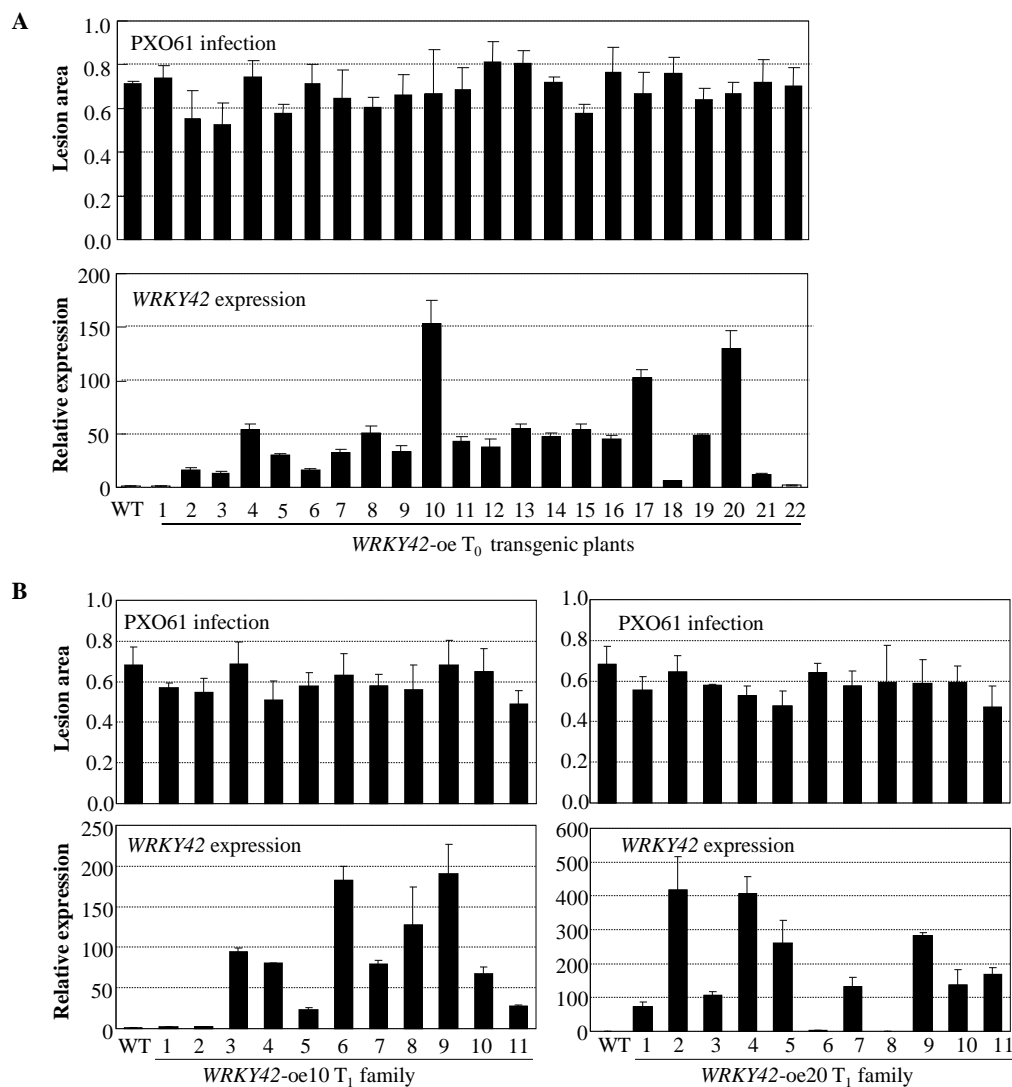


The WRKY45-2–WRKY13–WRKY42 Transcriptional Regulatory Cascade Is Required for Rice Resistance to Fungal Pathogen

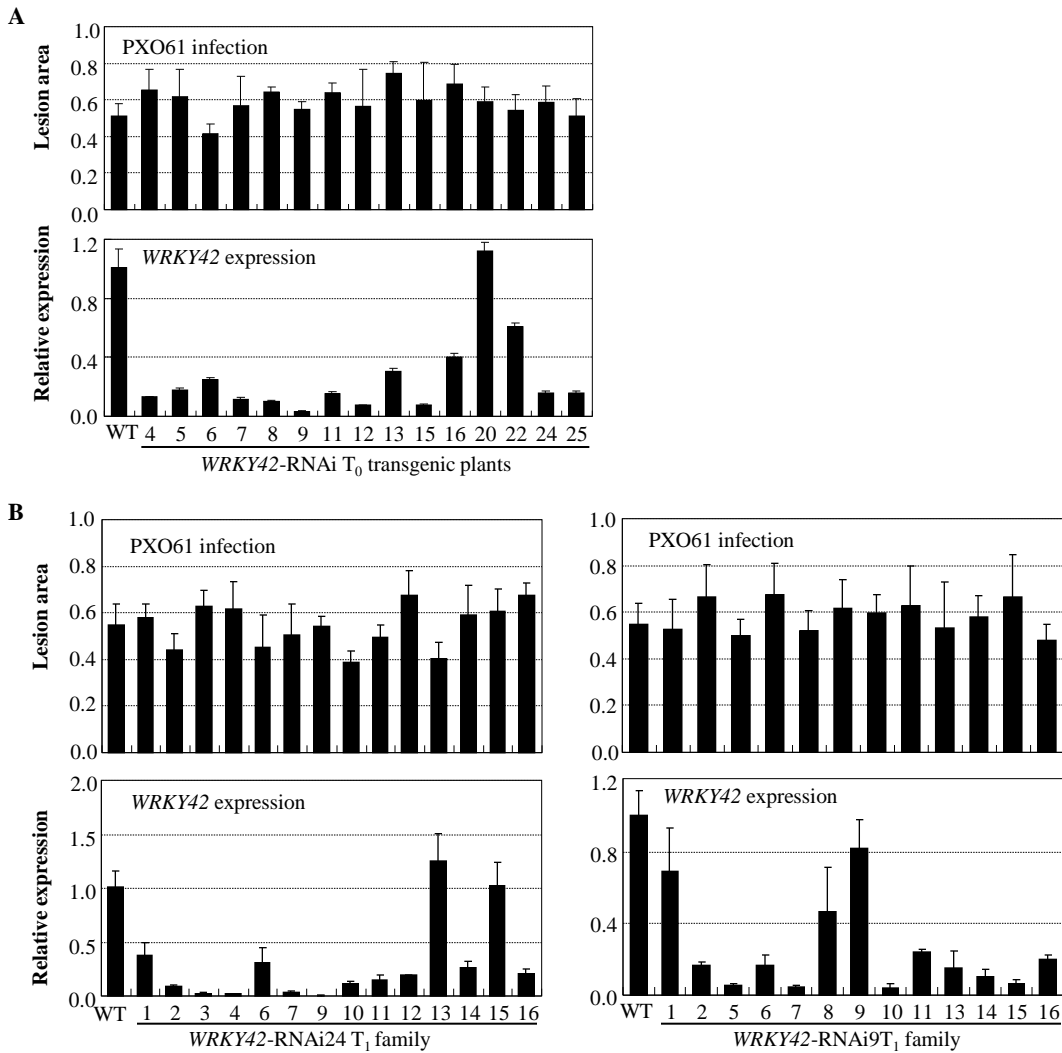
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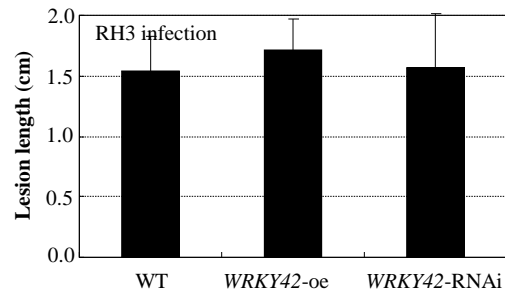
Supplemental Data



Supplemental Figure S1. WRKY42-overexpressing (oe) plants at both T₀ (A) and T₁ (B) generations showed no obvious difference from wild-type (WT; Mudanjiang 8) plants in response to the infection of *Xoo* strain PXO61. Plants were inoculated with PXO61 at the booting (panicle development) stage. Data represent mean (three replicates from one plant for gene expression and two to five replicates from one plant for disease area) \pm standard deviation.



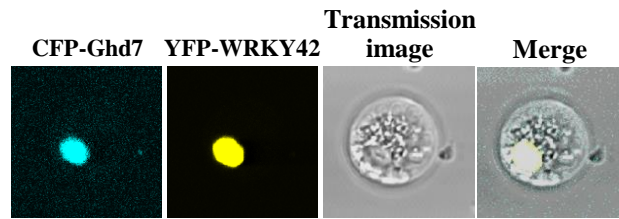
Supplemental Figure S2. *WRKY42*-RNAi plants at both T₀ (A) and T₁ (B) generations showed no obvious difference from wild-type (WT; Mudanjiang 8) plants in response to the infection of *Xoo* strain PXO61. Plants were inoculated with PXO61 at the booting stage. Data represent mean (three replicates from one plant for gene expression and two to five replicates from one plant for disease area) \pm standard deviation.



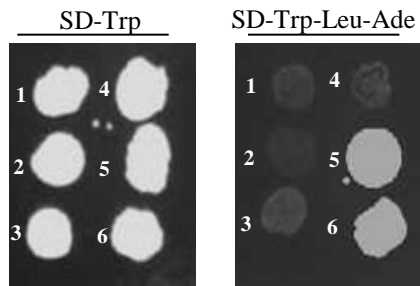
Supplemental Figure S3. *WRKY42*-transgenic plants showed no obvious difference from wild-type (WT; Mudanjiang 8) plants in response to the infection of *Xoc* strain RH3. Plants were inoculated with RH3 at the booting stage. Data represent mean (nine to 18 replicates from three to six plants) \pm standard deviation. *WRKY42*-oe, homozygous T₃ line D153UM20-5; *WRKY42*-RNAi, homozygous T₃ line D153RM9-6.



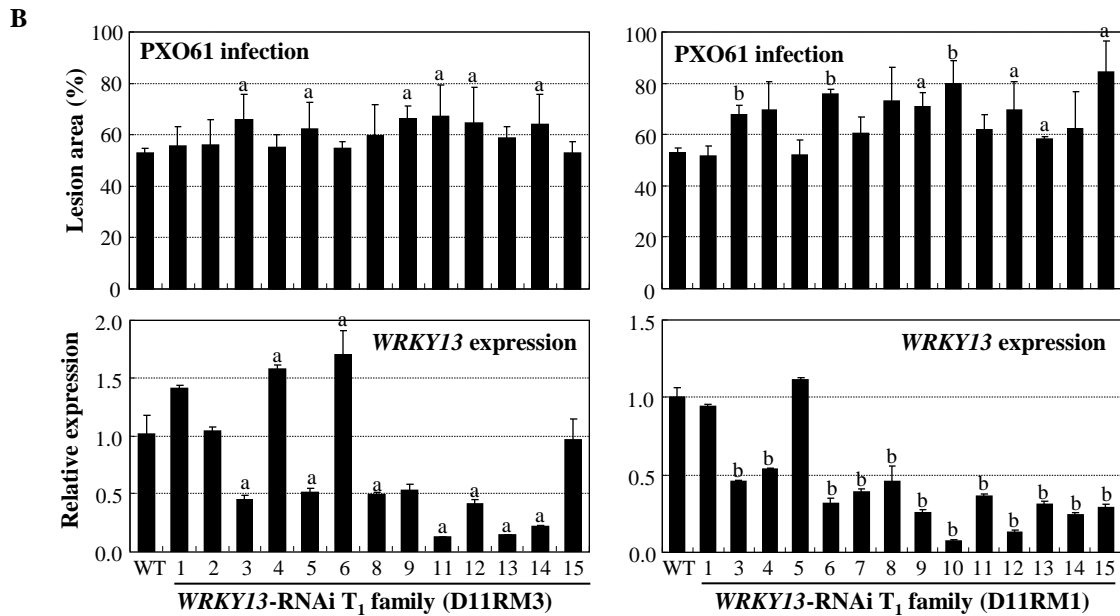
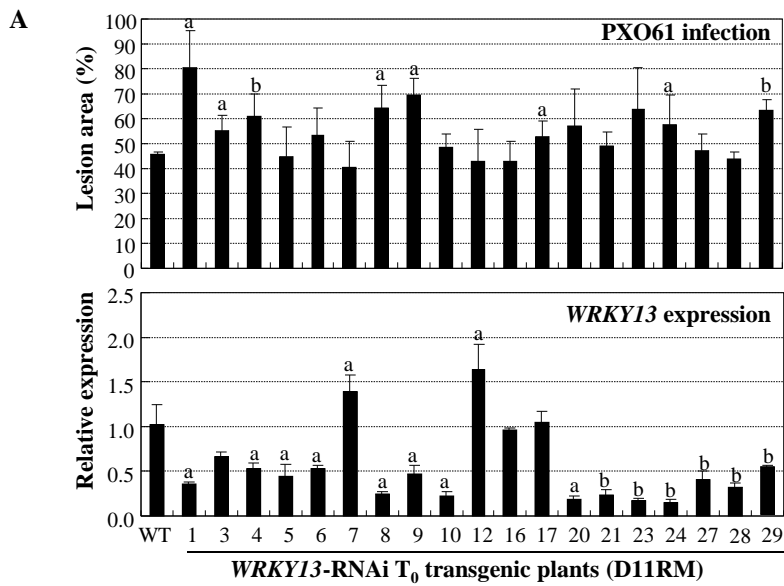
Supplemental Figure S4. The schematic diagram of rice WRKY42 protein structure. NLS, nuclear localization signal; WRKY, WRKY domain; zinc finger, zinc finger motif; X, any amino acid.



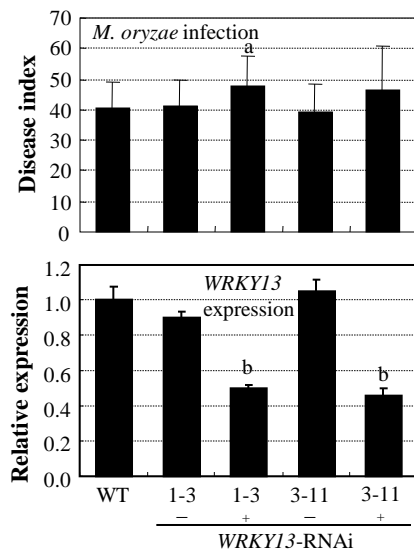
Supplemental Figure S5. WRKY42 co-localized with transcription factor Ghd7 in the nucleus of rice protoplast. Rice Ghd7 has been used as a marker since it was reported as a transcription factor localized in the nucleus.



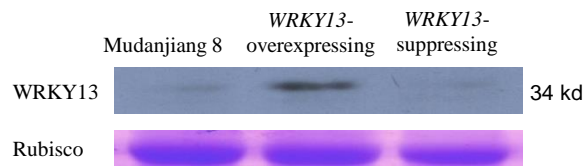
Supplemental Figure S6. WRKY42 displayed no transactivation activity as compared to the positive control OsbZIP23. The transactivation activity was analyzed by growing yeast cells carrying transgene on plates lacking tryptophan (Trp), leucine (Leu), and adenine (Ade). 1, complete WRKY42; 2, the N terminal part of WRKY42; 3, the C terminal part of WRKY42; 4, negative control (empty pGBKT7 vector); 5 and 6, positive control (rice transcription factor OsbZIP23).



Supplemental Figure S7. Increased susceptibility to *Xoo* is associated with suppressed expression of *WRKY13*. Rice plants were inoculated with *Xoo* strain PXO61 at the booting stage. Bars represent mean (three to five replicates for lesion area and three replicates for gene expression) \pm standard deviation. The “a” and “b” indicate that a significant difference between transgenic plants and wild-type (WT) Mudanjiang 8 was detected at $P < 0.05$ and $P < 0.01$, respectively. A, Increased susceptibility to *Xoo* strain PXO61 was associated with suppression of *WRKY13* in *WRKY13*-RNAi plants (T₀ generation). The correlation coefficient was -0.463 (significant at $\alpha = 0.05$; $n = 19$). B, Increased susceptibility to *Xoo* strain PXO61 was associated with suppression of *WRKY13* in two *WRKY13*-RNAi T₁ families. The correlation coefficients of the two families were -0.77 (significant at $\alpha = 0.01$; $n = 13$) and -0.67 (significant at $\alpha = 0.01$; $n = 14$), respectively.



Supplemental Figure S8. Modulating *WRKY13* expression influenced rice response to *M. oryzae* infection. *WRKY13*-suppressing (RNAi) plants (T_2 generation) were inoculated with *M. oryzae* isolate N2-2 at the three-leaf to four-leaf stage. WT, wild-type (Mudanjiang 8); +, positive transgenic plant; -, negative transgenic plant. Bar represents mean (three replicates for gene expression and 16 to 21 plants for disease index) \pm standard deviation. Increased susceptibility of *WRKY13*-RNAi plants to *M. oryzae* was associated with suppressed expression of *WRKY13*. The “a” and “b” indicate that a significant difference between transgenic plants and WT Mudanjiang 8 was detected at $P < 0.05$ and $P < 0.01$, respectively.



Supplemental Figure S9. The specificity of anti-WRKY13 antibody was examined using WRKY13-overexpressing and *WRKY13*-suppressing plants.

-1000 TTATGAAAATGGCCTAAATAACTTCGTTGCCCTATATTTATGGAAATAATGAGTCGATAATGTACACTCT

-930 TTGATTAAAAAACTCGTTTTAGAAATCTGTAGTCAATCTGAGTTAATTTTGATCATAATTATTGTTTTG
WBOX11 N3F WBOX10

-860 ACTTATAGCGTTTGGATATAGGTAAATTACATGGAATAACATAGCCAAGTTTGATACTGTATAAGAAAAGC

-790 TTCTATAATACTCTTATTATACTTTTTTCGACTATTTAAAAGATATGTAAAATAATTATGTAATCAAAAAT

-720 ATGCCTTATTGCCTTTGAAAATTCAAACAATATTCTTTTGTGACTAGATCAAGCCGTAGTGTTTATTTGG

-650 CAAGAAAAAAGTAAATATAAAAAGTCACCTAGGAGTTTTAAAAAAGCTTTTAATTTTTTTGGCAGGTAAC

-580 TAATATTTTAAAGATTTGAGAAGACAGATATAACTTATGTTTTGAATCGCTGGTATATCATACTGTTATG
N3R N2F

-510 TATACACAGATTACTCAAAATTGTTGTACTGTCAAAAAAAAAACAACAAAAGATATCACCTCACATAAA
WBOX9

-440 CACACTGGATCGCTGTTTCCTCCACGTGGTGCATTGTCCTTGGCTAATCACGGTCAAACTGCTTCGGTCA
WBOX8 N2R WBOX7 WBOX6 WBOX5

-370 AACTTCACGTCCACGTAAAGGAACCGTAAACATCAGCCCGTTCGTTCGGCCGAGGGCGCACCACCGGCCG
N1F

-300 GCCGGCCTGCTAATCCTCGCCGTGCAATCGCCCCCCCACGGGCACCAGGTAACCCCTTCCCCGCCGTCCG

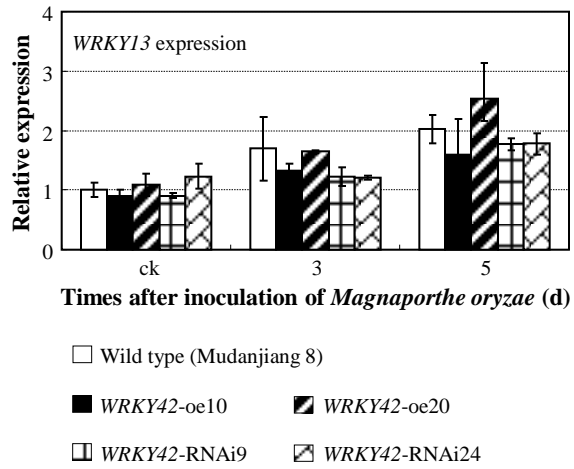
-230 ATCGATCCAGCCGTCTCCCTCACTCGCTTTTTGACTCGAACCAGTCCGCTCTCTGACCGGTGCGCGTCCG
WBOX4 WBOX3 WBOX2

-160 TCGCGTCGCGCCCCCTACAAACCCGCCCTCTCCCGCCGTTCTACGTTTCCCCCATTCGGTTTACGCGCG

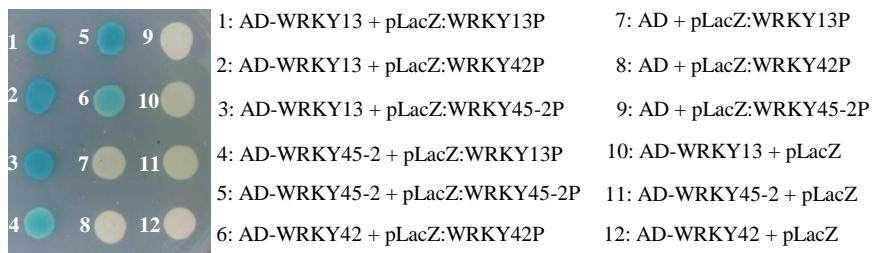
-90 AGCGCGCTACGATACACGACGCGTACGTACGTCCCAGTTCTTGGCTAAGCTTCAATTTTGCTTTGCTTGCT
WBOX1

-20 TCTCGCCGCGATCGCGCGC⁻¹ATG
N1R

Supplemental Figure S10. The promoter region of rice *WRKY42*. The nucleotide immediately upstream of the translation start codon is numbered “-1”. Arrows indicate the positions of PCR primers used for chromatin immunoprecipitation (ChIP) assay. The putative W or W-like boxes for WRKY protein binding in the segments for ChIP assay are underlined and named WBOX1 to WBOX11.



Supplemental Figure S11. Transcriptionally modulating *WRKY42* did not influence *WRKY13* expression. Plants were inoculated with *M. oryzae* isolate N2-2 at the three-leaf to four-leaf stage. Data represent mean (three replicates) \pm standard deviation. ck, before inoculation.



Supplemental Figure S12. WRKY45 binds to the *WRKY13* promoter, and WRKY45-2 and WRKY42 bind to their own promoters, analyzed by yeast one-hybrid assays. The interactions were assessed by examination of the β -galactosidase (LacZ) activity in yeast cells. 1, interaction between WRKY13 and *WRKY13* promoter (P); 2, interaction between WRKY13 and *WRKY42* promoter; 3, interaction between WRKY13 and *WRKY45-2* promoter; 4, interaction between WRKY45-2 and *WRKY13* promoter; 5, interaction between WRKY45-2 and *WRKY45-2* promoter; 6, interaction between WRKY13 and *WRKY42* promoter; 7, interaction between pB42AD empty vector and *WRKY13* promoter; 8, interaction between pB42AD empty vector and *WRKY42* promoter; 9, interaction between pB42AD empty vector and *WRKY45-2* promoter; 10, interaction between WRKY13 and p8op-lacZ empty vector; 11, interaction between WRKY45-2 and p8op-lacZ empty vector; 12, interaction between WRKY42 and p8op-lacZ empty vector.