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, 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 (TaRLP1$_{738}$), JX198220 (TaRLP1$_{800}$-1), JX198221 (TaRLP1$_{800}$-1), 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$_{813}$).

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* (EES01889.1*7*). 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.

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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 H_2O as the template. (C) Expression analysis of *TaRLP1.1* in the identified positive transgenic plants. *Tubulin* gene was used as the internal control.

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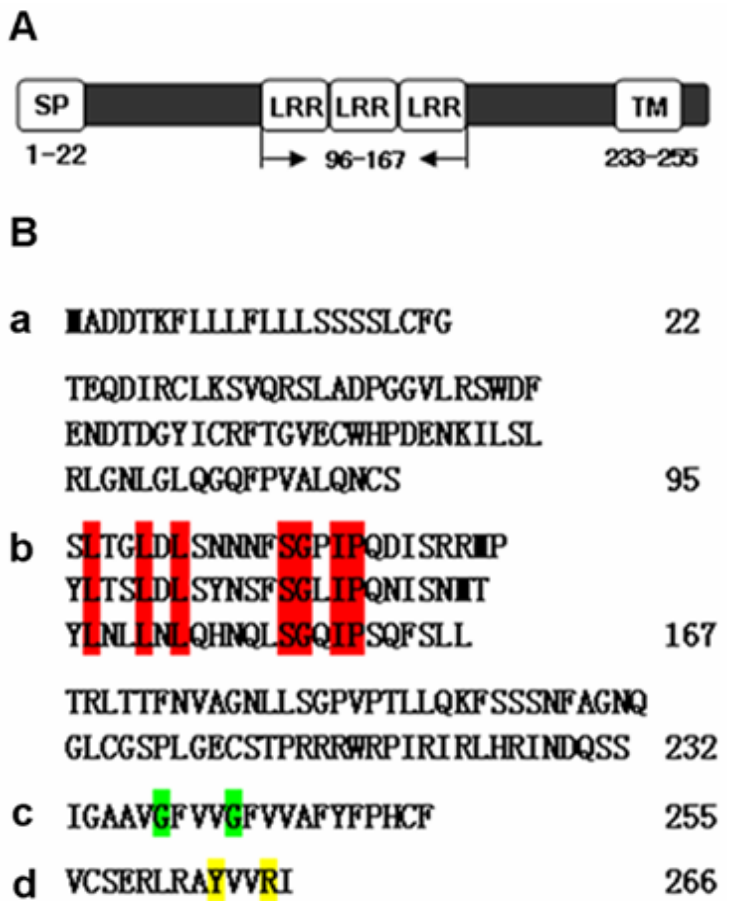
inoculated leaves. 723-3 and 723-7 indicated T_1 generation plants of two individual T_0 plants, Y158 indicated the susceptible control cv. Yang158, and 92R137 indicates the resistant control line.

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.

Supplementary Fig. S1



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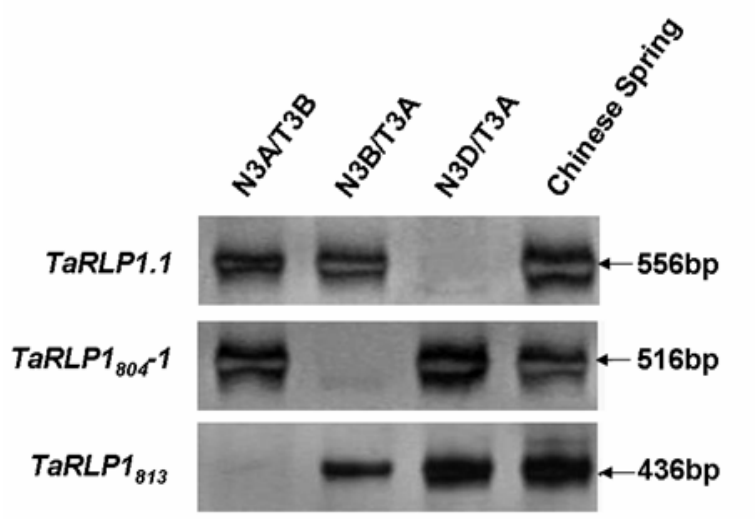
Supplementary Fig. S2



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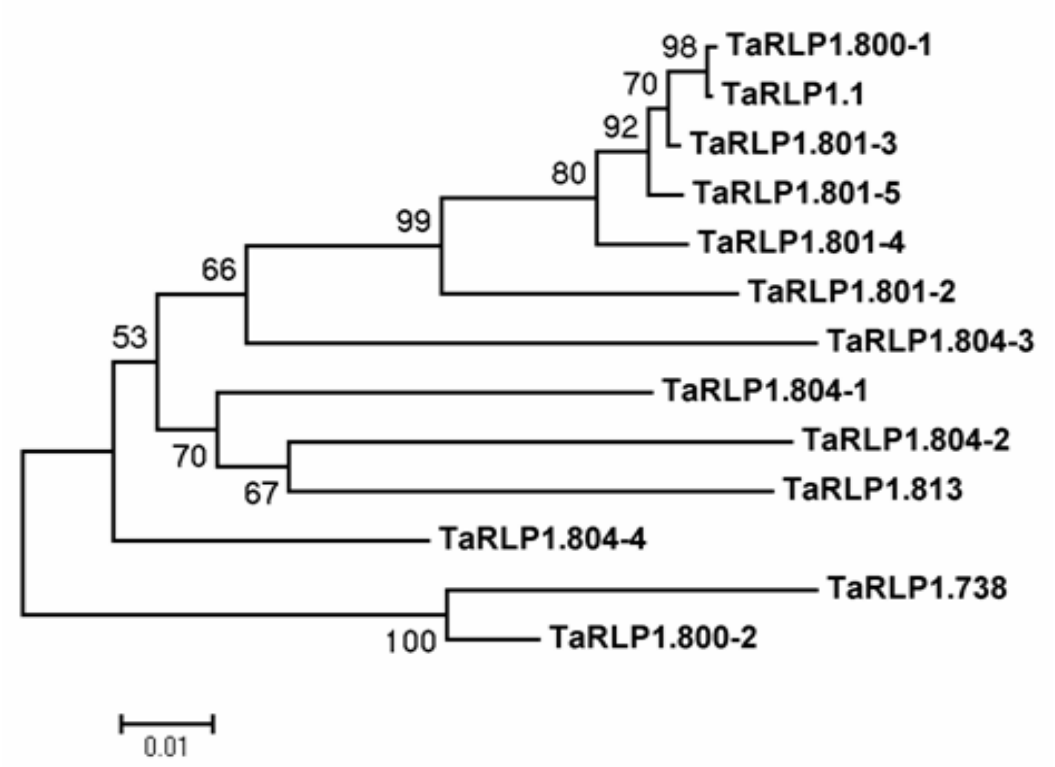

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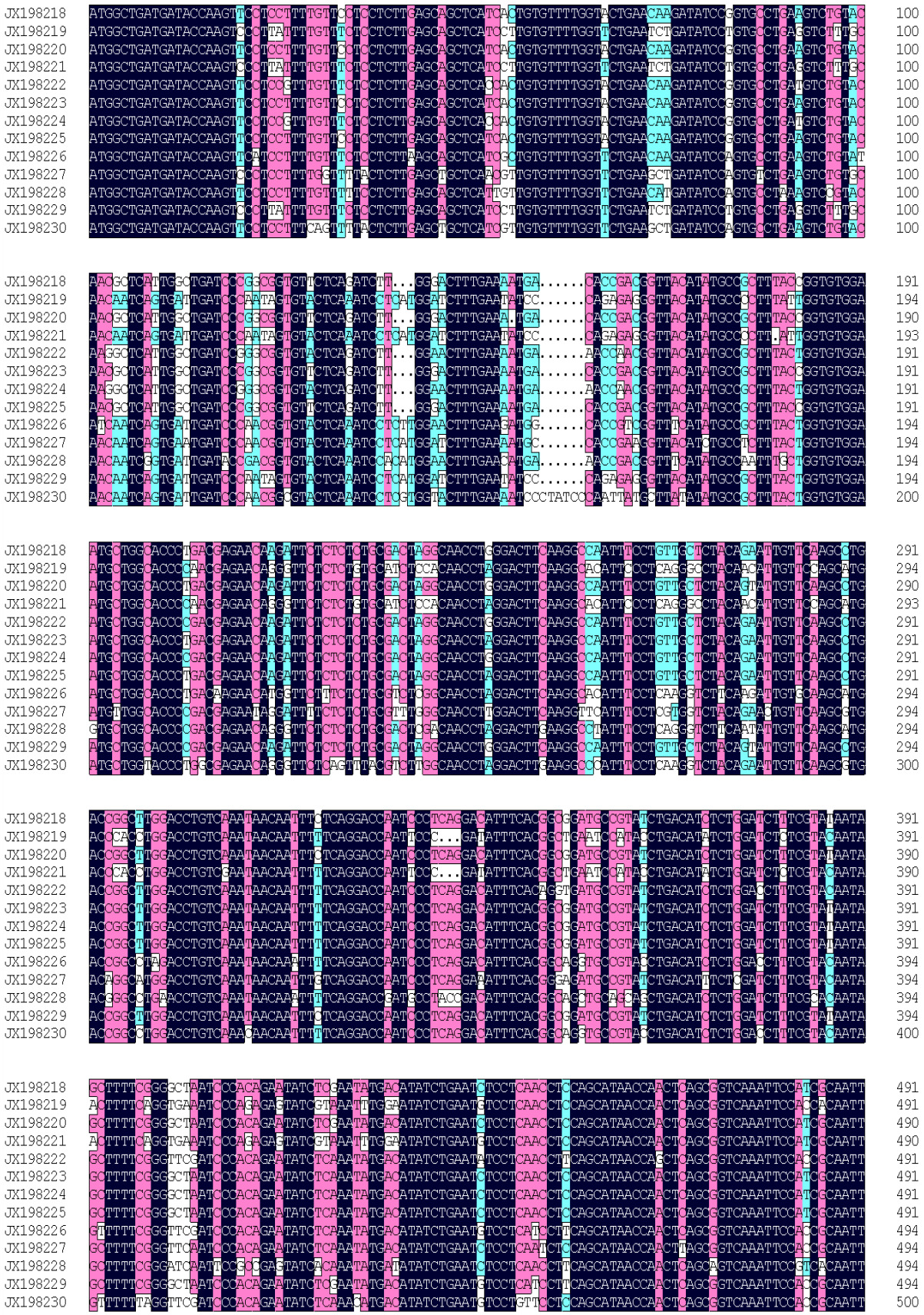
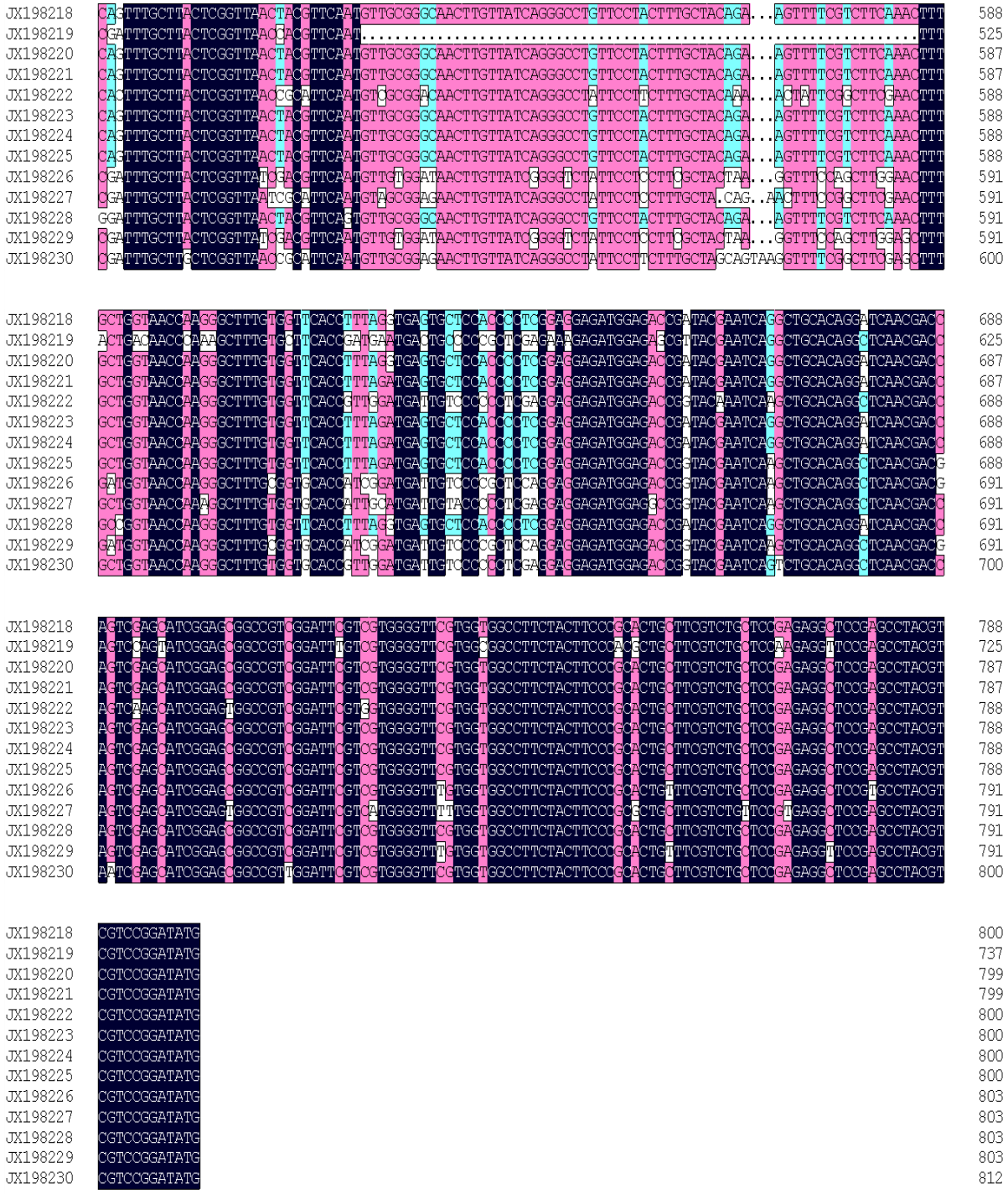
Supplementary Fig. S4





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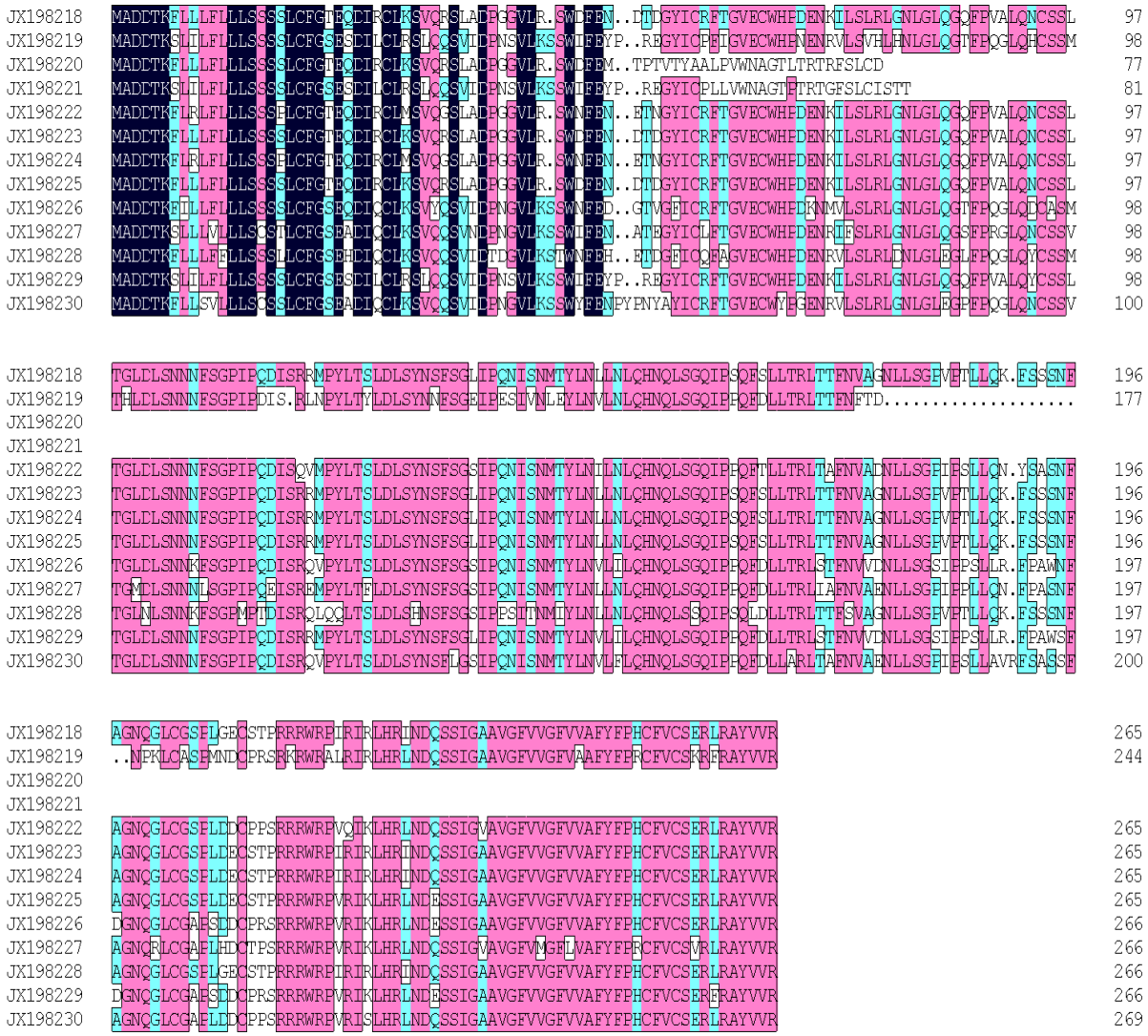

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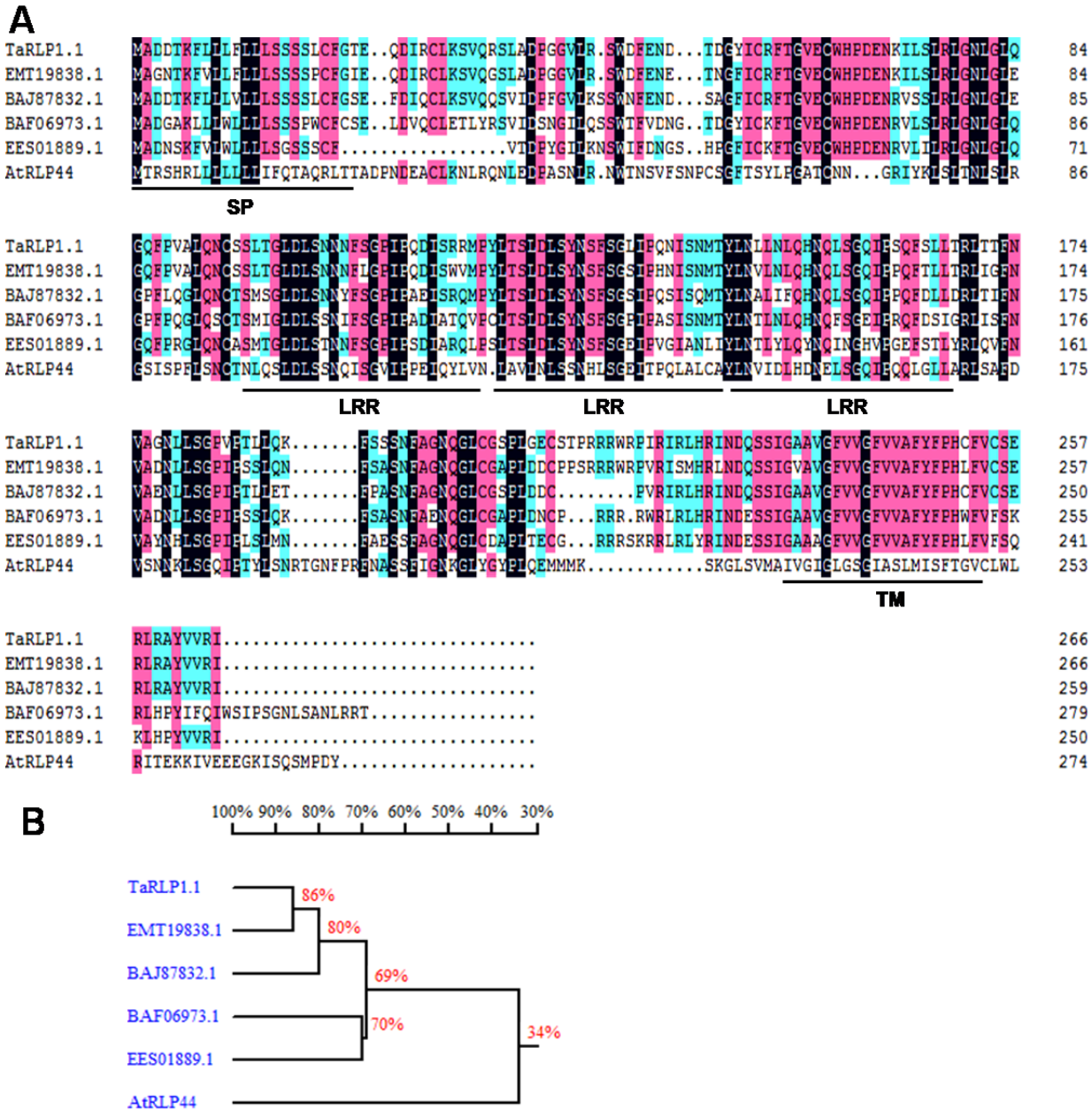
Supplementary Fig. S6



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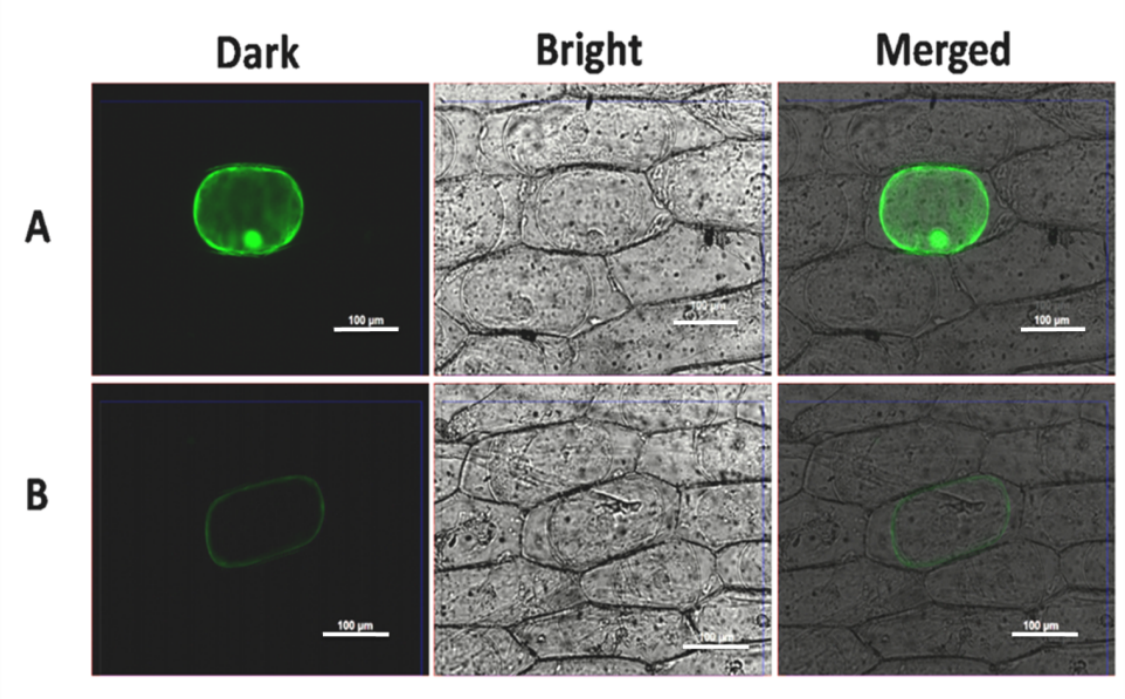

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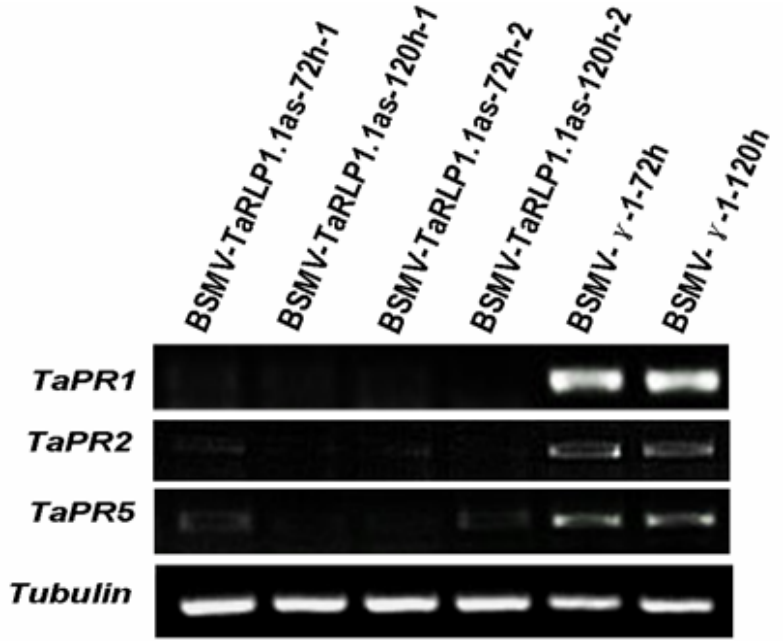

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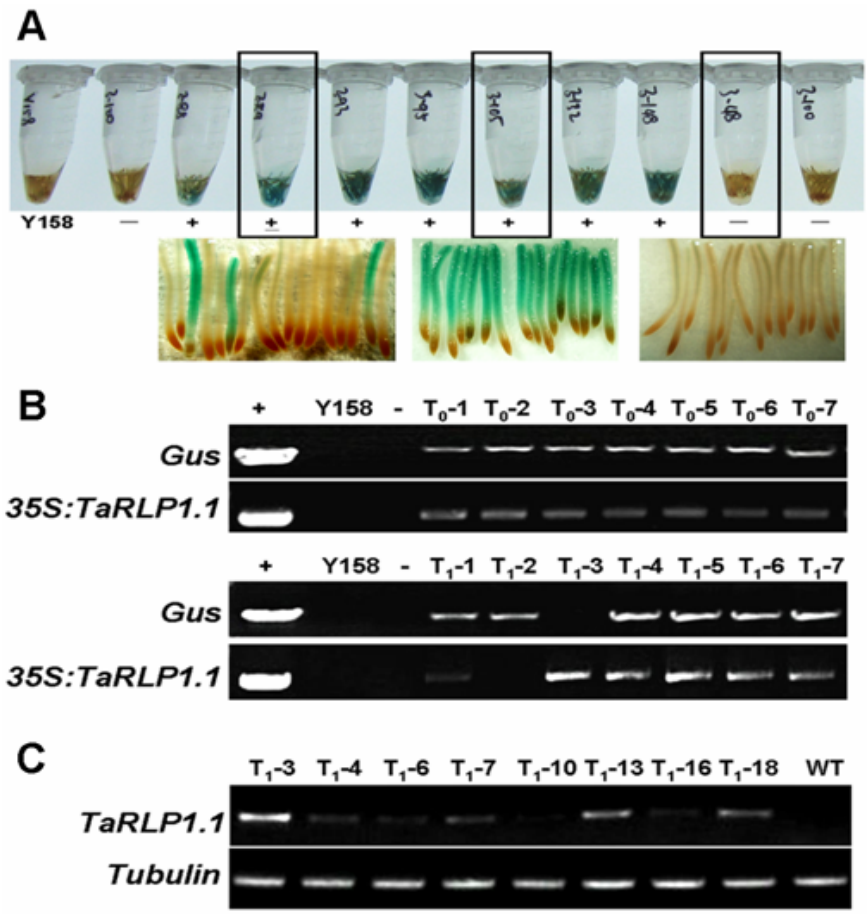

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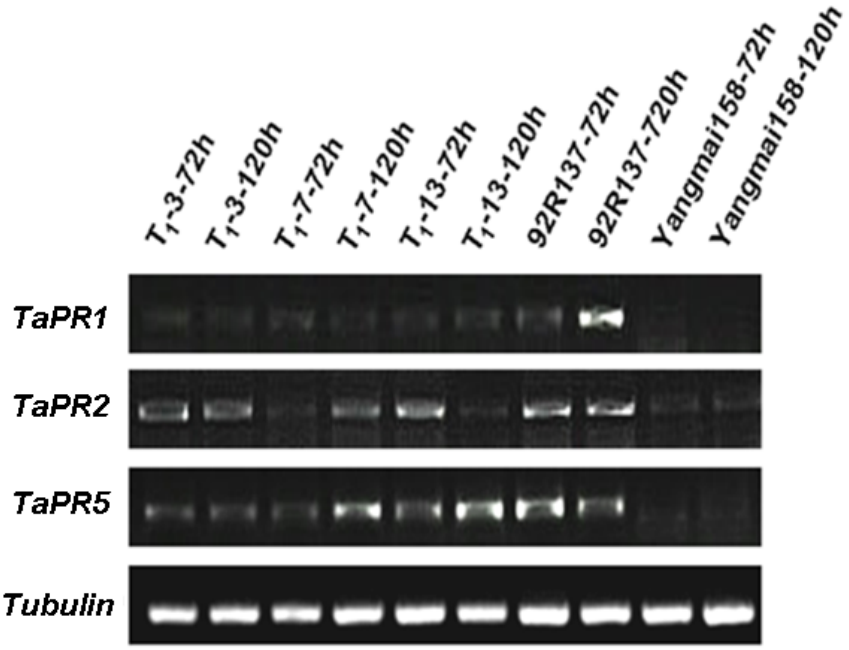

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Supplementary Table

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	*TaRLP*-3'RACE-F	TTGTTATCAGGGCCTGTTCC
	TaRLP-5'RACE-R	GAAGTAGAAGGCCACCACGA
Primers for full length sequence amplification	*TaRLP1.1*-FL-F	TGGAATTGACAAGCTGCAAG
	TaRLP1.1-FL-R	GGGGTAGCTATGCCAGACAA
Primers for ORF amplification	*TaRLP1.1*-ORF-F	GATGGCTGATGATACCAAGT
	TaRLP1.1-ORF-R	GAATCATATCCGGACGACGT
Semi-quantitative RT-PCR primers	*TaRLP1.1*-RT-F	CATGGGGATGGCGGCTTCTG
	TaRLP1.1-RT-R	GTAGAGTTTCACTGTGTTCC
Primers for chromosome location of the *TaRLP1* family genes	*TaRLP1.1*-DW-F	TGCCTGAAGTCTGTACAACGC
	TaRLP1.1-DW-R	CTTCTGTAGCAAAGTAGGAAC
	TaRLP1$_{804}$-1-DW-F	CTCCTCTTGAGCAGCTCATCA
	TaRLP1$_{804}$-1-DW-R	CTTCTGTAGCAAAGTAGGAAC
	TaRLP1$_{813}$–DW-F	GGTACTTTGAAAATCCCTATCC
	TaRLP1$_{813}$- DW-R	TACTGCTAGCAAAGAAGGAAT
Prmers for Subcellular localization of TaRLP1.1	*TaRLP1.1*-SL-F	GCGTCGACCTGGAATTGACAAGCTGCAAG
	TaRLP1.1-SL-R	CCCATGGAGGCTCGGAGCCTCTCGGAGCA
Primers for VIGS vector construction	*TaRLP1.1*-VIGS-F	GCTGCTAGCGATACGAATCAGGCTGCACA
	TaRLP1.1-VIGS-R	GCTGCTAGCGGGGTAGCTATGCCAGACAA
Primers for over-expression vector	*TaRLP1.1*-WT-F	CGCGGATCCATGGCTGATGATACCAAG

construction	*TaRLP1.1*-WT-R	CGAGCTCTCATATCCGGACGACGTA
Primers for *TaRLP1.1* detection in the transgenic plants	*35S*-F	AGTTCATTTCATTTGGAGAGAACAC
	TaRLP1.1-R	GTTTGAAGACGAAAACTTCTGTAGC
Primers for *Gus* detection in the transgenic plants	*Gus*-F	AGTGTACGTATCACCGTTTGTGTGAAC
	Gus-R	ATCGCCGCTTTGGACATACCATCCGTA
Primers for qRT-PCR of *TaRLP1.1*	*TaRLP1.1*-qPCR-F	AGCTTTGTGGTTCACCTTTAGA
	TaRLP1.1-qPCR-R	CTCGGAGCCTCTCGGAGCAGAC
Semi-quantitative RT-PCR primers for *TaPRs* gene	*TaPR1*-F（AAK60565）	GAGAATGCAGACGCCCAAGC
	TaPR1-R（AAK60565）	CTGGAGCTTGCAGTCGTTGATC
	TaPR2-F（DQ090946）	GCAGCTCTACAGGTCCAAGG
	TaPR2-R（DQ090946）	CGGCGATGTACTTGATGTTG
	TaPR5-F（FG618781）	CAAGCAGTGGTATCAACGCAGAG
	TaPR5-R（FG618781）	GTGAAGCCACAGTTGTTCTTGATG
Primers for the internal control gene	*Tubulin*-RT-F	AGAACACTGTTGTAAGGCTCAAC
	Tubulin-RT-R	GAGCTTTACTGCCTCGAACATGG
	18sRNA-RT-F	AACACTTCACCGGACCATTCA
	18sRNA-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.