

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 ‘Yxxφ’ 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*, *TaRLP1₈₀₄₋₁* and *TaRLP1₈₁₃* with specific primers using the nulli-tetrasomic (NT) lines derived from cv. Chinese Spring. *TaRLP1.1* was located to the chromosome 3D, *TaRLP1₈₀₄₋₁* to 3B, and *TaRLP1₈₁₃* 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 (*TaRLP1₈₀₁₋₅*), 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 = 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: TaRLP1.1 as infected plants and the BSMV infected control plants. *Tubulin* gene was used as the internal control.

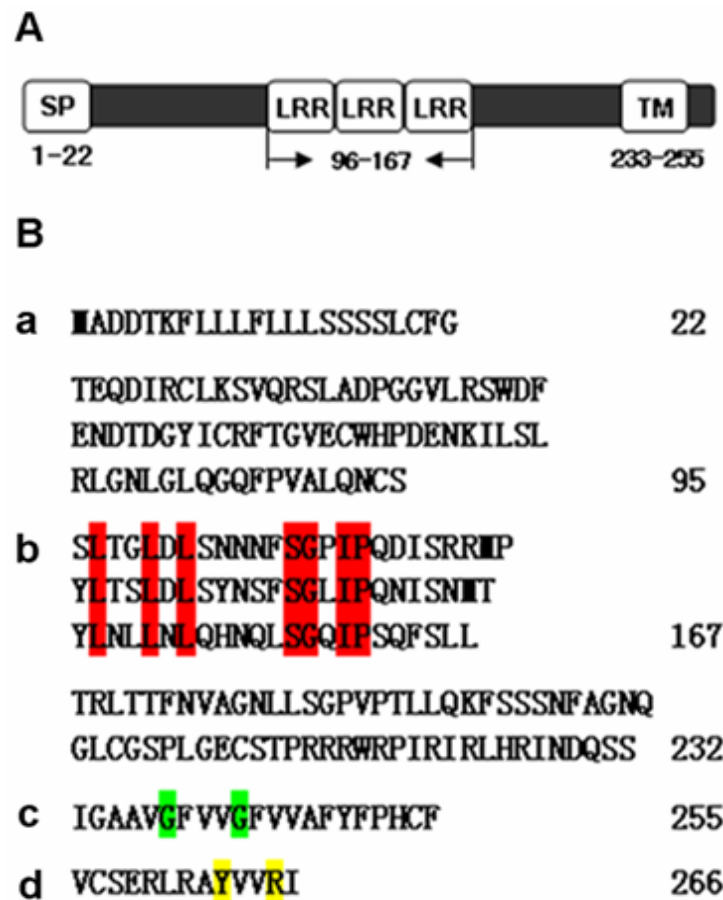
Supplementary Fig. S9. Identification of positive transgenic plants by GUS staining, gene detection and gene expression analysis. (A) GUS staining of the root tips of the T₁ generation transgenic lines. '+' indicates positive GUS staining and '-' indicates negative GUS staining. (B) *Gus* and *TaRLP1.1* detection in some of the T₀ and T₁ 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 T₁ generation plants of two individual T₀ plants, Y158 indicated the susceptible control cv. Yang158, and 92R137 indicates the resistant control line.

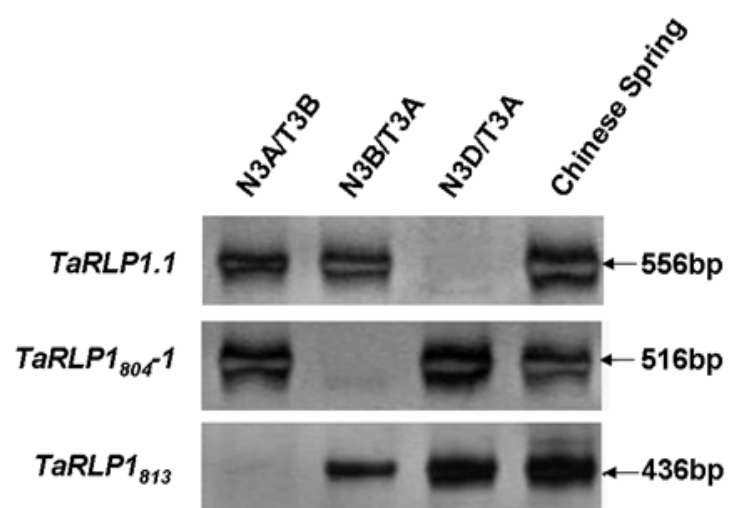
Supplementary Fig. S11. Expression analysis by semi-quantitative RT-PCR of three pathogenesis-related genes, including *TaPR1*, *TaPR2* and *TaPR5*, at both 72 and 120 h after CYR32 inoculation in *TaRLP1.1* over-expressed transgenic plants, the *Yr26* containing cv. 92R137 and the susceptible recipient.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

Supplementary Fig. S1



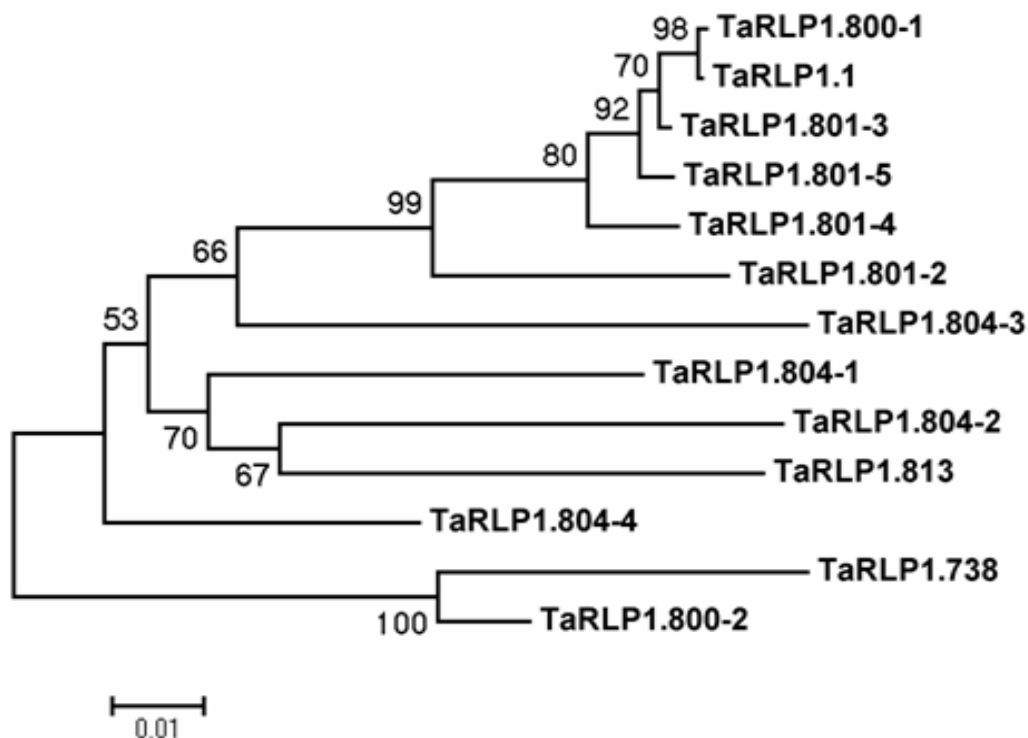
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Supplementary Fig. S2



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The Genbank accession numbers are JX198219 (*TaRLP1*₇₃₈), JX198220 (*TaRLP1*₈₀₀₋₁), JX198221 (*TaRLP1*₈₀₀₋₂), JX198222 (*TaRLP1*₈₀₁₋₂), JX198223 (*TaRLP1*₈₀₁₋₃), JX198224 (*TaRLP1*₈₀₁₋₄), JX198218 (*TaRLP1.1*), JX198225 (*TaRLP1*₈₀₁₋₅), JX198226 (*TaRLP1*₈₀₄₋₁), JX198227 (*TaRLP1*₈₀₄₋₂), JX198228 (*TaRLP1*₈₀₄₋₃), JX198229 (*TaRLP1*₈₀₄₋₄), JX198230 (*TaRLP1*₈₁₃).

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JX198223	AGTCGAGCATGAGAGCGGCCGTGGATTGTCGTGGGGTTCTGTGTGCCCTTCTACTTCCCGACTGCTTTCGTCTGTCCGAGAGGCTCCGAGCCTACGT	788
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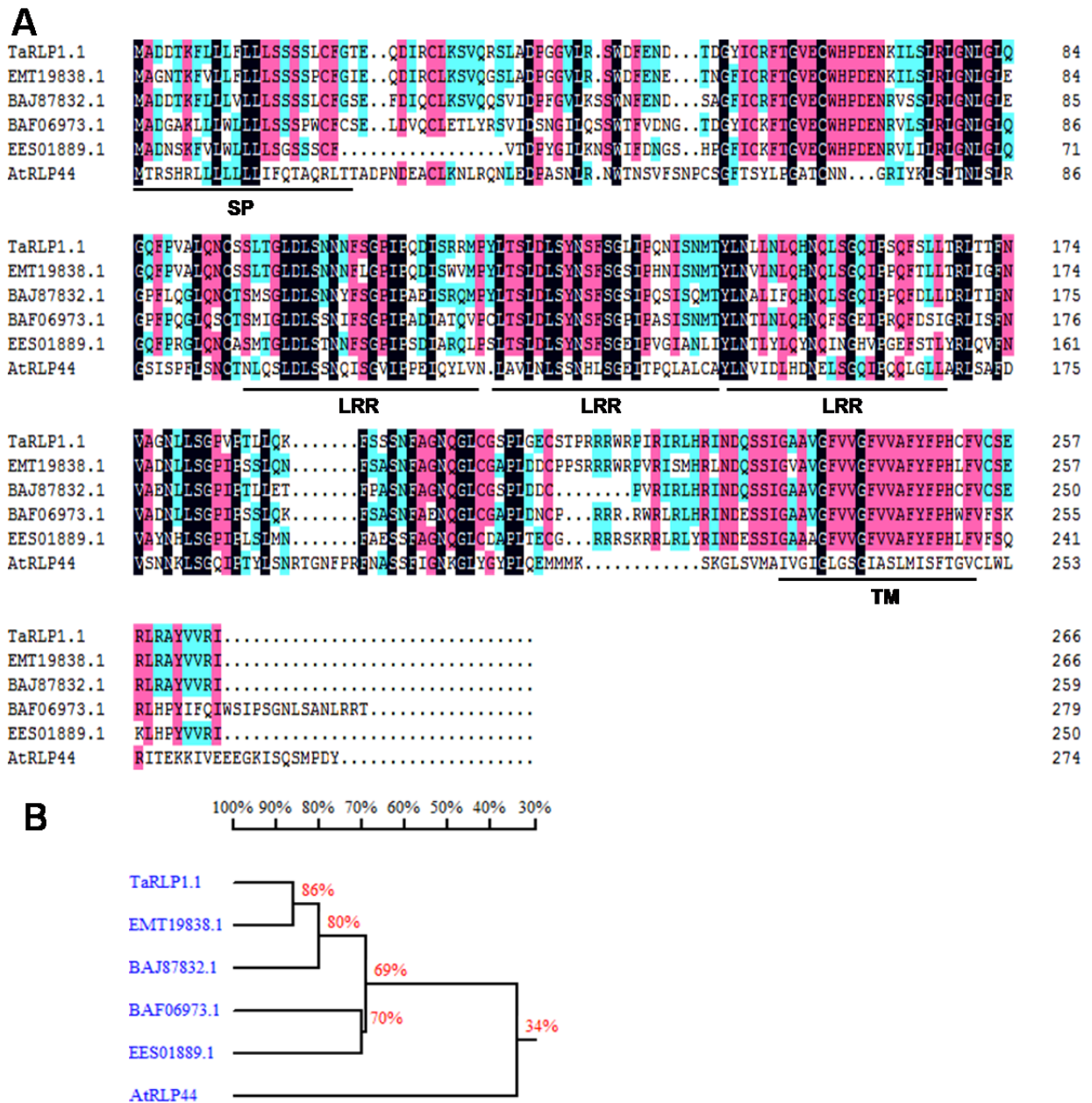
Supplementary Fig. S4. The multi-sequence alignment of the identified *RLP* genes from the stripe rust resistant wheat cv. 92R137 and susceptible wheat cv. Yangmai158.

Supplementary Fig. S5

JX198218	MADDTKFLLLFLLSSSSLCFGTEODTRCLKSVCFSLADPGGVLR, SWDFEN, .LTDGYICRFTGVCEWHPDENKILSLRLGNLGLQGFVVALQNCSSL	97
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JX198224	MADDTKFLRLFLLSSSSLCFGTEODTRCLM SVQSSLADPGGVLR, SWDFEN, .ETNGYICRFTGVCEWHPDENKILSLRLGNLGLQGFVVALQNCSSL	97
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JX198227	MADDTKFLLLFLLSSSSLCFGSEDTLCLKSVYCSVIDPNSVLKSWIFEN, .ATEGYICRFTGVCEWHPDENRVLVHLGNLGLQGFVVALQNCSSM	98
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JX198230	TGLLELNNNFGSGPIPCDISRQVPEYLYLDLSYNSFSGLTIPQNI SNMTYINLNLCHNQLSGQIPQFDLLLRLLTRLTTFNVAGNLLSGFVETLLQK, FPAWNE	200
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JX198221		
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JX198223	AGNQLCGSEFLDEGPTFRRRWRPVRIRLHRLNDQSSIGAAVGFVVGFFVAFYFPHCFVCSERLRAYVVR	265
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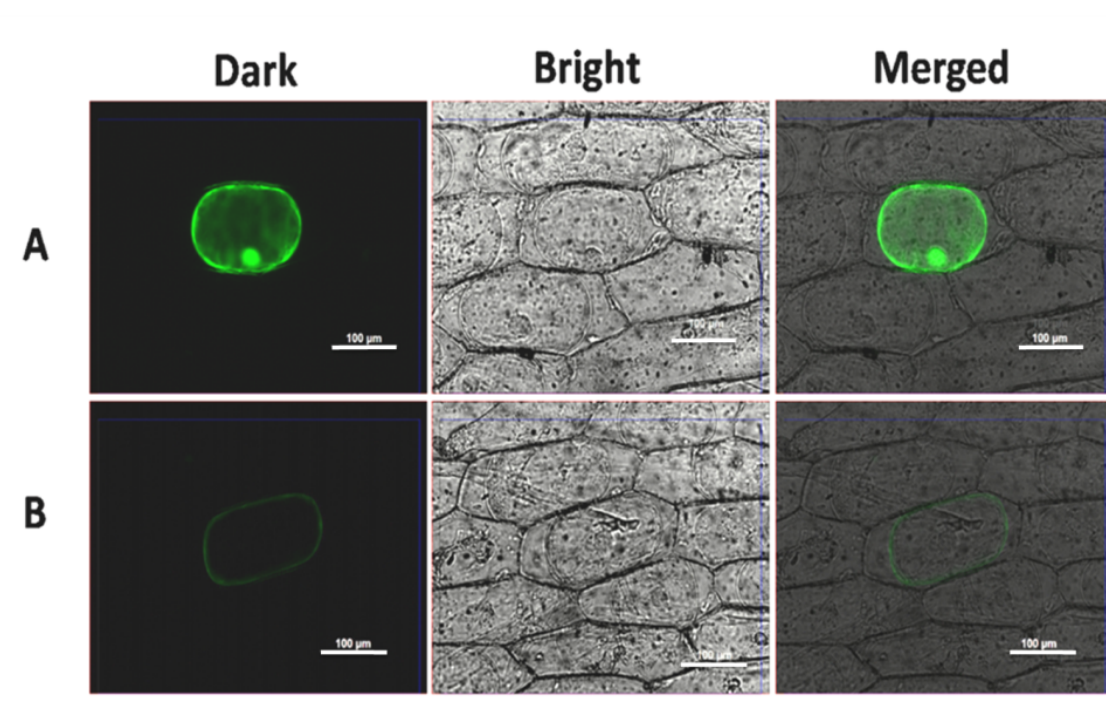
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Supplementary Fig. S6



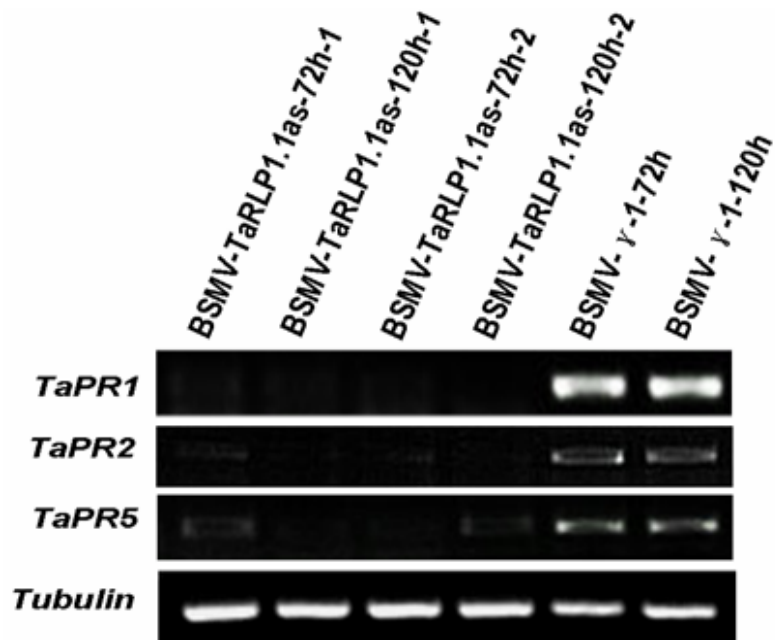
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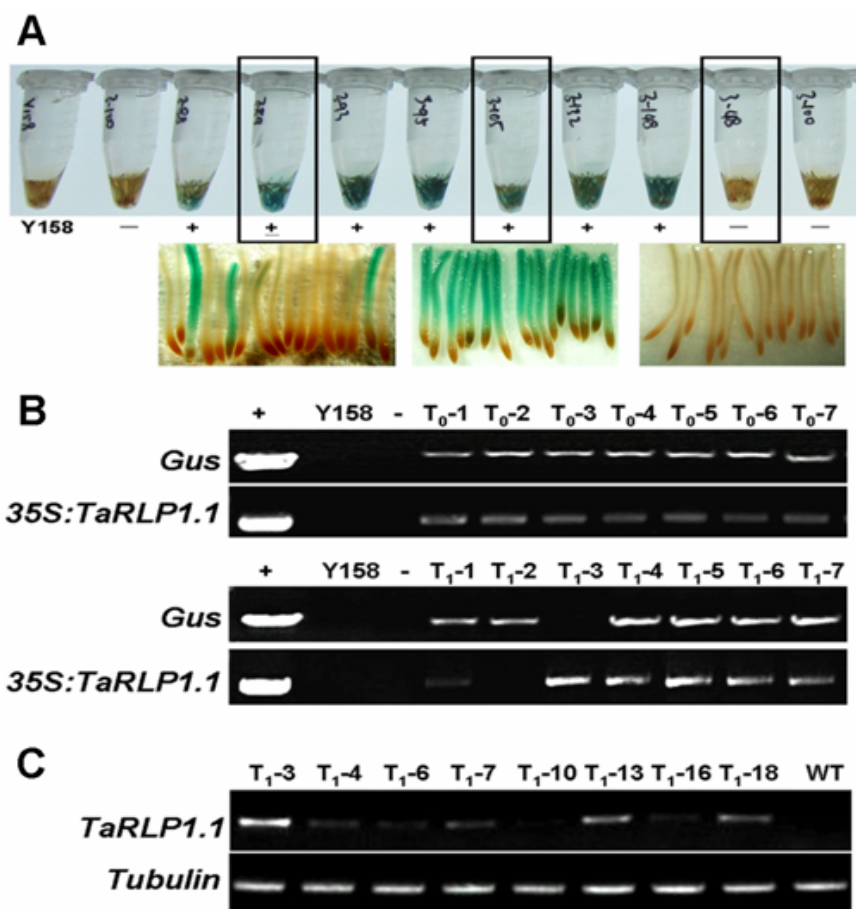
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Supplementary Fig. S8



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Supplementary Fig. S9



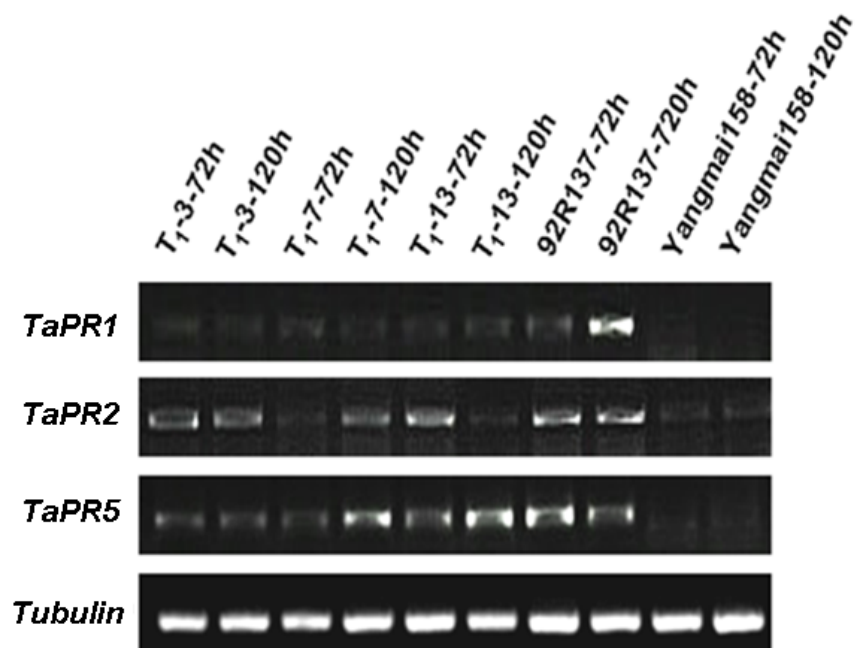
Supplementary Fig. S9. Identification of the positive transgenic plants by GUS staining, gene detection and gene expression analysis. (A) GUS staining of the root tips of the T₁ generation transgenic lines. ‘+’ means positive GUS staining and ‘-’ means negative GUS staining. (B) *Gus* and *TaRLP1.1* detection in some of the T₀ and T₁ 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



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 T₁ generation plants of two individual T₀ plants, Y158 indicated the susceptible control Yang158, and 92R137 indicated the resistant control line.

Supplementary Fig. S11



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 homolog cloning	Ta.22666.2.S1-EST-F	CAGCTGACATCTCTGGATCT
	Ta.22666.2.S1-EST-R	CAGACGAAGCAGTGCGGAAG
Primers for RACE	<i>TaRLP</i> -3'RACE-F	TTGTTATCAGGGCCTGTTCC
	<i>TaRLP</i> -5'RACE-R	GAAGTAGAAGGCCACCACGA
Primers for full length sequence amplification	<i>TaRLP1.1</i> -FL-F	TGGAATTGACAAGCTGCAAG
	<i>TaRLP1.1</i> -FL-R	GGGGTAGCTATGCCAGACAA
Primers for ORF amplification	<i>TaRLP1.1</i> -ORF-F	GATGGCTGATGATACCAAGT
	<i>TaRLP1.1</i> -ORF-R	GAATCATATCCGGACGACGT
Semi-quantitative RT-PCR primers	<i>TaRLP1.1</i> -RT-F	CATGGGGATGGCGGCTTCTG
	<i>TaRLP1.1</i> -RT-R	GTAGAGTTTCACTGTGTTCC
Primers for chromosome location of the <i>TaRLP1</i> family genes	<i>TaRLP1.1</i> -DW-F	TGCCTGAAGTCTGTACAACGC
	<i>TaRLP1.1</i> -DW-R	CTTCTGTAGCAAAGTAGGAAC
	<i>TaRLP1</i> ₈₀₄₋₁ -DW-F	CTCCTCTTGAGCAGCTCATCA
	<i>TaRLP1</i> ₈₀₄₋₁ -DW-R	CTTCTGTAGCAAAGTAGGAAC
	<i>TaRLP1</i> ₈₁₃ -DW-F	GGTACTTTGAAAATCCCTATCC
	<i>TaRLP1</i> ₈₁₃ -DW-R	TACTGCTAGCAAAGAAGGAAT
Primers for Subcellular localization of <i>TaRLP1.1</i>	<i>TaRLP1.1</i> -SL-F	GCGTCGACCTGGAATTGACAAGCT GCAAG
	<i>TaRLP1.1</i> -SL-R	CCCATGGAGGCTCGGAGCCTCTCGG AGCA
Primers for VIGS vector construction	<i>TaRLP1.1</i> -VIGS-F	GCTGCTAGCGATACGAATCAGGCTG CACA
	<i>TaRLP1.1</i> -VIGS-R	GCTGCTAGCGGGGTAGCTATGCCAG ACAA
Primers for over-expression vector	<i>TaRLP1.1</i> -WT-F	CGCGGATCCATGGCTGATGATACCA AG

construction	<i>TaRLP1.1</i> -WT-R	CGAGCTCTCATATCCGGACGACGTA
Primers for <i>TaRLP1.1</i> detection in the transgenic plants	35S-F	AGTTCATTTCAATTTGGAGAGAACAC
	<i>TaRLP1.1</i> -R	GTTTGAAGACGAAAACCTTCTGTAGC
Primers for <i>Gus</i> detection in the transgenic plants	<i>Gus</i> -F	AGTGTACGTATCACCGTTTGTGTGA AC
	<i>Gus</i> -R	ATCGCCGCTTTGGACATACCATCCGT A
Primers for qRT-PCR of <i>TaRLP1.1</i>	<i>TaRLP1.1</i> -qPCR-F	AGCTTTGTGGTTCACCTTTAGA
	<i>TaRLP1.1</i> -qPCR-R	CTCGGAGCCTCTCGGAGCAGAC
Semi-quantitative RT-PCR primers for <i>TaPRs</i> gene	<i>TaPR1</i> -F (AAK60565)	GAGAATGCAGACGCCCAAGC
	<i>TaPR1</i> -R (AAK60565)	CTGGAGCTTGCAGTCGTTGATC
	<i>TaPR2</i> -F (DQ090946)	GCAGCTCTACAGGTCCAAGG
	<i>TaPR2</i> -R (DQ090946)	CGGCGATGTACTIONTGTGTTG
	<i>TaPR5</i> -F (FG618781)	CAAGCAGTGGTATCAACGCAGAG
	<i>TaPR5</i> -R (FG618781)	GTGAAGCCACAGTTGTTCTTGATG
Primers for the internal control gene	<i>Tubulin</i> -RT-F	AGAACACTGTTGTAAGGCTCAAC
	<i>Tubulin</i> -RT-R	GAGCTTTACTGCCTCGAACATGG
	<i>18sRNA</i> -RT-F	AACACTTCACCGGACCATTCA
	<i>18sRNA</i> -RT-R	CGTCCCTGCCCTTTGTACAC

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

Probes	Controls	Tests			
		92R137-12h	R236-12h	92R137-36h	R236-36h
Ta.22666.2.S1	R236-0h	/	4.59	/	8.00
	Yangmai158-12h	16.00	7.46	/	/
	Yangmai158-36h	/	/	4.00	4.92
TaAffx.55699.1.S1_at	R236-0h	/	3.73	/	19.69
	Yangmai158-12h	6.49	3.73	/	/
	Yangmai158-36h	/	/	3.03	2.83

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

Length	Gene	Accession No.	92R137		Yangmai158	
738 bp	TaRLP1 ₇₃₈	JX198219	+		+	
800 bp	TaRLP1 ₈₀₀₋₁	JX198220	+	*	+	*
	TaRLP1 ₈₀₀₋₁	JX198221			+	
801 bp	TaRLP1 ₈₀₁₋₂	JX198222	+		+	*
	TaRLP1 ₈₀₁₋₃	JX198223	+			
	TaRLP1 ₈₀₁₋₄	JX198224	+			
	TaRLP1.1	JX198218	+	*		
	TaRLP1 ₈₀₁₋₅	JX198225			+	
804 bp	TaRLP1 ₈₀₄₋₁	JX198226	+		+	
	TaRLP1 ₈₀₄₋₂	JX198227	+			
	TaRLP1 ₈₀₄₋₃	JX198228	+			
	TaRLP1 ₈₀₄₋₄	JX198229			+	
813 bp	TaRLP1 ₈₁₃	JX198230	+	*	+	

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.