

A pair of orthologs of a leucine-rich repeat receptor kinase-like disease resistance gene family regulates rice response to raised temperature

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Additional file 1: Supplemental table and figures

Table S1: PCR primers used for plasmid construction and gene expression analysis

Gene name (GenBank accession number)	Primer name	Forward primer (5'-3')	Reverse primer (5'-3')	Use
<i>NRKe</i> (JN176870) <i>9RKe</i> (JN176871)	NRKe-F/N RKeR	GCGGATCC ^a AAGCCAATCA TCCCTTGAAATAG	GCGGATCC ^a GAAGCAAATA TGGATGCAGAGG	Amplifying <i>9RKe</i> , <i>NRKe</i> , <i>NRKe-K</i> , <i>NRKe-ΔK</i> , <i>Bel</i> , and <i>Be2</i> for plasmid construction
<i>NRKe</i>	RKe-6F	AGCGGATCC ^a ATGGATTTC AGTGATGATAATATGTTGG G		Amplifying <i>NRKe-K</i> for plasmid construction
	B+E-2	ACCCCAACATATTATCATC ACTGAAATCATCGGTTGC ACGAAGAAG		Amplifying <i>Bel</i> for plasmid construction
	B+E-4	TGAGCTTGCTCATGCTACT AATGATTTTCAGCGATGATA GCATGTTGG		Amplifying <i>Be2</i> for plasmid construction
<i>NRKe</i> <i>9RKe</i>	RKe-RF/RR	GAGCAGCGGTTTGGTGGT T	GTCGAGCCATTCGAAGAA CAC	Quantitative RT-PCR
<i>Xa3/Xa26</i>	MKb-F/MK	TAGGATCC ^a ATGGCTCTTG	ATGGATCC ^a ACCACGAGAG	Amplifying <i>Bel</i> and <i>Be2</i> for

(DQ355952)	b-R	TTCGATTGCC	AGCGATGAAT	plasmid construction
	B+E-1	TTCAGTGATGATAATATGT TGGGGT		Amplifying <i>Be1</i> for plasmid construction
	B+E-3	ATCATTAGTAGCATGAGCA AGCTCA		Amplifying <i>Be2</i> and <i>NRKe-ΔK</i> for plasmid construction
<i>Actin</i> (X15865)	Actin-F/R	TGTATGCCAGTGGTCGTAC CA	CCAGCAAGGTCGAGACG AA	Quantitative RT-PCR

^aThe underlined nucleotides are the digestion site of *Bam*HI.

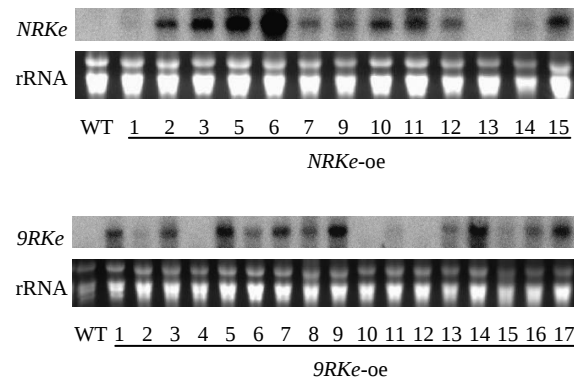
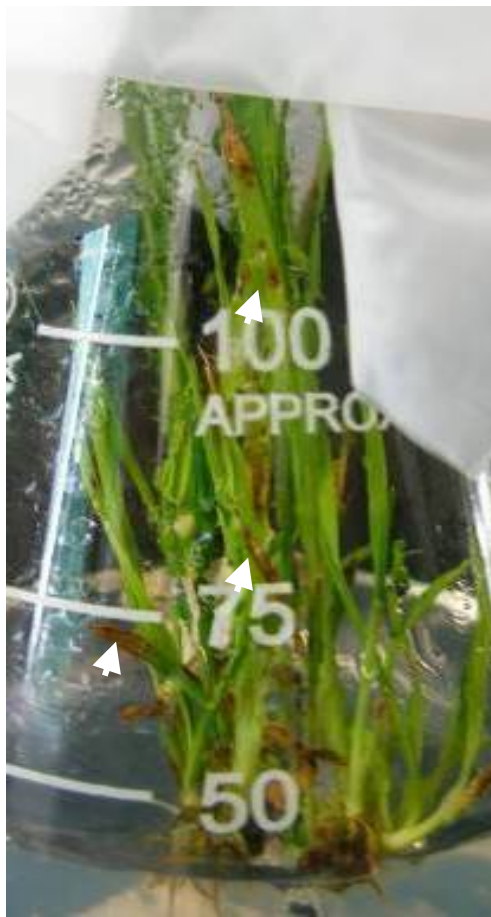
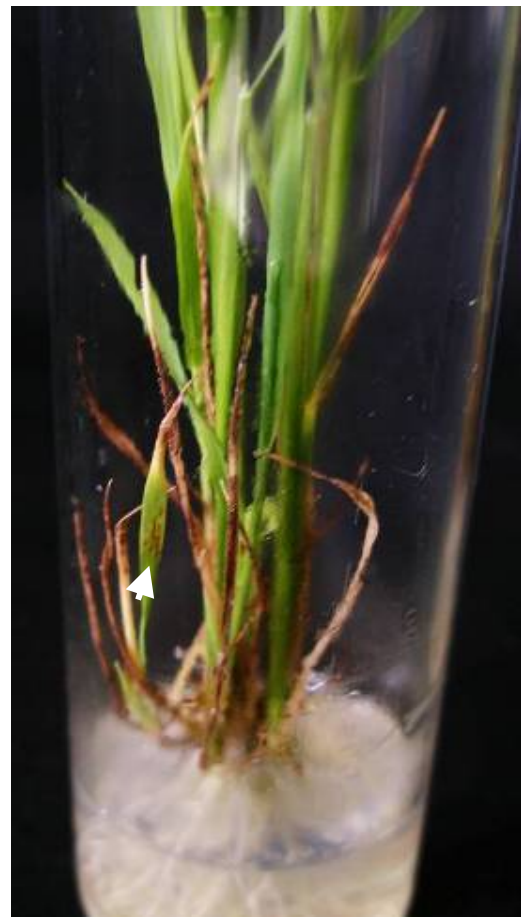


Figure S1: Expression of *NRKe* and *9RKe* in transgenic plants (T_0 generation) analyzed by RNA gel blot. *NRKe*-oe, *NRKe*-overexpressing plants; *9RKe*-oe, *9RKe*-overexpressing plants; WT, wild-type Mudanjiang 8.



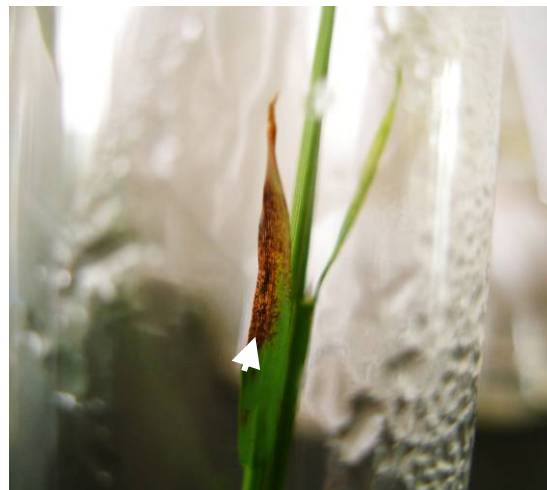
NRKe-oe plants



NRKe-oe plants



NRKe-oe plant



Be2-oe plant

Figure S2: *NRKe*-overexpressing and *Be2*-overexpressing plants formed lesion mimics spontaneously in sterilized container during tissue culture.

9RKe	GTGGCATATCCTATTAAATTCGTGCGATGGCTGGTAATAGGATGCCACGCCCTCGTCTTGTGAAACAGCCACGTCATCTCGCGGGTGAATCCTTTTCAT	-1933
NRKe	GTGGCATATCCTATTAAATTCGTGCGATGGCTGGTAATAGGATGCCACGCCCTCGTCTTGTGAAACAGCCACGTCATCTCGCGGGTGAATCCTTTTCAT	-1963
9RKe	TGATAGTGTGGTTTTGTGAAACTACACTACCAAAATATATGTACTTAAATGTCAAGTGTCAAAGTGTGTGGTTTTCTGCAACTTGGACCACAAAACGTAT	-1833
NRKe	TGATAGTGTGGTTTTGTGAAACTACACTACCAAAATATATGTACTTAAATGTCAAGTGTCAAAGTGTGTGGTTTTCTGCAACTTGGACCACAAAACGTAT	-1863
9RKe	AGTTTTCTGAAATTTACTCTTTAAAAAATAACAGCCAAATAACACGGTATTCACTATTTAGTGCTTAAAAAAAAGATCCAGAGTATTTCAATCACAAAA	-1733
NRKe	AGTTTTCTGAAATTTACTCTTTAAAAAATAACAGCCAAATAACACGGTATTCACTATTTAGTGCTTAAAAAAAAGATCCAGAGTATTTCAATCACAAAA	-1763
9RKe	ATTTCCGAAAAGTTTCCACCGATTCTGAGTTCACACGAAAATTGCCCTTATCATTTTCAATCCGTTTCCGAGAAAATATTTCCGAATTGTTTACGTT	-1633
NRKe	ATTTCCGAAAAGTTTCCACCGATTCTGAGTTCACACGAAAATTGCCCTTATCATTTTCAATCCGTTTCCGAGAAAATATTTCCGAATTGTTTACGTT	-1663
9RKe	TCCGAAAAAATTACGACTGATAGATTCCGTTTTCGAAAAATAAGTCTGGAATCCGGAAGTTTCTATATCGTTTTACCCTTATCTCACACGGATTAGGAT	-1533
NRKe	TCCGAAAAAATTACGACTGATAGATTCCGTTTTCGAAAAATAAGTCTGGAATCCGGAAGTTTCTATATCGTTTTACCCTTATCTCACACGGATTAGGAT	-1563
9RKe	CCTCTGAAGTAAATAAGAGGAACATGGTGGCTGACATGAGGGCCCATCGCTTGCAGTTGCGGGCCACCTATCAGTGATCAGCTTAGTAGGGAGTTCA	-1433
NRKe	CCTCTGAAGTAAATAAGAGGAACATGGTGGCTGACATGAGGGCCCATCGCTTGCAGTTGCGGGCCACCTATCAGTGATCAGCTTAGTAGGGAGTTCA	-1463
9RKe	TATTAGAGGTTCTGCTTCGCTTTACACTCAGCACAGGCGTGTGACCACCGTGATTGTGAAGATTGCAACCGCTGGCGACCGGCGGACGGCGCGGACA	-1333
NRKe	TATTAGAGGTTCTGCTTCGCTTTACACTCAGCACAGGCGTGTGACCACCGTGATTGTGAAGATTGCAACCGCTGGCGACCGGCGGACGGCGCGGACA	-1363
9RKe	TATTTCTGTAACCGAAGGATGTAGTACTCAATTGAGGATACATATAATCTGAACCTGAACATTACTGTCA	-1263
NRKe	TATTTCTGTAACCGAAGGATGTAGTACTCAATTGAGGATACATATAATCTGAACCTGAACATTACTGTCA	-1263
HSE (nGAAn core)		
9RKe	GACAACGGAGATGCATACCTTTTACCAGAATGCCTAGATTGACTAGTTATTCCTTGAAAACAGTTTATAATTGATGGCGCCAAATTAATGGTTGCATGC	-1163
NRKe	GACAACGGAGATGCATACCTTTTACCAGAATGCCTAGATTGACTAGTTATTCCTTGAAAACAGTTTATAATTGATGGCGCCAAATTAATGGTTGCATGC	-1163
9RKe	AAGGCATAAATTAATGGAAGCTGAATAAATCTTAACCCAAATCATCCCTGATATAGTGACATTTTCTTGTTGGTTGACCTTTTCACCTCTGGCTGCTGG	-1063
NRKe	AAGGCATAAATTAATGGAAGCTGAATAAATCTTAACCCAAATCATCCCTGATATAGTGACATTTTCTTGTTGGTTGACCTTTTCACCTCTGGCTGCTGG	-1063
CCAAT box		
9RKe	TCGGCAGATTGACACTGCCCAAATTTATACTCCCTCATCTCTAAATATTTGACGCGTTAACTTTTTAAACATATTTGACCGTTGCTTATTCAAAA	-963
NRKe	TCGGCAGATTGACACTGCCCAAATTTATACTCCCTCATCTCTAAATATTTGACGCGTTAACTTTTTAAACATATTTGACCGTTGCTTATTCAAAA	-963
9RKe	ACTTTTACGAAATAGTAAACTATATGTATACATAAAAGTACATTTAACAATGAATCAAATGGTAAGAAAAAATTAATAATTGCTTAAATTTTTTCAA	-863
NRKe	ACTTTTACGAAATAGTAAACTATATGTATACATAAAAGTACATTTAACAATGAATCAAATGGTAAGAAAAAATTAATAATTGCTTAAATTTTTTCAA	-863
HSE (PlantCARE)		
9RKe	TAAGACGAACGGTCAAACATGTTTAAAAAATCAACGGTGTCAAACATTTAGGGATGGAGGGAGTACAATGTAGGCGAAAAAATTAATAATTGAAGGCAC	-763
NRKe	TAAGACGAACGGTCAAACATGTTTAAAAAATCAACGGTGTCAAACATTTAGGGATGGAGGGAGTACAATGTAGGCGAAAAAATTAATAATTGAAGGCAC	-763
9RKe	CGGATGGATGCAATCAACAAAAAATGACACGTTTCACTAGATATAGAGAAAAAATGAATAATTCTCATGCCAATCATAAATAATGACATATTGGTTGACT	-663
NRKe	CGGATGGATGCAATCAACAAAAAATGACACGTTTCACTAGATATAGAGAAAAAATGAATAATTCTCATGCCAATCATAAATAATGACATATTGGTTGACT	-663
CCAAT box		
9RKe	TTTTGCCGGGTCCACCGATTGGCACTGCCCGAATGTATAGAACGATGGAGGGAAAAACAGTTTCATAAATGAAGGAGCCAGATGGTTGCATCCACAAAGAA	-563
NRKe	TTTTGCCGGGTCCACCGATTGGCACTGCCCGAATGTATAGAACGATGGAGGGAAAAACAGTTTCATAAATGAAGGAGCCAGATGGTTGCATCCACAAAGAA	-563
9RKe	TGGAGAACTCCATAAATCTCAGCGCTAATCATCCTCTGAAATAATGTCATGTTCTTGATTGACTTTTTTCGCCGGCCGCGGATTGACACCCAAATTTATAG	-463
NRKe	TGGAGAACTCCATAAATCTCAGCGCTAATCATCCTCTGAAATAATGTCATGTTCTTGATTGACTTTTTTCGCCGGCCGCGGATTGACACCCAAATTTATAG	-463
HSE (nGAAn core)		
9RKe	AGCATATGGGAAGTGAACAACTTTTACAATTGAAGGCACCATACCATTGGTTGCAAGGGCAGGGTAATGTTGAGCGCTGAATACATATCAAGCCAATCA	-363
NRKe	AGCATATGGGAAGTGAACAACTTTTACAATTGAAGGCACCATACCATTGGTTGCAAGGGCAGGGTAATGTTGAGCGCTGAATACATATCAAGCCAATCA	-363
CCAAT box		
9RKe	TCCGCTAAATAATGCCATATTCTTGTTGATTCTTCTCACCGCACCAGACCAGCAGATTGATACCAACAAATTTATTGAGCAATGGAGGGAAAAACAATTT	-263
NRKe	TCCGCTAAATAATGCCATATTCTTGTTGATTCTTCTCACCGCACCAGACCAGCAGATTGATACCAACAAATTTATTGAGCAATGGAGGGAAAAACAATTT	-263
9RKe	ATAATTGAAGGTGGCAGATGTTTACTTGAGCAGTGAATAAATCTCAAGCTAATCATCCCTGAAATTATGGCATCTTCTTGTTGACTTCATCACCGAG	-163
NRKe	ATAATTGAAGGTGGCAGATGTTTACTTGAGCAGTGAATAAATCTCAAGCTAATCATCCCTGAAATTATGGCATCTTCTTGTTGACTTCATCACCGAG	-163
9RKe	CAACCGATTGACGCCGACAAGATTGATTAAGCAAGGTAGGGAAAAAGATTTACAATTGAAGGCGATGACTGCATGCACAGCGGAATGATGTATTGGATA	-63
NRKe	CAACCGATTGACGCCGACAAGATTGATTAAGCAAGGTAGGGAAAAAGATTTACAATTGAAGGCGATGACTGCATGCACAGCGGAATGATGTATTGGATA	-63
9RKe	AATATAAGCCAATCATCCCTTGAAATAGCGCCATCTTGTGGCAATGCATCAGAGCCCAAAC	-1
NRKe	AATATAAGCCAATCATCCCTTGAAATAGCGCCATCTTGTGGCAATGCATCAGAGCCCAAAC	-1
CCAAT box (Place)		

Figure S3: Alignment of promoter regions of NRKe and 9RKe. The nucleotides immediately upstream of the translation start codon ATG are numbered as “-1”. The putative heat-responsive *cis*-elements are underlined. HSE (nGAAn core), heat shock element consisting of repeated core nGAAn in alternate orientation; HSE (PlantCARE), heat shock element found in PlantCARE database; CCAAT box (Place), CCAAT box found in PLACE database.

NRKe	(797)	DFSDDNMLGSGSFGKVFRGQLSGLVVAIKVIHQHLEHAIRSFDT	ECRVLRMARHRNLIK	
Xa3/Xa26	(807)	DFSDDNMLGSGSFGKVFRGRLSNGVVAIKVIHQHLEHAMRS	FDTECRVLRMARHRNLIK	
		I	II	III
NRKe	(857)	ILNTCSNLDFRALVLQYMPNGSLEALLHSDQRMQLGFLERLDIMLDVSLAMEYLHHEHCE		
Xa3/Xa26	(867)	ILNTCSNLDFRALVLQYMPNGSLEALLHSDQRMQLGFLERLDIMLDVSLAMEYLHHEHCE		
		IV	V	VI
NRKe	(917)	VVLHCDLKPSNVLFDDDMTAHVSDFGIARLLLGDDNSIISASMPGTVGYMAPEYGLGKA		
Xa3/Xa26	(927)	VVLHCDLKPSNVLFDDDMTAHVSDFGIARLLLGDDNSIISASMPGTVGYMAPEYGLGKA		
		VII	VIII	
NRKe	(977)	SRKSDVFSYGIMLLEVFTAKRPTDAMFVGELNIRQWVLAQFPANLVHVVDGQLLQDSSSS		
Xa3/Xa26	(987)	SRKSDVFSYGIMLLEVFTAKRPTDAMFVGELNIRQWVLAQFPANLVHVVDGQLLQDSSSS		
		IX		
NRKe	(1037)	SSSDAFLMPVFELGLLCSDSPEQRMVMSDVVITLKKIRKEYVKSSTATMGRDENCTAV		
Xa3/Xa26	(1047)	SSSNHDFLMPVFELGLLCSDSPEQRMMSDVVITLKKIRKEYVKLMATTVSVVQC---		
		X	XI	
NRKe	(1096)	FF		
Xa3/Xa26	(1104)	---		

Figure S4: Alignment of kinase domains of NRKe and Xa3/Xa26. The solid black shade indicates different amino acid residues and the gray shade indicates residues with similarity. Asterisks (*) indicate conserved amino acid residues of protein kinase (Hanks SK et al. *Science* 1998, **241**:42-52). The conserved subdomains are numbered and underlined according to Cao et al. (Cao Y et al. *Theor Appl Genet* 2007, **115**:887-895)..

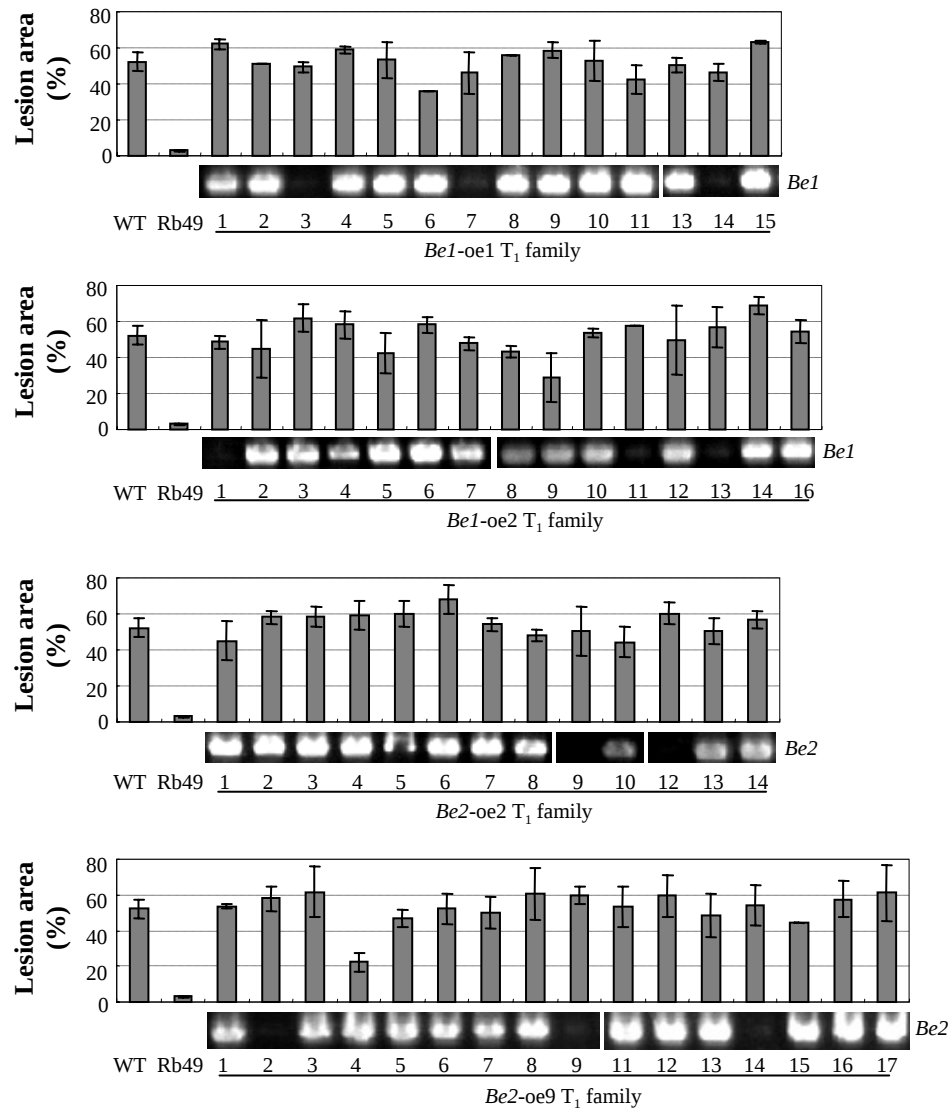


Figure S5: Overexpression of *Be1* or *Be2* could not influence rice response to *Xoo* strain PXO61. Positive transgenic plants were determined by PCR amplification of *Be1* or *Be2* using gene-specific primers (Additional file 1, Table S1). Wild type (WT) is Mudanjiang 8. Rb49 is a transgenic line carrying *Xa3/Xa26* driven by its native promoter in Mudanjiang 8 background.