.316618512	osa-miR399b	P15 and PC
LOC_Os02g17910.1	Os02g0280400	Similar to casein kinase I isoform delta-like.	5E-164	GO:0016310	phosphorylation	0.046977778	1.316618512	osa-miR399c	P15 and PC
LOC_Os02g17910.1	Os02g0280400	Similar to casein kinase I isoform delta-like.	5E-164	GO:0016310	phosphorylation	0.046977778	1.316618512	osa-miR399d	P15 and PC
LOC_Os04g41100	Os04g0488000	Similar to PITSLRE serine/threonine-protein kinase CDC2L1 (EC 2.7.1.37) (Galactosyltransferase associated protein kinase p58/GTA) (Cell division cycle 2-like protein kinase 1).	0	GO:0016310	phosphorylation	0.046977778	1.316618512	osa-miR530-3p	P15 and PC
LOC_Os01g59360.1	Os01g0808400	Similar to Calcium-dependent protein kinase (Fragment).	0	GO:0016310	phosphorylation	0.046977778	1.316618512	osa-miR6249a	P15 and PC
LOC_Os03g60810.1	Os03g0823000	Resistance protein candidate (Fragment).	0	GO:0016310	phosphorylation	0.046977778	1.316618512	osa-miR6249a	P15 and PC
LOC_Os01g59360.1	Os01g0808400	Similar to Calcium-dependent protein kinase (Fragment).	0	GO:0016310	phosphorylation	0.046977778	1.316618512	osa-miR6249b	P15 and PC
LOC_Os03g60810.1	Os03g0823000	Resistance protein candidate (Fragment).	0	GO:0016310	phosphorylation	0.046977778	1.316618512	osa-miR6249b	P15 and PC
LOC_Os05g34390.1	LOC_Os05g34390.1		2E-70	GO:0016310	phosphorylation	0.046977778	1.316618512	osa-miR166k-5p	PC

Supplemental Table S5. Primers used for qRT-PCR and transformation of rice protoplasts.

Primers used for qRT-PCR.

Primer Name	Primer Sequences
SL1-miR166a-3p	GTCGTATCCAGTGCAGGGTCCGAGGTATTGCACGGATACGACGGGAAT
Q1-miR166a-3p	cctCGGACCAGGCTTCATT
SL2-miR169h	GTCGTATCCAGTGCAGGGTCCGAGGTATTGCACGGATACGACCAAGGCAA
Q2-miR169h	cggcTAGCCAAGGATGACTT
SL3-miR168a-3p	GTCGTATCCAGTGCAGGGTCCGAGGTATTGCACGGATACGACATTCACT
Q3-miR168a-3p	ATGATCCCGCCTTGACCAA
SL4-miR396c-5p	GTCGTATCCAGTGCAGGGTCCGAGGTATTGCACGGATACGACAAGTTCA
Q4-miR396c-5p	ccgcTTCCACAGCTTCTTG
SL5-miR399d	GTCGTATCCAGTGCAGGGTCCGAGGTATTGCACGGATACGACCAAGGGCA
Q5-miR399d	gctTGCCAAAGGAGAGTTG
SL6-miR444e	GTCGTATCCAGTGCAGGGTCCGAGGTATTGCACGGATACGACAAGCTTG
Q6-miR444e	TTCCTTGCAGTTGCTGCCTC
SL7-miR827	GTCGTATCCAGTGCAGGGTCCGAGGTATTGCACGGATACGACTGTTGC
Q7-miR827	gcggTTAGATGACCATCAGC
SL8-miR1425-5p	GTCGTATCCAGTGCAGGGTCCGAGGTATTGCACGGATACGACAGCAGCA
Q8-miR1425-5p	gcgcTAGGATTCAATCCTT
SL9-MIR8155-y	GTCGTATCCAGTGCAGGGTCCGAGGTATTGCACGGATACGACTGGTATC
Q9-MIR8155-y	gcgcAACCTGGCTCTGA
SL10-MIR5168-y	GTCGTATCCAGTGCAGGGTCCGAGGTATTGCACGGATACGACGAAGGGA
Q10-MIR5168-y	atgTCGGACCAGGCTTCAATC
SL11-MIR8175-y	GTCGTATCCAGTGCAGGGTCCGAGGTATTGCACGGATACGACTGGCGCC
Q11-MIR8175-y	atgcCCCCGGCAACGG
SL12-novel-m0131-5p	GTCGTATCCAGTGCAGGGTCCGAGGTATTGCACGGATACGACTAAGACG
Q12-novel-m0131-5p	ggggTAACGTTGACTACTCG
SL13-novel-m0269-3p	GTCGTATCCAGTGCAGGGTCCGAGGTATTGCACGGATACGACACTCCCT
Q13-novel-m0269-3p	ggggTTTTTTCTAGGACAGAG
SL14-novel-m0465-3p	GTCGTATCCAGTGCAGGGTCCGAGGTATTGCACGGATACGACGTTTTA
Q14-novel-m0465-3p	ggggTCAAACGTTGCAAGTA
SL15-novel-m0017-3p	GTCGTATCCAGTGCAGGGTCCGAGGTATTGCACGGATACGACAAGAGCA
Q15-novel-m0017-3p	ggggTCAAGGACCGTAGAATTG
SL16-novel-m0073-5p	GTCGTATCCAGTGCAGGGTCCGAGGTATTGCACGGATACGACGGATAAT
Q16-novel-m0073-5p	atgcTCATTGGCGATCGGAT
SL17-novel-m0546-3p	GTCGTATCCAGTGCAGGGTCCGAGGTATTGCACGGATACGACAGTCAA
Q17-novel-m0546-3p	ggcccggtATTATAAGACGTTT
SL18-novel-m0494-3p	GTCGTATCCAGTGCAGGGTCCGAGGTATTGCACGGATACGACAGTTTT
Q18-novel-m0494-3p	ggggTTAAACGTTGCAAGTAA
U6-F	TACAGATAAGATTAGCATGGCCCC
U6-R	GGACCATTCTCGATTGTACGTG
SL-universal R	GTGCAGGGTCCGAGGTATT

Primers used for transformation of rice protoplasts.

Primer Name	Primer Sequences
miR160f-5pF	TACCACTGCTACTCTGCCTG
miR160f-5pR	AGTGCAAAATAGAGGATCGCA
miR167a-5pF	GCCAAAGCCTGAAACACTTTG
miR167a-5pR	ACATACACTGAGAAATCCAGCA
ARF16-FSpe1	GAATAGTATGATCACGTTCGCGGACCT
ARF16-RSpe1	GAATAGTGTCCCTAACGTTGTCGCTCC
Os07g29820FXmaJ1	CCCTAGGATGGAGACAATTCTAGCTGG
Os07g29820RXmaJ1	CCCTAGGATGTGAAGAAGCATGCGTGG