RESEARCH PAPER

Title:

TaRLP1.1, a Novel Wheat Receptor-Like Protein Gene, is Involved in the Defense Response against *Puccinia striiformis* f. sp. *tritici*

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Supplementary Data

Supplementary data are available at JXB online

Supplementary Table S1. Designed primers

Supplementary Table S2. The up-regulated folds of Ta.22666.2.S1_at and TaAffx.55699.1.S1_at between the different samples of tests and the controls
Supplementary Table S3. The genes identified in resistant cv. 92R137 and susceptible cv. Yangmai158

Supplementary Fig. S1. The primary structure and conserved domains of TaRLP1.1. (A) Predicted protein structure of TaRLP1.1. (B) The sequence (1-22aa) represents a signal peptide (SP) (a). The sequence (96-167aa) represents the LRRs domain (b). In the LRR domains there are several 23–25-aa plant-specific extracellular LRR motifs 'LxxLxLxxNxLt/SGxIP'; conserved L, S, G, I and P marked with red . The sequence (233-255aa) represents the transmembrane (TM) region that contains the 'GxxxG' motifs (c), and the conserved G in the GxxxG-type motifs are marked with green. A short cytoplasmatic tail (d) contains the 'Yxx ϕ ' motif (' ϕ ' represents any bulky, hydrophobic amino acid), and the conserved 'Y' and ' ϕ ' are marked with yellow.

Supplementary Fig. S2. Chromosomal location of *TaRLP1.1, TaRLP1*₈₀₄-1 and *TaRLP1*₈₁₃ with specific primers using the nulli-tetrasomic (NT) lines derived from cv. Chinese Spring. *TaRLP1.1* was located to the chromosome 3D, *TaRLP1*₈₀₄-1 to 3B, and *TaRLP1*₈₁₃ to 3A.

Supplementary Fig. S3. Phylogenetic analysis of the DNA sequence of TaRLP1 genes from common wheat using the MEGA 4.0. Bootstrap values are shown above nodes. Scale bar represents 0.05 nucleotide substitutions per site. The Genbank accession numbers are JX198219 (TaRLP1₇₃₈), JX198220 (TaRLP1800-1), JX198221 (TaRLP1800-1), JX198222 (TaRLP1801-2), JX198223 (TaRLP1801-3), JX198224 (TaRLP1₈₀₁-4), JX198218 (TaRLP1.1), JX198225 $(TaRLP1_{801}-5),$ JX198226 (TaRLP1804-1), JX198227 (TaRLP1804-2), JX198228 (TaRLP1804-3), JX198229 (TaRLP1804-4), JX198230 (TaRLP1813).

Supplementary Fig. S4. The multi-sequence alignment of the identified *RLP* genes from the stripe rust resistant wheat cv. 92R137 and susceptible cv. Yangmai158.

Supplementary Fig. S5. The multi-sequence alignment of the deduced proteins of the identified *RLP* genes from the stripe rust resistant wheat cv. 92R137 and susceptible cv. Yangmai158.

Supplementary Fig. S6. Sequence alignment and phylogenetic analysis of TaRLP1.1 and other putative RLP proteins using DNAMAN. (A) Alignment of the amino acid sequence of *TaRLP1.1* with other putative RLP proteins from *Oryza sativa* (BAF06973.1), *Hordeum vulgare* (BAJ87832.1), *Aegilops tauschii* (EMT19838.1) *Arabidopsis* (AtRLP44, AEE78586.1) and *Sorghum bicolor* (EES01889.17). Identical residues in all organisms are shaded. Underlines indicate sequence domains involved RLPs. (B) A representative phylogenetic tree of TaRLP1.1 and selected putative RLP proteins.

Supplementary Fig. S7. Subcellular localization of TaRLP1.1 protein. Onion epidermal cells were transformed with plasmids expressing the fusion protein and green fluorescent protein (GFP) by bombardment. All images were observed with a confocal microscope. (A) Onion epidermal cells expressing the GFP alone driven by the 35 S promoter. (B) Onion epidermal cells expression the TaRLP1.1-GFP fusion protein (Bars = $100\mu m$).

Supplementary Fig. S8. Expression analysis by the semi-quantitative RT-PCR of three pathogenesis-related genes, including *TaPR1*, *TaPR2* and *TaPR5*, at both 72 and 120 h after CYR32 inoculation in the BSMV: TaRLP1.1as infected plants and the BSMV infected control plants. *Tubulin* gene was used as the internal control.

Supplementary Fig. S9. Identification of positive transgenic plants by GUS staining, gene detection and gene expression analysis. (A) GUS staining of the root tips of the T_1 generation transgenic lines. '+' indicates positive GUS staining and '-' indicates negative GUS staining. (B) *Gus* and *TaRLP1.1* detection in some of the T_0 and T_1 plants by PCR. '+': the positive control using the vector as the template; '-': the negative control using H₂O as the template. (C) Expression analysis of *TaRLP1.1* in the identified positive transgenic plants. *Tubulin* gene was used as the internal control. **Supplementary Fig. S10.** Positive *TaRLP1.1* transgenic plants showed robust hypersensitive response to *Pst* 15 days after inoculation. The red arrows indicated the

inoculated leaves. 723-3 and 723-7 indicated T_1 generation plants of two individual T_0 plants, Y158 indicated the susceptible control cv. Yang158, and 92R137 indicates the resistant control line.

Supplementary Fig. S11. Expression analysis by semi-quantitative RT-PCR of three pathogenesis-related genes, including *TaPR1*, *TaPR2* and *TaPR5*, at both 72 and 120 h after CYR32 inoculation in *TaRLP1.1* over-expressed transgenic plants, the *Yr26* containing cv. 92R137 and the susceptible recipient. Yangmai158. *Tubulin* gene was used as the internal control.



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JX198218 JX198219 JX198220 JX198221 JX198222 JX198223 JX198223 JX198224 JX198226 JX198227 JX198227 JX198227 JX198228 JX198229 JX198229 JX198230	ATGCTGATGATACCAAGTTCCTCTTTGTTCCTCTTCAGCAGCTCATCATCATCTTTTGGTTCTGAACAAGATATCCCGGTGCTGAAGTCTGTAC ATGCTGATGATACCAAGTTCCTCTTTGTTCTCCTCTTCAGCAGCTCATCCTTGTGTTTTTGGTTCTGAACTATCCTGTGCCTGAGGTCTGAGGTCTGAGGTCGTGTTTGGTTCTGAACTAGGTGCTGAGGTCTGAGGTCTGAGGTCGTGTGTGT	100 100 100 100 100 100 100 100 100 100
JX198218 JX198219 JX198220 JX198221 JX198222 JX198223 JX198224 JX198224 JX198226 JX198226 JX198227 JX198227 JX198228 JX198229 JX198230	AAGGCTCATTESCTGATCCGGCGTGTTCTCACATCTGATGCTGTTGAAATGA	191 194 190 193 191 191 191 194 194 194 200
JX198218 JX198219 JX198220 JX198221 JX198222 JX198223 JX198224 JX198224 JX198225 JX198226 JX198227 JX198227 JX198228 JX198229 JX198229 JX198230	ATGOTGGOACCOTGACGAGAGAGAGAGATTOTOTOTOGOGACTAGGCAACCTGGGACTTOAAGGOCAATTTOCTGTTGCTCTAGAGAATTGTTGAGGOTG ATGOTGGOACCOTGACGAGAGAGAGAGATTOTOTOTOTOGOGACTAGGCAACCTGGGACTTOAAGGOCAATTTOCTGTTGCTCTAGAGAATTGTTGAGGCTG ATGOTGGOACCOTGACGAGAAGAGAGATTOTOTOTOTOTOGOGACTAGGCAACCTGGGACTTOAAGGCAATTTOCTGTTGCTCTAGAGAATTGTTGAGGCTG ATGOTGGOACCOCGACGAGAAGAGAGATTOTOTOTOTOTOGOGACTAGGCAACCTGGGACTTOAAGGCAATTTOCTGTTGCTCTACAGAATTGTTGAGGCTG ATGOTGGOACCOCGACGAGAAGAGAGATTGTCTCTCTCTGTGCGACTAGGCCAACCTGGGACTTCAAGGCAATTTOCTGTTGCTCTACAGAATTGTTCAAGGCTG ATGOTGGOACCOCGACGAGAAGAAGAGATTGTCTCTCTCTGCGACTAGGCAACCTGGGACTTCAAGGCCAATTTOCTGTTGCTCTACAGAATTGTTCAAGGCTG ATGOTGGOACCOCGACGAGAAGAAGAGATTGTCTCTCTCTGCGACTAGGCAACCTGGGACTTCAAGGCCAATTTOCTGTTGCTCTACAGAATTGTTCAAGGCTG ATGOTGGOACCOCGACGAGAAGAAGAGATTGTCTCTCTGCGACTAGGCAACCTGGGACTTCAAGGCCAATTTOCTGTTGCTCTACAGAATTGTTCAAGGCTG ATGOTGGOACCOCGACGAGAAGAAGATTGTCTCTCTCTGCGACTAGGCAACCTGGGACTTCAAGGCCAATTTOCTGTTGCTCTACAGAATTGTTCAAGGATG ATGOTGGOACCOCGACGAGAAGAAGATTGTCTCTCTCTGCGACTAGGCAACCTGGGACTTCAAGGCCAATTTOCTGGTGCTCTACAGAATTGTTCAAGGATG ATGOTGGOACCOCGACGAGAACAAGATTGTCTCTCTCTGCGACTAGGCAACCTGGGACTTCAAGGCCAATTTOCTCTGTGCTCTACAGAATTGTTCAAGGATG ATGOTGGOACCCGACGAGAAGAAGATGGTTCTTTCTCGGACTAGGCAACCTGGGACTTCAAGGCCAATTTOCTCTGCGTCTCAAGGATTGAAGCCTG ATGOTGGOACCCGACGAGAAGAAGATGGTTCTTTCTCTCTGCGACTTGGACCTAGGACCTGGACCTAGGACCTGGACCTGGGACTTCCAGGGCCAATTTOCTCTGCGACTTGAAGGCTG ATGOTGGOACCCGACGAGAAGAAGAGATTGTCTCTCTCTGCGACTTGGCCAACCTGGGACTTCAAGGGTCATTTCCTCCGGGCCTTTCAAGGACTG GTGCTGCACCCGACGAGAAGAAGAGAGATTGTCTCTCTCT	291 294 290 293 291 291 291 294 294 294 300
JX198218 JX198219 JX198220 JX198221 JX198222 JX198223 JX198224 JX198224 JX198226 JX198227 JX198227 JX198228 JX198229 JX198229 JX198230	ACCGCT TGGACCTGTCRAATAACAATTTC TCAGGACCAATCCCTCAGGACATTCACGCCGGATCCCGTATCTGACATCTCTGATCTTGATCTTTCTGAATAACAATTTCTCCGATCTTTCCGATCTTCCGATCTTTCCTGATCTTGAATAACAATTTTTTCAGGACCATTCACGCCGGATCCGGATCTGACATTCTCGATCTTGATCTTGATCTTGAATAACAATTTTTTCAGGACCATTCCACGCGCGGATCCGATCTGACATTCTCGATCTTGATCTTGATCTTGAATAACAATTTTTTCAGGACCAATCCGGCGGATTCCACGCGGATCCGACATTCTGACATCTTGGATCTTGATCTTGATCTTGAATAACAATTTTTTCAGGACCAATCCGGCGGGATCCGACGTATCTGACATCTTGGATCTTTCCAGACCATTTCACGCGCGGATCCGATCTGACATCTTGGACTTTTCTGAGACCAATTAACAATTTTTTCAGGACCAATTCCACGGCGGATTTCCACGGCGGATCTGACATCTTGGACTCTTGGACTTTCCCGACTTTCCAGACCAATTAACAATTTTTTCAGGACCAATCCCTCAGGACATTTCCACGCGGGATGCCGGTATCTGACATCTTCGGACCTTTCCTAGAATAACAATTTTTTCCAGGACCAATCCCTCAGGACATTTCCACGCGCGGATCCTGACATCTCTGGACTTTTCCTGATCTTTCTAGGACCAATCACGCCGGGCTTGGACCTGCGATCTGGACCTGTCGAATAATTTTTTCCAGGACCAATCCCCTCAGGACATTTCCACGCGGGATGCCGGTATCTGACACTCTTGGACTTTTCCTAGAATTAACAATTTTTTCCAGGACCAATCCCCTCAGGACATTTCCACGCGGGATGCCGTATCTGACATCTCTGGACTTTTCCTGATATAAAAAAAA	391 390 390 391 391 391 394 394 394 394
JX198218 JX198219 JX198220 JX198221 JX198222 JX198223 JX198223 JX198225 JX198227 JX198227 JX198227 JX198228 JX198229 JX198230	GCTTTTCGGGGCTAATCOCACAGAATATCTCGAATATGACATATCTGAATCTCCTCAACCTCCAGCATAACCARCTCAGCGTCAAATTCCATCGCAATT ACTTTTCGGGGCTAATCOCACAGAATATCTCGAATATGTGGATATCTGAATCTCCTCAACCTCCAGCATAACCARCTCAGCGGTCAAATTCCATCGCAATT GCTTTTCGGGGCTAATCOCACAGAATATCTCGAATATGTGGATATCTGAATCTCCTCAACCTCCAGCATAACCAACTCAGCGGTCAAATTCCATCGCAATT GCTTTTCGGGGCTAATCOCACAGAATATCTCAAATATGACATATCTGAATCTCCTCAACCTCCAGCATAACCAACTCAGCGGTCAAATTCCATCGCAATT GCTTTTCGGGGCTAATCCCACAGAATATCTCAAATATGACATATCTGAATCTCCTCAACCTCCAGCATAACCAACTCAGCGGTCAAATTCCATCGCAATT GCTTTTCGGGGCTAATCCCACAGAATATCTCAAATATGACATATCTGAATCTCCTCAACCTCCAGCATAACCAACTCAGCGGTCAAATTCCATCGCGCATT GCTTTTCGGGGCTAATCCCACAGAATATCTCAAATATGACATATCTGAATCTCCTCAACCTCCAGCATAACCAACTCAGCGGCACAAATTCCATCGCAATT GCTTTTCGGGGCTAATCCCACAGAATATCTCAAATATGACATATCTGAATCTCCTCAACCTCCAGCATAACCAACTCAGCGGCACAAATTCCATCGCAATT GCTTTTCGGGGGCTAATCCCACAGAATATCTCAAATATGACATATCTGAATCTCCTCAACCTCCAGCATAACCAACTCAGCGGCACAAATTCCATCGCAATT GCTTTTCGGGGCTAATCCCACAGAATATCTCAAATATGACATATCTGAATCTCCTCAACCTCCAGCATAACCAACTCAGCGGTCAAATTCCATCGCACATT GCTTTTCGGGTTCAATCCCACAGAATATCTCAAATATGACATATCTGAATCTCCTCAACCTCCAGCATAACCAACTCAGCGCTCAAATTCCACCGCACATT GCTTTTCGGGGTCAATTCCCACAGAATATCTCAAATATGACATATCTGAATCTCCCAACTTCCAGCATAACCAACTTAGCGGTCAAATTCCACCGCACATT GCTTTTCGGGGTCAATTCCCACAGAATATCTCAAATATGACATATCTGAATCTCCCCAACTTCCAGCATAACCAACTTAGCGGTCAAATTCCACCGCACATT GCTTTTCGGGGATCAATCCCACAGAATATCTCAAATATGACATATCTGAATCTCCCCACCTCCAGCATAACCAACTTAGCGGTCAAATTCCACCGCCACATT GCTTTTCGGGATCAATTCCGCACAGAATATCTGAATATCTGAATCTCCCCCCACCTTCCAGCATAACCAACTTAGCGGTCAAATTCCACCGCCACATT GCTTTTTCGGGATCAATTCCGCAAATTCCGCACACTTCCACCTCCAGCATAACCAACTGAGCGCTCAAATTCCACCGCCACATT GCTTTTTCGGGGCTCAATTCCGCAGAATATCCTGAATGTCCTCCACCTCCAGCCATAACCAACTGAGCGCCAAATTCCACCGCCACATT GCTTTTTCGGGGCTCAATTCCGCGGATATCCGCCCCCCCC	491 490 490 491 491 491 494 494 494 494 500

JX198218	CASTTIGCTTACTCGGTTAACTACGTTCAATGTIGCGGGCAACTT	GTTATCAGGGCCT <mark>G</mark> TTCCT <mark>A</mark> CTTTGCTAC <mark>AG</mark> A.	.AGTTTTCGTCTTCAAACTTT 588
JX198219	OGATTTGCTTACTCGGTTAAOOAOGTTCAAT.		
JX198220	CASHINGCINACICGGINAACTACGINCAAIIGTIGCGGGCAACTT	GTTATCAGGGCCTGTTCCTACTTTGCTACAGA.	. AGTITITCGICTICAAACIIII 587
JX198221			AGTITITCGICTICAAAOIIII 587
JA198222			
UA198223		GTTATCAGGGCCTGTTCCTACTTTGCTACAGA.	AGTTTTCGTCTTCAAACHIII 588
UA190224			ACTETICGICITICAAACIII 588
TV198226			demmedademmedaacomm 501
TX198227			AACTTTCCCCCCTTCCAACTTT 591
TX198228	GGATTTGCTTACTCGGTTAACTACGTTCACTGCGGGGGGGG	GTTATCAGGGCCTGTTCCTACTTGCTACAGA.	AGTTTTCGTCTTCAAACTTT 591
JX198229	GGATTTGCTTACTCGGTTATOGACGTTCAATGTTGTGGGATAACTT	GTTATOGGGGTCTATTCCTCCTTOGCTACTAA.	GGTTTCCAGCTTGGAGCTTT 591
JX198230	CGATTTGCTTCCTCCGCTTAACCCCATTCAATGTTGCGGAGAACTT	GTTATCAGGGCCTATTCCTTCTTTGCTAGCAGT.	AAG <mark>GTTTT</mark> CGGCTTGGAGCTTT 600
TV100010			A C C C C C C C C C C C C C C C C C C C
TV100210		ACCOLOGOACAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	AGCIGCACAGGAICAACGACG
TX198220			ACCTCCACACCACCACCACCACCACCACCACCACCACCAC
TX198221			AGCTCCACAGGATCAACGACG
TX198222	GCTCGTAACCAAGGCCTTTCTCTCGTTCACCGTTCGCATGATTCTCCC		AAGCTGCACAGGCTCAACGACG 688
JX198223	GCTCGTAACCAAGCCCTTTCTCTCGTTCACCTTTAGATCAC		AGGCTGCACAGGATCAACGACG 688
JX198224	GCTCGTAACCAAGGGCTTTCTCTCGTCACCTTTAGATCACC	ACCCCTCCCACCACCACCCCATACCAATCC	AGGCTGCACAGGATCAACGACC 688
JX198225	GCTCGTAACCAAGGGCTTTGTCGTTCACCTTTAGATGAGTGCTCC	ACCOTOG CACGAGATGGAGACCGCTACCAATC	AAGCTGCACAGGCTCAACGACG 688
JX198226	CATGCTAACCTAGCCTTTCCCCTCCACCATCCCATCATTCCCC	CCCTCCACCACGAGAGATGGAG <mark>AC</mark> CG <mark>G</mark> TACCAATC.	AAGCTGCACAGGCTCAACGACG 691
JX198227	GCTGGTAACCAAAGGCTTTGTGGTGCACCATTGCATGATTGTACC	C <mark>CCTCGACGACGAGATGGAG<mark>GC</mark>CG<mark>C</mark>TAC<mark>G</mark>AATC.</mark>	aa <mark>g</mark> ctgcacagg <mark>c</mark> tcaacgac <mark>g</mark> 691
JX198228	GOCGETAACCAAGECTTTETEETTCACCTTTAGETGAETGCCCC	ACCCC <mark>CTC</mark> CCACGAGATGGAG <mark>AC</mark> CG <mark>A</mark> TACCAATC.	A <mark>GG</mark> CTGCACAGG <mark>A</mark> TCAACGAC <mark>C</mark> 691
JX198229	GATCGTAACCAAGCCCTTTGCCCTCCACCATCCCATCATTG	CCCTCCACCACCACCACACACACCACACACACACACACA	A <mark>AG</mark> CTGCACAGG <mark>C</mark> TCAACGAC <mark>G</mark> 691
JX198230	GCTEGTAACO <mark>AAGG</mark> ECTTTE <mark>TEGTE</mark> CACC <mark>GTTEGA</mark> TGA <mark>T</mark> TETCCC	C <mark>CCTCGACGAC</mark> GAGATGGAC <mark>AC</mark> CG <mark>G</mark> TAC <mark>G</mark> AATC.	a <mark>gt</mark> ctgcacagg <mark>c</mark> tcaacgac <mark>c</mark> 700
.TX198218	A GROBAC DA TOCCA COCCORDICA ATTRET DE TOCCACATTRET CO		TORREACCOMPACET 788
TX198219		GGCCTTCTACTTCCCACCTCCTCCTCCTCCTCCTC	CORRECTION ACCORDING TO THE TRANSPORT
JX198220		TGGCCTTCTACTTCCCCCCACTGCTTCGTCTCCT	COGAGAGGCTCCCGAGCCTACGT 787
JX198221		TGGCCTTCTACTTCCCCCCACTGCTTCGTCTCCT	COGAGAGGOTCOGAGCCTACGT 787
JX198222	ACTOZAGOATOGGAG <mark>I</mark> GGOOGTOGGATTOGT <mark>OG</mark> TGGGGTT <mark>OC</mark> TGG	TGGCCTTCTACTTCCC <mark>C</mark> OACTG <mark>C</mark> TTCGTCTG <mark>C</mark> T	CCGAGAGGCTCCGAGCCTACGT 788
JX198223	A <mark>G</mark> TC <mark>E</mark> AG <mark>O</mark> ATCGGAG <mark>O</mark> GGCCGT <mark>O</mark> GGATT <mark>O</mark> GTGGGGTT <mark>CG</mark> TGG	TGGCCTTCTACTTCCC <mark>GCA</mark> CTG <mark>C</mark> TTCGTCTG <mark>C</mark> T	CC <mark>GA</mark> GAGG <mark>C</mark> TCCG <mark>A</mark> GCCTACGT 788
JX198224	A <mark>G</mark> TO <mark>C</mark> AG <mark>O</mark> ATOGGAG <mark>O</mark> GGCCGT <mark>O</mark> GGATT <mark>O</mark> GT <mark>OG</mark> TGGGGTT <mark>OG</mark> TGG	TGGCCTTCTACTTCCC <mark>G</mark> C <mark>A</mark> CTG <mark>C</mark> TTCGTCTG <mark>C</mark> T	CC <mark>GA</mark> GAGG <mark>C</mark> TCCG <mark>A</mark> GCCTACGT 788
JX198225	A <mark>G</mark> TC <mark>G</mark> AG <mark>O</mark> ATCGGAG <mark>C</mark> GGCCGT <mark>O</mark> GGATT <mark>C</mark> GTGGGGTT <mark>CG</mark> TGG	<mark>T</mark> GGCCTTCTACTTCCC <mark>G</mark> C <mark>A</mark> CTG <mark>C</mark> TTCGTCTG <mark>C</mark> T	CO <mark>GA</mark> GAGG <mark>C</mark> TCCG <mark>A</mark> GCCTACGT 788
JX198226	A <mark>G</mark> TO <mark>G</mark> AG <mark>O</mark> ATOGGAG <mark>O</mark> GGOCGT <mark>O</mark> GGATT <mark>O</mark> GTGGGGTT <mark>TG</mark> TGG	T <mark>GGCCTTCTACTTCCC<mark>G</mark>C<mark>A</mark>CTG<mark>T</mark>TTCGTCTG<mark>O</mark>T</mark>	co <mark>ga</mark> gage <mark>c</mark> tccg <mark>t</mark> gcctacgt 791
JX198227	A <mark>G</mark> TC <mark>G</mark> AG <mark>C</mark> ATCGGAG <mark>T</mark> GGCCGT <mark>C</mark> GGATT <mark>C</mark> GT <mark>CA</mark> TGGGGTT <mark>TT</mark> TGG	T <mark>GGCCTTCTACTTCCC<mark>G</mark>C<mark>G</mark>CTG<mark>C</mark>TTCGTCTG<mark>T</mark>T</mark>	co <mark>g</mark> igagg <mark>o</mark> tocg <mark>a</mark> gcctacgt 791
JX198228	A <mark>G</mark> TC <mark>G</mark> AG <mark>C</mark> ATCGGAG <mark>C</mark> GGCCGT <mark>C</mark> GGATT <mark>C</mark> GTCG <mark>TGGGGTTCC</mark> TGG	T <mark>GGCCTTCTACTTCCC<mark>G</mark>C<mark>A</mark>CTG<mark>C</mark>TTCGTCTG<mark>C</mark>T</mark>	co <mark>ga</mark> gagg <mark>o</mark> tcog <mark>a</mark> gcctacgt 791
JX198229	A <mark>G</mark> TC <mark>G</mark> AG <mark>O</mark> ATCGGAGCGGCCGT <mark>O</mark> GGATT <mark>O</mark> GTGGGGTT <mark>T</mark> GTGG	TGGCCTTCTACTTCCC <mark>G</mark> CACTG <mark>T</mark> TTCGTCTG <mark>C</mark> T	co <mark>ga</mark> gagg <mark>t</mark> tccg <mark>a</mark> gcctacgt 791
JX198230	A <mark>R</mark> TC <mark>C</mark> AG <mark>O</mark> ATCGGAG <mark>O</mark> GGCCGT <mark>T</mark> GGATT <mark>C</mark> GT <mark>OC</mark> TGGGGTT <mark>CC</mark> TGG	<mark>T</mark> GGCCTTCTACTTCCC <mark>G</mark> C <mark>A</mark> CTG <mark>C</mark> TTCGTCTG <mark>C</mark> T	CO <mark>GA</mark> GAGE <mark>C</mark> TCCG <mark>A</mark> GCCTACGT 800
JX198218	CGTCCGGATATG		800
JX198219	CGTCCGGATATG		737
JX198220	CGTCCGGATATG		799
JX198221	CGTCCGGATATG		799
JX198222	CGTCCGGATATG		800
JX198223	CGTCCGGATATG		800
JX198224	CGTCCGGATATG		800
JX198225	CGTCCGGATATG		800
JX198226	CGTCCGGATATG		803
JX198227			803
UX198228	CGTCCGGATATG		803
TX100030	CCTCCCCATATC		010
OVTAQTON	COICCOGATAIG		812

Supplementary Fig. S4. The multi-sequence alignment of the identified *RLP* genes from the stripe rust resistant wheat cv. 92R137 and susceptible wheat cv. Yangmai158.

JX198218	MADDINELLELLELSS <mark>SLCEGTEODIRCINSVORS</mark> LADEGGVIR.SWDEEMDTDGYICRETGVECWHEDEMKILSLRLGNLGL <mark>O</mark> GOFFVALONCSSL	97
JX198219	MADLTKGULLFILLGSSSLCFCSSSULLCFRSLQCSVLLPNSVLKSSNIEDYPREGYLGPUIGVBCWHBNENRVLSVHUHVLGLQGTFBCGLQFCSSM	98
JX198220	MADLTKFILLFILLSSSLCEGTDODIRCLKSVQESLADPGQVLR.SWDEDMTPTVTYAALPVWNAGTLTRTRESLCD	77
JX198221	MADLTK <mark>SLILFILLSSS</mark> LCFCSBSDILCFRSLCCSVILFSNSVLKSSWIFBYPREGYICPLLVWNAGTFTRGFSLCISTT	81
JX198222	MADETRFIELFILLS <mark>SELCFGTEODIRCLY</mark> SVQS <mark>LADPGCVLR.SWNFENETNGVICRFTGVECWHFDENKI</mark> LSLRLGNLGLQGQFFVALQNCSSL	97
JX198223	MADETKFILLFILLSE <mark>SSLEFGTEQEIRELK</mark> SVQRSLA <mark>BFGGVLR.SWDFENDTDGYICRFTGVECWHFDENKI</mark> LSLRLGNLGLQGQFFVALQNCSSL	97
JX198224	MADETKFIRLFILLSSELCFGTEQCIRCLASVQCELAEPGGVLR.SWAFEAETAGVICRETGVECWHEDEAKILSLRLGALGQQFEVALQACSSL	97
JX198225	MADETKFLLLFLLSSSSLCFGTEQDIRCLKSVCRSLAFGGVLR.SWDFDNDTDGYICRFTGVECWHEDENKILSLRLCNLGLCCCFPALQNCSSL	97
JX198226	MADETKETILLFILLS <mark>SSLCFGSEODICCIKSVYQSVIE</mark> PNGVLKSSWNFDDGTMGFICRFTGVECWHFDRMMVLSLRLGNLGLQGTFPOGLQDASM	98
JX198227	MADETK <mark>SELIVELLSESTLEFESEADIGEIKSVQSVNEPNEVEKSSWIFENATEGYICEFTEVECWHFDEMRIFSLRECNEGEGEFFRELONESS</mark> V	98
JX198228	MADETK <mark>ELLEELLSE</mark> SLLCFESEHDIGELKSVCCSVLETDEVLKSTWNEDHETDEFICDEAGVECWHEDENRVLSLRLENLGLEEDCEFPCLCYCSSM	98
JX198229	MADETKSLILFILLSS <mark>SSLCFGSBSDILCLRSLCCSVII</mark> FNSVLKSSWIFPYPREGYICRFTGVECWHEDRNKILSLRLCNLGLCCGFFVALQYCSSL	98
JX198230	MADETNELLSVILLS <mark>ES</mark> SLOFGSBADICCL <mark>KSVCCSVIL</mark> PNGVLKSSWYEDNY ENYAYICRFTGVECWYEGENRVLSLRLGNLGLEGPFPCGLONCSSV	100
JX198218	TGLELSWNNFSGPIPCDISREMPYLTSLDLSYNSFSGLIPCNISNMTYLNLLNLCHNQLSGQIPSDFSLLTRLTTENVAGNLLSGEVETLLCK.FSSSNF	196
JX198219	THLCLSNNNFSGPIEDIS.RLNPYLTYLDLSYNNFSGEIFESTVNLEYLNVLNLCHNQLSGQIEFQEDLLTRLTTFNFTD	177
JX198220		
JX198221		
JX198222	ŢĠĿĊĿŚŊŊŊŦŚĠŖIJŦŷĊŨĬŚĊŃŊŶĽŦŚĿĊĿŚŊŊĸŦŚĠŚIJŦĊŊĬĔŊŊŢĬIJŊĿĊŊŊĊĿŚĠĊIJŦŖĹŦŔĿŢŔĔſŊŊĂĹŊĿĿŚĠĔIJŦŚĿĹŴĿŶſŚŔĸŊŦ	196
JX198223	TGLDLSNNNFSGPIPQDISRRMPYLTSLDLSYNSFSGLIPQNISNMTYLNLLNLCHNQLSGQIFSQFSLLTRLTTFNNAGNLLSGEVITLLQK.FSSSNF	196
JX198224	TGLDLSNNNFSGPIPQDISRRMPYLTSLDLSYNSFSGLIPQNISNMTYLNLLNLCHNQLSGQIFSQRSLLTRLTTFNNAGNLLSGEVITLLQK.FSSSNF	196
JX198225	TGLDLSNNNFSGPIPQDISRRMPYLTSLDLSYNSFSGLIPQNISNMTYLNLLNLCHNQLSGQIFSQRSLLTRLTTFNNAGNLLSGEVITLLQK.FSSSNF	196
JX198226	TGLCLSNNRFSGPIPCDISRCVPYLTSLDLSYNSFSGSIPCNISNNTYLNVLILCHNQLSGQIPFCPFDLLTRLSTFNNVINLLSGSIPPSLLR.FPANNF	197
JX198227	TGYDLSWNYLSGPIPGEISREMPYLTFLDLSYNSFSGSIPGNISNYTYLNLLNLGHQLSGQIPEQFDLLTRUIAFNVAENLLSGFIFFLLQN. FPASNF	197
JX198228	ŢĠĨŊĿŚŊŊŔŦŚĠĘŴĘŢĎĬĸŔĊĬĊġĊĿŢĔĹŊĹŚĔŊĸĔſĠĠĬŦĔſĠĬŢŊŊĨŢŶĿŊĹĿŊĹĊĔŊŎĹĠŶŎŢĔŎŶĹĹĬŢŔĸŢĔĠĸŎĸĔ	197
JX198229	TGLËLSNNN FSGPI PÇDI SRRMPYLTSLDLSYN SFSGLI PONISNY UNVLILCHNOLSGO I PFORDLLTRLGTFN VUNLLSGSI PPSLER. PPAWSF	197
JX198230	ŢĠĿĊĿŚŊŊŊĔŚĠŖĬŖĊŊĿĸĸĸĸĸĸĸĸĸĸĸĸĸĸĸĸĸĸĸĸĸĸĸĸĸĸĸĸĸĸĸĸĸĸĸĸ	200
JX198218	AGNQGLCGSELGEGSTFRRRWREIRIRLHRINDCSSIGAAVGFVVAFYFEHCFVCSERLRAYVVR	265
JX198219	. NFKLGASEMNDOPRSRKWRAIRIRLHRLNDOSSIGAAVGFVVGFVAAFYFEROFVCSKRFRAYVVR	244
JX198220		
JX198221		
JX198222	AGNQGLCGSFLLDGPPSRRRWREVQIIKLHRLNDQSSIGVAVGFVVAFYFPHCFVCSERLRAYVVR	265
JX198223	AGNQGLCGSPLEECSTFRRRWRPIRIRLHRINDCSSIGAAVGFVVAFYFPHCFVCSERLRAYVVR	265
JX198224	AGNQGLCGSFLDEQSTFRRRWRFIRIRLHRINDCSSIGAAVGFVVAFVFPHCFVCSERLRAYVVR	265
JX198225	AGNQGLCGSELEECSTFRRWRPVRIKLHRLNEESSIGAAVGFVVGFVVAFYFPHCFVCSERLRAYVVR	265
JX198226	DENÇELCEAE <mark>SED</mark> EPRSERRWEPVRIKLEHRLNEESSIGAAVGEVVAEVFPECEVCSERLEAYVVR	266
JX198227	AGNCRLCCAPTEDCTPSRRWRPVRIKLHRLNDCSSIGVAVGFVMGFLVAFYFFRCFVCSVRLRAYVVR	266
JX198228	AGNÇĞLCGS <mark>EL</mark> GECSTFRRRWRFIRIRLHRINDCSSIGAAVGFVVGFVVAFYFPECFVCSERLRAYVVR	266
JX198229	DGNQGLCGAFSDDDPRSRRWRPVRIKLHRINDESSIGAAVGEVVAEVFPFHCFVCSERFRAYVVR	266
JX198230	AGNQGLCGAELLDCPPSRRWRPVRISLHRLNDCSSIGAAVGFVVGFVVAFYFPHCFVCSERLRAYVVR	269

Supplementary Fig. S5. The multi-sequence alignment of the deduced proteins of the identified *RLP* genes from the stripe rust resistant wheat cv. 92R137 susceptible wheat cv. Yangmai158.



Supplementary Fig. S6. Sequence alignment and phylogenetic analysis of TaRLP1.1 and other putative RLP proteins using DNAMAN. (A) Alignment of the amino acid sequence of *TaRLP1.1* with other putative RLP proteins from *Oryza sativa* (BAF06973.1), *Hordeum vulgare* (BAJ87832.1), *Aegilops tauschii* (EMT19838.1) *Arabidopsis* (AtRLP44, AEE78586.1 and *Sorghum bicolor* (EES01889.17). Identical residues in all organisms are shaded. Underlines indicate sequence domains involved RLPs. (B) A representative phylogenetic tree of TaRLP1.1 and selected putative RLP proteins.



Supplementary Fig. S7. Subcellular localization of TaRLP1.1 protein. Onion epidermal cells were transformed with plasmids expressing the fusion protein and green fluorescent protein (GFP) by bombardment. All images were observed with a confocal microscope. (A) Onion epidermal cells expressing the GFP alone driven by the 35 S promoter. (B) Onion epidermal cells expression the TaRLP1.1-GFP fusion protein (Bars=100µm).



Supplementary Fig. S8. Expression analysis by the semi-quantitative RT-PCR of three pathogenesis-related genes, including *TaPR1*, *TaPR2* and *TaPR5*, at both 72 and 120 h after CYR32 inoculation in the BSMV: TaRLP1.1as infected plants and the BSMV infected control plants. *Tubulin* gene was used as the internal control.



Supplementary Fig. S9. Identification of the positive transgenic plants by GUS staining, gene detection and gene expression analysis. (A) GUS staining of the root tips of the T_1 generation transgenic lines. '+'means positive GUS staining and '-' means negative GUS staining. (B) *Gus* and *TaRLP1.1* detection in some of the T_0 and T_1 plants by PCR. '+': the positive control using the vector as the template; '-': the negative control using H₂O as the template. (C) Expression analysis of the *TaRLP1.1* in the identified positive transgenic plants. *Tubulin* gene was used as the internal control.



Supplementary Fig. S10. The positive *TaRLP1.1* transgenic plants showed robust hypersensitive response to the *Pst* 15 days after inoculation. The red arrowed indicated the inoculated leaves. 723-3 and 723-7 indicated T_1 generation plants of two individual T_0 plants, Y158 indicated the susceptible control Yang158, and 92R137 indicated the resistant control line.



Supplementary Fig. S11. Expression analysis by the semi-quantitative RT-PCR of three pathogenesis-related genes, including *TaPR1*, *TaPR2*, and *TaPR5*, at both 72 and 120h after CYR32 inoculation in the *TaRLP1.1* over-expressed transgenic plants, the *Yr26* containing 92R137 and the susceptible recipient Yangmai158. *Tubulin* gene was used as the internal control.

Supplementary Table

Table S1. Designed primers

	Primer Name	Primer Sequence (5' to 3')		
RT-PCR primers for	Ta.22666.2.S1-EST-F	CAGCTGACATCTCTGGATCT		
homolog cloning	Ta.22666.2.S1-EST-R	CAGACGAAGCAGTGCGGAAG		
	TaRLP-3'RACE-F	TTGTTATCAGGGCCTGTTCC		
Primers for RACE	TaRLP-5'RACE-R	GAAGTAGAAGGCCACCACGA		
Primers for full length	TaRLP1.1-FL-F	TGGAATTGACAAGCTGCAAG		
sequence amplification	TaRLP1.1-FL-R	GGGGTAGCTATGCCAGACAA		
Primers for ORF	TaRLP1.1-ORF-F	GATGGCTGATGATACCAAGT		
amplification	TaRLP1.1-ORF-R	GAATCATATCCGGACGACGT		
Semi-quantitative	<i>TaRLP1.1-</i> RT-F	CATGGGGGATGGCGGCTTCTG		
RT-PCR primers	TaRLP1.1-RT-R	GTAGAGTTTCACTGTGTTCC		
	TaRLP1.1-DW-F	TGCCTGAAGTCTGTACAACGC		
	TaRLP1.1-DW-R	CTTCTGTAGCAAAGTAGGAAC		
Primers for	<i>TaRLP1</i> 804-1-DW-F	CTCCTCTTGAGCAGCTCATCA		
of the <i>TaRLP1</i> family	<i>TaRLP1</i> 804-1-DW-R	CTTCTGTAGCAAAGTAGGAAC		
genes	TaRLP1 ₈₁₃ –DW-F	GGTACTTTGAAAATCCCTATCC		
	TaRLP1 ₈₁₃ - DW-R	TACTGCTAGCAAAGAAGGAAT		
Prmers for Subcellular	TaRLP1.1-SL-F	GC <u>GTCGAC</u> CTGGAATTGACAAGCT GCAAG		
TaRLP1.1	<i>TaRLP1.1-</i> SL-R	C <u>CCATGG</u> AGGCTCGGAGCCTCTCGG AGCA		
Primers for VIGS	TaRLP1.1-VIGS-F	GCT <u>GCTAGC</u> GATACGAATCAGGCTG CACA		
vector construction	TaRLP1.1-VIGS-R	GCT <u>GCTAGC</u> GGGGTAGCTATGCCAG ACAA		
Primers for over-expression vector	TaRLP1.1-WT-F	CGC <u>GGATCC</u> ATGGCTGATGATACCA AG		

construction	TaRLP1.1-WT-R	C <u>GAGCTC</u> TCATATCCGGACGACGTA			
Primers for <i>TaRLP1.1</i>	<i>35S</i> -F	AGTTCATTTCATTTGGAGAGAACAC			
transgenic plants	<i>TaRLP1.1-</i> R	GTTTGAAGACGAAAACTTCTGTAGC			
Primers for <i>Gus</i>	Gus-F	AGTGTACGTATCACCGTTTGTGTGA AC			
transgenic plants	Gus-R	ATCGCCGCTTTGGACATACCATCCGT A			
Primers for qRT-PCR	TaRLP1.1-qPCR-F	AGCTTTGTGGTTCACCTTTAGA			
of TaRLP1.1	TaRLP1.1-qPCR-R	CTCGGAGCCTCTCGGAGCAGAC			
	<i>TaPR1-</i> F (AAK60565)	GAGAATGCAGACGCCCAAGC			
Somi quantitativa	<i>TaPR1</i> -R (AAK60565)	CTGGAGCTTGCAGTCGTTGATC			
RT-PCR primers for	<i>TaPR2</i> -F (DQ090946)	GCAGCTCTACAGGTCCAAGG			
TaPRs gene	<i>TaPR2</i> -R (DQ090946)	CGGCGATGTACTTGATGTTG			
	<i>TaPR5-</i> F (FG618781)	CAAGCAGTGGTATCAACGCAGAG			
	<i>TaPR5-</i> R (FG618781)	GTGAAGCCACAGTTGTTCTTGATG			
	Tubulin-RT-F	AGAACACTGTTGTAAGGCTCAAC			
Primers for the internal	Tubulin-RT-R	GAGCTTTACTGCCTCGAACATGG			
control gene	18sRNA-RT-F	AACACTTCACCGGACCATTCA			
	18sRNA-RT-R	CGTCCCTGCCCTTTGTACAC			

Table S2. The upregulated folds of Ta.22666.2.S1_at and TaAffx.55699.1.S1_atbetween the different samples of tests and the controls

D 1	Controls	Tests				
Probes		92R137-12h	R236-12h	92R137-36h	R236-36h	
	R236-0h	/	4.59	/	8.00	
Ta.22666.2.S1	Yangmai158-12h	16.00	7.46	/	/	
	Yangmai158-36h	/	/	4.00	4.92	
	R236-0h	/	3.73	/	19.69	
TaAffx.55699.	Yangmai158-12h	6.49	3.73	/	/	
1.51_at	Yangmai158-36h	/	/	3.03	2.83	

Length	Gene	Accession No.	92R137		Yangmai158	
738 bp	TaRLP1738	JX198219	+		+	
900 hr	TaRLP1800-1	JX198220	+	*	+	*
800 bp	TaRLP1800-1	JX198221			+	
	TaRLP1801-2	JX198222	+		+	*
	TaRLP1 ₈₀₁ -3	JX198223	+			
801 bp	TaRLP1 ₈₀₁ -4	JX198224	+			
	TaRLP1.1	JX198218	+	*		
	TaRLP1 ₈₀₁ -5	JX198225			+	
	TaRLP1804-1	JX198226	+		+	
904 hr	TaRLP1804-2	JX198227	+			
804 Up	TaRLP1804-3	JX198228	+			
	TaRLP1804-4	JX198229			+	
813 bp	TaRLP1 ₈₁₃	JX198230	+	*	+	

 Table S3.
 The genes identified in the resistant 92R137 and Yangmai158

Note: The '+' indicated that the gene was existed in the genome of 92R137 or Yangmai158, and '*' indicated that the gene was expressed in the corresponding materials respectively.