Two Different Transcripts of a LAMMER Kinase Gene Play Opposite Roles in Rice Disease Resistance

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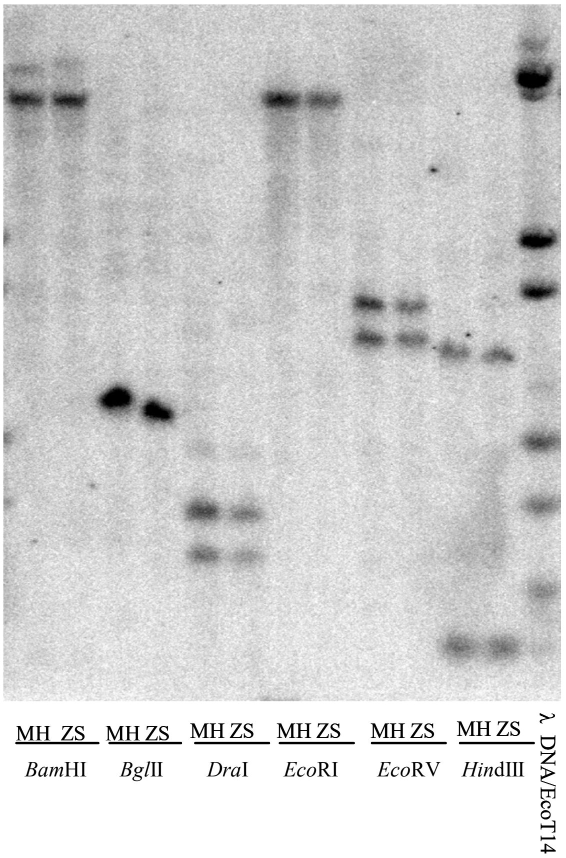
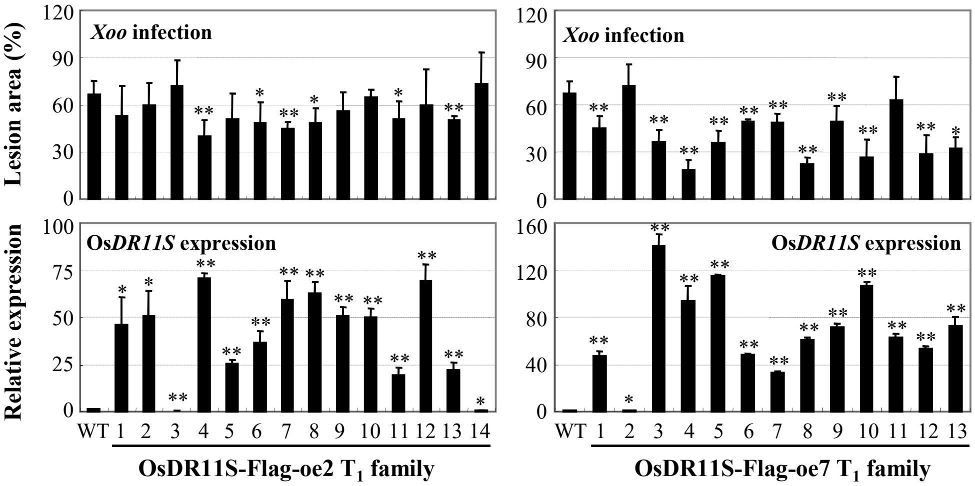
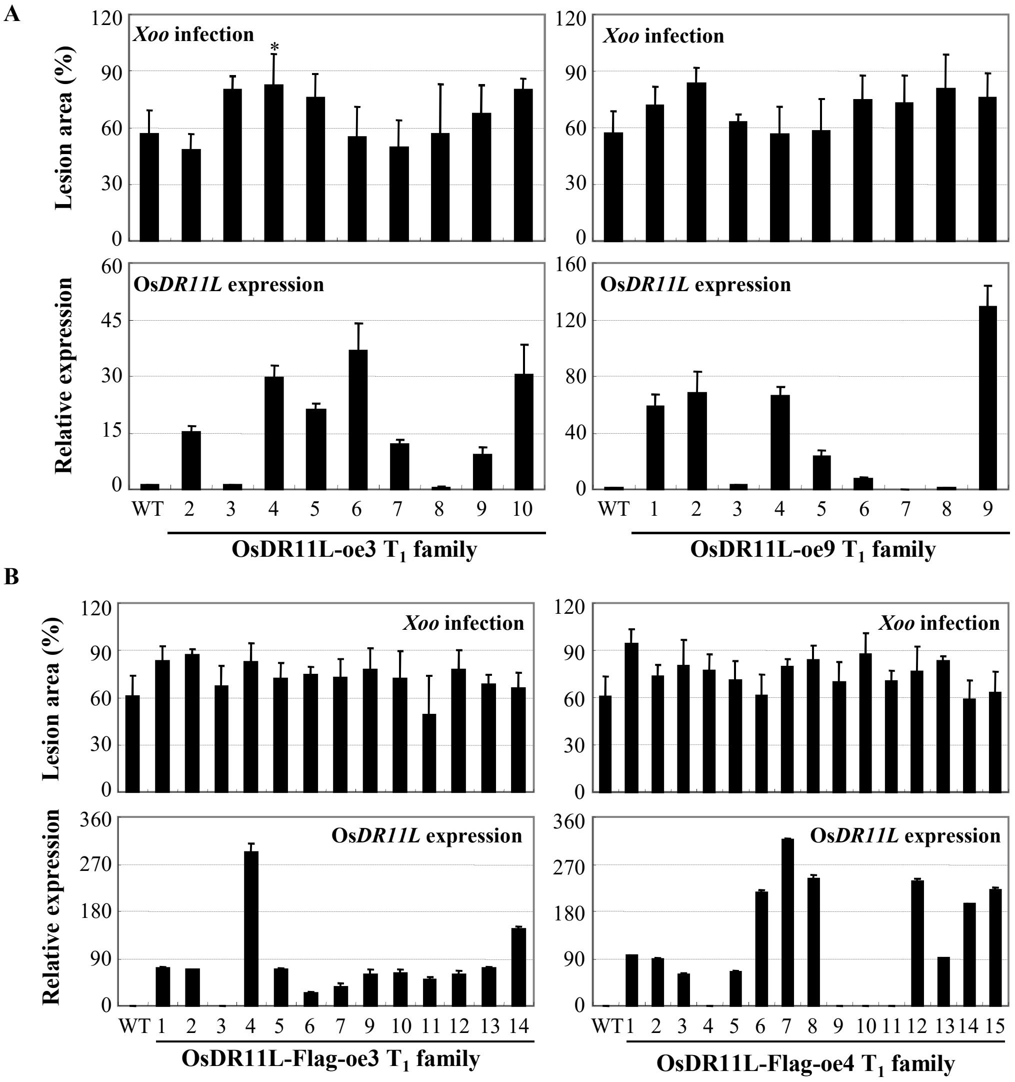
Supplemental Data



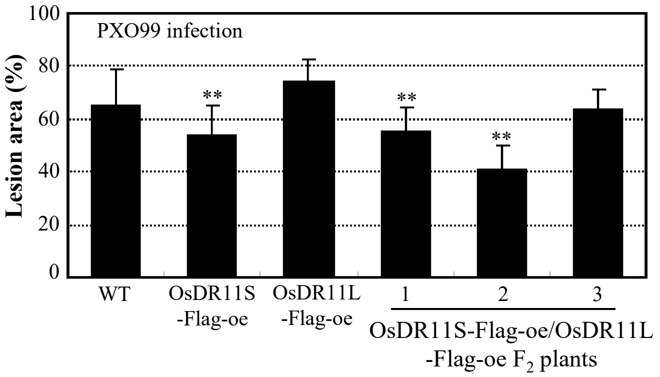
Supplemental Figure S1. DNA gel blot analysis of Os*DR11* gene in rice varieties Minghui 63 (MH) and Zhenshan 97 (ZS).



Supplemental Figure S2. Enhanced resistance to *Xoo* was associated with overexpression of *OsDR11S* in two T_1 families. Plants were inoculated with *Xoo* strain PXO99 at the booting (panicle development) stage. Bars represent mean (three to six replicates from one plant for l area and three replicates from one plant for gene expression) ± standard deviation. Asterisks indicate significant difference between transgenic and wild-type (WT) plants at $^{**}P < 0.01$ or $^{*}P < 0.05$.



Supplemental Figure S3. The response of OsDR11L-oe (A) and OsDR11L-Flag-oe (B) plants to infection by *Xoo* strain PXO99 was not closely associated with overexpression of Os*DR11L*, respectively. The plants were inoculated with PXO99 at the booting stage. Bars represent means (three replicates) ± standard deviation..



Supplemental Figure S4. The F_2 plants overexpressing both Os*DR11S-Flag* and *OsDR11L-Flag* showed the phenotype of OsDR11S-Flag-oe line in response to *Xoo* infection. Plants were inoculated with *Xoo* strain PXO99 at the booting stage. The F_2 plants were generated from a cross between OsDR11S-Flag-oe and OsDR11L-Flag-oe lines. WT, wild-type Mudanjiang 8. 1, F_2 plants carrying both Os*DR11S-Flag* and *OsDR11L-Flag* constructs; 2, F_2 plants carrying only Os*DR11S-Flag* construct; 3, F_2 plants carrying only Os*DR11L-Flag* construct. Data represent mean (30 to 32 plants for F_2 carrying both Os*DR11S-Flag* and *OsDR11L-Flag* constructs, 15 to 20 plants for OsDR11S-Flag-oe line and OsDR11L-Flag-oe line, and three to four plants for the F_2 carrying only *OsDR11S-Flag* or *OsDR11L-Flag* construct) ± standard deviation. Asterisks indicate significant difference between transgenic or F_2 and WT at $^{**}P < 0.01$ or $^{*}P < 0.05$.

Supplemental Table S1. The phenotype of Os*DR11S*-overexpressing T_0 plants after inoculation with *Xoo* strain PXO99

Rice material	**Lesion area (%)**[a]	***P*-value**[b]
OsDR11S-oe31	37.4±11.7	0.061
OsDR11S-oe32	36.1±14.3	0.083
OsDR11S-oe33	14.2±9.3	0.000
OsDR11S-oe34	46.0±6.8	0.126
OsDR11S-oe35[c]	64.0±15.6	0.429
OsDR11S-oe36	68.0±9.0	0.087
OsDR11S-oe37	39.0±4.7	0.004
OsDR11S-oe38	42.4±12.9	0.168
OsDR11S-oe51[c]	61.9±4.2	0.099
OsDR11S-oe52	31.6±16.2	0.048
OsDR11S-oe53	51.2±21.8	0.731
OsDR11S-oe54	25.0±8.7	0.001
OsDR11S-oe55	55.7±21.5	0.986
OsDR11S-oe56	18.4±8.1	0.000
OsDR11S-oe57[c]	52.0±15.8	0.699
OsDR11S-oe58	26.2±7.5	0.002
OsDR11S-oe59	67.7±10.9	0.143
OsDR11S-oe60	21.2±14.7	0.008
OsDR11S-oe61[c]	68.0±7.0	0.038
OsDR11S-oe62[c]	76.7±8.3	0.008
OsDR11S-oe63[c]	72.4±21.2	0.244
Mudanjiang 8 (wild type)	59.8±11.0	

[a] Data were from four to five flag leaves of each plant at the booting (panicle development) stage. Disease on leaves was scored at 14 days after infection.

[b] Each *P*-value was calculated in comparison with wild-type Mudanjiang 8.

[c] The negative transgenic plants detected by PCR analysis.

Supplemental Table S2. The phenotype of Os*DR11S*-suppressing T_0 plants after inoculation with *Xoo* strain PXO99

Rice material	Lesion area (%)[a]	*P*-value[b]
OsDR11-ami6[c]	53.3 ± 4.1	0.117
OsDR11-ami7[c]	53.6 ± 7.9	0.239
OsDR11-ami8	66.9 ± 8.7	0.046
OsDR11-ami9	62.6 ± 4.4	0.040
OsDR11-ami10	69.5 ± 15.4	0.036
OsDR11-ami16	67.1 ± 7.1	0.007
OsDR11-ami17	60.8 ± 14.5	0.283
OsDR11-ami18	56.9 ± 19.1	0.492
OsDR11-ami19	68.6 ± 14.5	0.029
OsDR11-ami20	75.8 ± 18.1	0.038
OsDR11-ami21 [c]	62.5 ± 14.8	0.139
OsDR11-ami27[c]	59.8 ± 6.5	0.201
OsDR11-ami28[c]	52.4 ± 14.8	0.334
OsDR11-ami29[c]	63.2 ± 9.4	0.107
OsDR11-ami30	70.9 ± 11.0	0.011
OsDR11-ami31 [c]	57.7 ± 16.3	0.450
OsDR11-ami32[c]	58.9 ± 6.6	0.261
OsDR11-ami33[c]	59.4 ± 8.0	0.282
OsDR11-ami39	78.3 ± 13.2	0.009
OsDR11-ami40	74.4 ± 7.3	0.001
OsDR11-ami41	74.3 ± 14.2	0.014
OsDR11-ami42	71.4 ± 6.6	0.002
OsDR11-ami43	73.1 ± 15.0	0.057
OsDR11-ami44	63.0 ± 5.4	0.034
Minghui 63 (wild type)	56.7 ± 10.0	

[a] Data were from four to five flag leaves of each plant at the booting stage. Disease on leaves was scored at 14 days after infection.

[b] Each *P*-value was calculated in comparison with wild-type Minghui 63.

[c] The negative transgenic plants detected by PCR analysis.

Supplemental Table S3. The phenotype of Os*DR11L*-overexpression T_0 plants inoculated with *Xoo* strain PXO99

Rice material	**Lesion area (%)**[a]	***P*-value**[b]
OsDR11L-oe1	50.9±9.3	0.942
OsDR11L-oe2	58.6±9.4	0.498
OsDR11L-oe3	76.2±4.5	0.012
OsDR11L-oe4	56.3±12.8	0.543
OsDR11L-oe5	31.4±18.6	0.127
OsDR11L-oe6	42.8±11.4	0.458
OsDR11L-oe7[c]	79.0±4.5	0.006
OsDR11L-oe8	66.3±10.2	0.150
OsDR11L-oe9	80.5±5.5	0.005
OsDR11L-oe10	44.3±5.5	0.528
OsDR11L-oe11	73.8±5.6	0.021
OsDR11L-oe12	47.5±11.0	0.781
OsDR11L-oe13	54.7±19.7	0.681
OsDR11L-oe14	48.2±8.3	0.834
OsDR11L-oe15	50.8±13.4	0.955
OsDR11L-oe16	60.8±15.5	0.315
OsDR11L-oe17	69.1±15.8	0.084
OsDR11L-oe18	62.8±17.6	0.289
OsDR11L-oe19	43.8±17.3	0.549
OsDR11L-oe20	64.2±15.2	0.227
Mudanjiang 8 (wild type)	50.2±19.7	

[a] Data were from four to five flag leaves of each plant at the booting stage. Disease on leaves was scored at 14 days after infection.

[b] Each *P*-value was calculated in comparison with wild type Mudanjiang 8.

[c] The negative transgenic plants detected by PCR analysis.

Supplemental Table S4. The phenotype of Os*DR11L-Flag*-overexpression T_0 plants inoculated with *Xoo* strain PXO99

Rice material	**Lesion area (%)**[a]	***P*-value**[b]
OsDR11L-Flag-oe1	62.0 ± 9.6	0.404
OsDR11L-Flag-oe2	81.6 ± 8.0	0.020
OsDR11L-Flag-oe3	84.8 ± 11.6	0.010
OsDR11L-Flag-oe4	74.3 ± 16.4	0.125
OsDR11L-Flag-oe5	77.2 ± 15.1	0.051
OsDR11L-Flag-oe6	56.1 ± 12.9	0.232
OsDR11L-Flag-oe7	82.2 ± 10.6	0.018
OsDR11L-Flag-oe8	66.0 ± 13.0	0.386
OsDR11L-Flag-oe9[c]	59.9 ± 13.2	0.331
OsDR11L-Flag-oe10[c]	60.8 ± 14.5	0.385
Mudanjiang 8 (wild type)	63.7 ± 13.6	

[a] Data were from four to five flag leaves of each plant at the booting stage. Disease on leaves was scored at 14 days after infection.

[b] Each *P*-value was calculated in comparison with wild type Mudanjiang 8.

[c] The negative transgenic plants detected by PCR analysis.

Supplemental Table S5. The phenotype of Os*DR11L*-suppressing T_0 plants after inoculation with *Xoo* strain PXO99

Rice material	**Lesion area (%)**[a]	***P*-value**[b]
OsDR11L-RNAi1	67.6 ± 3.5	0.023
OsDR11L-RNAi2	73.2 ± 9.6	0.002
OsDR11L-RNAi3	72.0 ± 11.0	0.009
OsDR11L-RNAi4	74.3 ± 15.7	0.015
OsDR11L-RNAi5	65.3 ± 12.7	0.001
OsDR11L-RNAi6	98.5 ± 2.1	0.185
OsDR11L-RNAi7	64.3 ± 10.3	0.003
OsDR11L-RNAi8	72.8 ± 8.5	0.001
OsDR11L-RNAi9	97.8 ± 2.7	0.368
OsDR11L-RNAi10	/	/
OsDR11L-RNAi1 [c]	94.9 ± 6.8	0.272
OsDR11L-RNAi12[c]	95.0 ± 10.0	0.344
Minghui 63 (wild type)	97.2 ± 3.5	

[a] Data were from four to five flag leaves of each plant at the booting stage. Disease on leaves was scored at 14 days after infection.

[b] Each *P*-value was calculated in comparison with wild-type Minghui 63.

[c] The negative transgenic plants detected by PCR analysis.

Supplemental Table S6. Primers used for PCR amplification

Gene name (GenBank accession number)	Primer name	Forward primer (5’-3’)	Reverse primer (5’-3’)	Use
Os*DR11* (KR107935)	39C8-GSP2/39C8-GSP3	CTTTCCTTTCCCTATCCCAAC	AGCCGAGAGAGAGAGAAAGA	Sequencing Os*DR11*
	39C8-8F/39C8-6R	GTCCCAGGAAATCCCTCGTT	CTGCGGTAGCTTTGGTAACTC	Sequencing Os*DR11*
	39C8-3F	TTGGATGGAGTTACCCCTGTG		Sequencing Os*DR11*
	39c8mir-1/2	AGAACGGGATGAAACCTGGATCTCAGGAGATTCAGTTTGA	TGAGATCCAGGTTTCATCCCGTTCTGCTGCTGCTACAGCC	Oligos for artificial microRNA vector of Os*DR11S*
	39c8mir-3/4	CTAGATCGAGGATTCATCCCGTTTTCCTGCTGCTAGGCTG	AAAACGGGATGAATCCTCGATCTAGAGAGGCAAAAGTGAA	Oligos for artificial microRNA vector of Os*DR11S*
	F3DSF1732/ F3DSR2253	AGACTAGTGGTACCTTGTGTTCAAATTCGGAACTGG	CGGAGCTCGGATCCTTTGTAATCAGGCACTTTAATG	Oligos for RNAi vector of Os*DR11L*
	39C8-5F/39C8-6R	TCCCTGTCAGCGTGATTATG	CTGCGGTAGCTTTGGTAACTC	Quantitative PCR for Os*DR11S* expression analysis
	39C8-6F/39C8-7R	GAATGTTGGGATAGGGAAAGG	ACGGGATGAAACCTGGATCTAC	Quantitative PCR for Os*DR11S* expression

				analysis
	83J4-2F/83J4-2R	CGGAACTGGTTTGACTATCG	CAGGCACTTTAATGTACTCCG	Quantitative PCR for Os*DR11L* expression analysis
	39C8-4F2/39C8pmalR-BamH1	ctggatccATGGAGTGCTTGGCCGagat	ctggatccTCACCTTAATAGCACTGGAC	Amplifying Os*DR11S* coding region
	39C8PET28F-NDE1/39C8-4R2	atcatatgATGGAGTGCTTGGCCGAGAT	CTGGATCCCCTTAATAGCACTGGACTTGG	Amplifying Os*DR11S* coding region
	39C8-4F4/39R-XBA1	ATTCTAGAATGGAGTGCTTGGCCGAGAT	CTTCTAGACCTTAATAGCACTGGACTTG	Amplifying Os*DR11S* coding region
	expF3-2R	ATGTCGACCTACATACAAGCAACAAAT		Amplifying Os*DR11L* coding region
	39C8-4F3/114F3-BamH1R	CTGAATTCATGGAGTGCTTGGCCGAGAT	atggatccCATACAAGCAACAAATGAGC	Amplifying Os*DR11L* coding region
	F3R-XBA1	CTTCTAGACATACAAGCAACAAATGAGC		Amplifying Os*DR11L* coding region
pMCG161 (AY572837)	pMCGF/R	GGCTCACCAAACCTTAAACAA	CTGAGCTACACATGCTCAGGTT	Identifying positive RNAi transgenic plants
IRS154	IRS154F/R	CTTGCGAAGGATAGTGGGAT	CCCTGCCTTCATACGCTA	Identifying positive artificial microRNA–suppressed transgenic plants
GUS	GUS-2F/2R	CCAGGCAGTTTTAACGATCAGTT	GAGTGAAGATCCCTTTCTT	Amplifying *GUS*

(AF234297)		CGC	GTTACCG	
LOX （D14000)	LOXF/R	GCATCCCCAACAGCACATC	AATAAAGATTTGGGAGTGACATATTGG	Quantitative PCR for gene expression analysis
AOS2 *(*AY062258)	AOS2F/R	CAATACGTGTACTGGTCGAATGG	AAGGTGTCGTACCGGAGGAA	Quantitative PCR for gene expression analysis
PAD4 (CX118864)	PAD4F/R	GCCAGCTCCCCTACGACTTC	CGTGTGCGGTGTAGGTTGTT	Quantitative PCR for gene expression analysis
PR1b (U89895)	PR1bF/R	GGCAACTTCGTCGGACAGA	CCGTGGACCTGTTTACATTTTCA	Quantitative PCR for gene expression analysis
pGADT7	5ADLDISA/3ADLDISA	CTATTCGATGATGAAGATACCCCACCAAACCCA	GTGAACTTGCGGGGTTTTTCAGTATCTACGATT	Sequencing
	T7/Sp6	TAATACGACTCACTATAGGG	TAATACGACTCACTATAGGG	Sequencing
Actin (X15865)	Actin-F/Actin-R	TGTATGCCAGTGGTCGTACCA	CCAGCAAGGTCGAGACGAA	Quantitative PCR for gene expression control