Rice

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OsRLCK109 Negatively Regulates Rice Immunity by Promoting *OsWRKY76's* Suppression of *PR1b*

Yue Zhang[1,2†], Yiwei Kang[1,5†], Guanqi Wang[1], Wenjing Duan[1], Yingxin Zhang[1], Weixun Wu[1], Daibo Chen[1], Yongbo Hong[1,3], Lianping Sun[1], Xihong Shen[1], Xiaodeng Zhan[1], Shihua Cheng[1], Lassois Ludivine[2*], Qunen Liu[1*] and Liyong Cao[1,3,4*]

Receptor-like cytoplasmic kinases (RLCKs) are essential components of plant signaling pathways, orchestrating various cellular processes associated with biotic and abiotic stress responses, as well as growth, development, and reproduction. The rice genome encompasses numerous OsRLCK members, totaling 379 identified entities (Vij et al. 2008). Notably, members of the RLCK-VII subfamily have been extensively documented to participate in plant immune responses. OsRLCK176 and OsRLCK118 have been reported to interact with the primary NADPH oxidase OsRbohB, which is responsible for ROS production in the rice plasma membrane (Fan et al. 2018). This interaction influences the regulation of reactive oxygen species and the immune response in rice. Additionally, it has been observed that OsCERK1 transmits immune signals from the cell membrane by phosphorylating OsRLCK185. The latter plays a role in the rice immune response triggered by chitin and peptidoglycan through the MAPK signaling cascade involving OsMAPKKKε-OsMAPKK4-OsMAPK3/6 (Wang et al. 2017).

Transcription factors can selectively bind specific DNA sequences, thereby playing an essential role in regulating gene expression within the cell. The WRKY transcription factors were initially considered to be unique to plants; however, subsequent research revealed that certain WRKY transcription factors are also present in protozoa and fungi (Song et al. 2023). These factors can bind to W-box cis-elements (TGACCA/TGACCT) within the promoters of target genes, thereby influencing their transcriptional activity (Javed and Gao 2023). WRKY transcription factors in rice have been documented to participate in a variety of biological processes, including responses to biotic stressors. *OsWRKY36* suppresses the expression of *OsPAL1* and *OsPAL6*. Knocking out *OsWRKY36* elevates lignin biosynthesis and increases the thickness of the leaf sheath sclerenchyma, thereby improving resistance to various pests and pathogens (Liu et al. 2025).*OsWRKY62* positively regulates the immune response mediated by *Pi9*. The knockout of *OsWRKY62* is expected to reduce rice immunity to the rice blast

†Yue Zhang and Yiwei Kang have contributed equally to this work.

*Correspondence:
Lassois Ludivine
Ludivine.Lassois@uliege.be
Qunen Liu
liuqunen@caas.cn
Liyong Cao
caoliyong@caas.cn
[1]State Key Laboratory of Rice Biology and Breeding, National Rice Research Institute, Hangzhou 311400, China
[2]Plant Genetics and Rhizosphere Processes Laboratory, Gembloux Agro-Bio Tech, TERRA Teaching and Research Center, University of Liege, Gembloux B-5010, Belgium
[3]National Nanfan Research Institute (Sanya), Chinese Academy of Agricultural Sciences, Sanya 572025, China
[4]Key Laboratory of Northern Japonica Rice Research of Heilongjiang Province, Baoqing 155600, China
[5]National Key Laboratory of Crop Genetic Improvement and National Center of Plant Gene Research, Huazhong Agricultural University, Wuhan 430070, Hubei, China

fungus (Shi et al. 2023). OsWRKY67 directly binds to W-box elements in the promoters of PR1a and PR10, thereby activating their transcription and, consequently, regulating rice blast resistance (Liu et al. 2018). This clearly indicates that WRKY transcription factors play a significant role in rice immunity.

Post-translational modification (PTM) is a fundamental process in regulating protein function. WRKY transcription factors have been documented to have their protein activities modulated by PTM. OsWRKY45 undergoes proteasome-mediated degradation following ubiquitination in the absence of pathogen infection, thereby preventing the premature activation of the defense response (Matsushita et al. 2013). OsWRKY31 can be phosphorylated by OsMPK3, OsMPK4, and OsMPK6, a process that enhances its DNA-binding activity and confers increased resistance to rice blast disease (Wang et al. 2023). OsWRKY76 has been reported to contribute to rice blast resistance and to drought and cold stress responses (Xu et al. 2022; Zhang et al. 2022, 2023). However, there are currently no reports indicating that it is subject to PTM.

In our previous study, we found that OsRLCK109 functions as a negative regulator of rice blast disease (Zhang et al. 2019). Considering that WRKY transcription factors are instrumental in conferring resistance to rice blast disease in rice, an investigation was conducted to examine the expression of certain WRKY transcription factors within the *OsRLCK109* knockout line (*Korlck109*). Notably, certain positive regulators associated with rice blast resistance exhibited elevated expression levels in *Korlck109* (Figure S1), aligning with its observed enhanced resistance to rice blast. Interestingly, the expression of a specific WRKY transcription factor, OsWRKY76, previously reported to be overexpressed and associated with reduced rice blast resistance (Yokotani et al. 2013), was also increased (Fig. 1A). This observation appears to contradict the disease-resistant phenotype of *Korlck109*. We hypothesize that a potential interaction may exist between *OsRLCK109* and *OsWRKY76*. Subsequently, we developed some experiments to verify this hypothesis. Initially, the interaction was verified in a yeast system, which demonstrated that yeast cells containing the AD-OsWRKY76 and BD-OsRLCK109 plasmids could grow on a selective medium (Figure S2), indicating an interaction between OsWRKY76 and OsRLCK109 in yeast. To further substantiate this interaction, both in vivo and in vitro experiments were conducted. The in vivo co-immunoprecipitation (Co-IP) experiments revealed that OsWRKY76-GFP could be immunoprecipitated alongside OsRLCK109-Myc, whereas the control GFP protein did not (Fig. 1B), thereby confirming an interaction within rice cells. Additionally, separate expression of GST-OsRLCK109 and His-OsWRKY76 proteins in vitro, followed by pull-down assays, showed that GST-OsRLCK109 could capture His-OsWRKY76. At the same time, the control failed to do so (Fig. 1C), indicating an in vitro interaction between OsWRKY76 and OsRLCK109. In conclusion, OsWRKY76 and OsRLCK109 physically interact.

In *Arabidopsis thaliana*, the cytoplasmic receptor kinase BIK1 can phosphorylate WRKY transcription factors, thereby modulating defense responses. An in vitro phosphorylation experiment was performed to determine whether OsRLCK109 can phosphorylate OsWRKY76. The results showed that GST-OsRLCK109 could phosphorylate His-OsWRKY76, while GST alone could not (Fig. 1D). Additionally, it was observed that OsRLCK109 is capable of auto-phosphorylation (Fig. 1D and S3).

Additionally, we generated a functionally deficient mutant of OsWRKY76 (*Kowrky76*) using CRISPR/Cas9 and subsequently assessed its resistance to rice blast disease. Inoculation assay results showed that knocking out *OsWRKY76* significantly enhanced rice's resistance to blast disease. (Fig. 1E, F, G). We measured the expression levels of pathogenesis-related (PR) genes in the mutants and found that *PR1a*, *PR1b*, and *PR10a* were significantly elevated in the mutants (Figure S4). This might be the reason for the increase in mutant resistance. In conclusion, *OsWRKY76*, similar to *OsRLCK109*, functions as a negative regulatory factor in rice blast resistance.

It has been reported that WRKY transcription factors can bind the W-box in the promoter of PR genes (Liu et al. 2018), thereby modulating their expression. Based on the observed increase in PR gene expression levels in *Kowrky76* and the utilization of the PlantCARE online tool for promoter analysis, it was revealed that all these PR genes contain the W-box (Figure S5). We hypothesize that OsWRKY76 can directly bind to the W-box elements in the promoters of these PR genes and regulate their expression. First, the yeast one-hybrid experiment (Y1H) was used to verify our hypothesis. The results demonstrated that OsWRKY76 directly binds to the *PR1b* promoter (Fig. 1H, I), whereas it did not bind to the *PR1a* and *PR10a* promoters (Figure S6). In addition, we conducted a transient luciferase transcriptional activity assay in rice protoplasts. The results showed that OsWRKY76 significantly reduced the activity of PR1b-p2-190Luc (Fig. 1J, S7). PR1b-p2 contains two W-boxes. Subsequently, we conducted an electrophoretic mobility shift assay (EMSA) to test the in vitro binding. The results showed that both W-boxes bound OsWRKY76 in vitro (Fig. 1K). In summary, OsWRKY76 directly binds to two W-boxes in the *PR1b* promoter and inhibits *PR1b* expression.

To evaluate whether OsRLCK109 affects the transcriptional activity of OsWRKY76, a dual-luciferase reporter assay was utilized to determine whether OsRLCK109

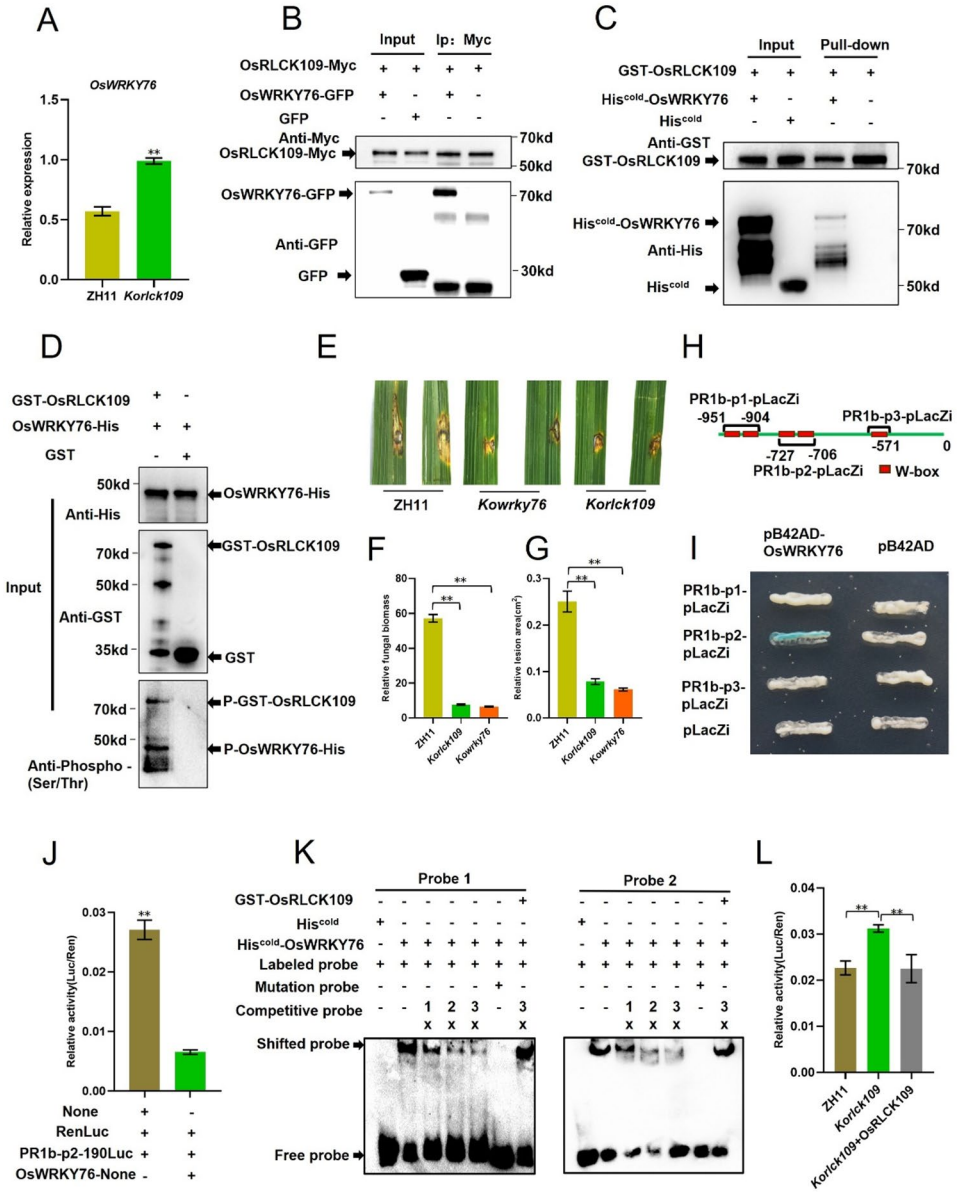

Fig. 1 The OsRLCK109-OsWRKY76-PR1b module regulates rice immunity. **A** The expression levels of *OsWRKY76* in the wild-type ZH11 and *Korlck109*. Data are presented as mean ± SD, $n = 3$. Statistical significance was determined using Student's t-test, with $^{**}p < 0.01$. **B** OsRLCK109 and OsWRKY76 interact within rice protoplasts. Plasmids encoding OsRLCK109-Myc and OsWRKY76-GFP fusion proteins were introduced into rice protoplasts. After a 20-hour incubation period, total proteins were extracted for the Co-IP assay. GFP protein served as a control. **C** OsRLCK109 and OsWRKY76 interact in vitro. The GST-OsRLCK109 and His^{cold}-OsWRKY76 fusion proteins were expressed and purified in vitro, and then used for the Pull-down experiment. **D** OsRLCK109 phosphorylates OsWRKY76 in vitro. **E**, **F**, **G** The performance of ZH11, *Korlck109*, and *Kowrky76* after being inoculated with *Magnaporthe oryzae* for 7 days. Data shown as mean ± SD, $n = 3$, $^{**}p < 0.01$, Student's t-test. **H** and **I** Y1H assay of OsWRKY76 binding to the promoter of *PR1b*. **J** Luc transient transcriptional activity assay in rice protoplast. Data shown as mean ± SD, $n = 3$, $^{**}p < 0.01$, Student's t-test. **K** The EMSA analysis demonstrated that OsWRKY76 interacts with the PR1b-p2. Additionally, OsRLCK109 can augment the binding affinity when a competing probe is introduced. Probe 1 and probe 2 were synthesized based on distinct W-box sequences situated on the *PR1b* promoter. **L** The inhibitory effect of OsWRKY76 on the luciferase transcriptional activity driven by the *PR1b* promoter was observed in protoplasts of both ZH11 and *korlck109*. Additionally, the OsRLCK109 protein was expressed in the *korlck109* background to complement the loss of endogenous OsRLCK109. Data shown as mean ± SD, $n = 3$, $^{**}p < 0.01$, Student's t-test

can enhance the transcriptional inhibitory effect of OsWRKY76 to *PR1b*. The results demonstrated that, within rice protoplasts, the transcriptional activity of *PR1b* in the absence of OsRLCK109 exceeds that of the wild-type ZH11. Subsequently, upon the manual addition of OsRLCK109, the transcriptional activity of *PR1b* was diminished relative to the control group lacking OsRLCK109. (Fig. 1L). Similarly, in the EMSA experiment, when the reaction system was additionally supplemented with OsRLCK109 and contained a

competitive probe, the binding of OsWRKY76 to the labeled probe was enhanced compared to not adding OsRLCK109 (Fig. 1K lane 7 vs. lane 6). This indicates that OsRLCK109 can enhance OsWRKY76's binding to the *PR1b* promoter. Previous studies have shown that OsWRKY76 acts as a transcriptional activator of *OsDREB1E* (Zhang et al. 2023) and as a repressor of *RSOsPR10* (Yamamoto et al. 2018). We further examined the expression levels of these genes in the *Korlck109* mutant. The findings revealed that *OsDREB1E* levels decreased, while *RSOsPR10* levels increased in *Korlck109* (Figure S8). Interestingly, it was previously observed that the transcriptional level of *OsWRKY76* in *Korlck109* was elevated (Fig. 1A); however, in *Korlck109*, the expression of these target genes did not correspond to the anticipated results of OsWRKY76 overexpression; instead, it aligned with the phenotype indicative of *OsWRKY76* being functionally deficient. This indicates that the absence of *OsRLCK109*, despite its capacity to augment OsWRKY76 expression, indeed reduces OsWRKY76's transcriptional ability, encompassing its functions in both repression and activation of target genes. Based on the above evidence, the interaction between OsRLCK109 and OsWRKY76 enhances OsWRKY76's transcriptional regulatory capacity. In other words, the expression of target genes regulated by OsWRKY76 requires OsRLCK109.

In the rice immune pathway, OsRLCKs have been extensively documented to mediate phosphorylation signals originating from pattern recognition receptors, thereby regulating multiple signaling nodes, including the MAPK cascade, NADPH oxidase, calcium ion channels, and heterotrimeric G proteins, to coordinate diverse immune responses. There are only a few reports about RLCK directly regulating WRKY transcription factors and, as a result, affecting the downstream PR genes that play a role in boosting immunity. This study has demonstrated that OsRLCK109 interacts with OsWRKY76 and phosphorylates it. Subsequent investigations have shown that OsWRKY76 can bind to the *PR1b* promoter and inhibit its expression. The in vitro experimental results indicate that OsRLCK109 can enhance the binding efficacy of OsWRKY76 to the *PR1b* promoter. Additionally, in vivo experiments suggest that the loss of OsRLCK109 function diminishes OsWRKY76-mediated transcriptional repression of *PR1b*. These findings collectively provide evidence that OsRLCK109 actively participates in regulating the transcriptional repression of *PR1b* by OsWRKY76. Although the phosphorylation of OsWRKY76 by OsRLCK109 has been observed in vitro, it remains uncertain whether RLCK109 exerts its regulatory role through this phosphorylation. Further research is therefore required. In conclusion, this study uncovers a novel immune regulatory mechanism in rice, namely OsRLCK109-OsWRKY76-PR1b, thereby contributing to the understanding of the complex immune system in rice.

Abbreviations

PTM	Post-translational modification
RLCK	Receptor-like cytoplasmic kinase
Co-IP	co-immunoprecipitation
Y1H	Yeast one-hybrid
PR	Pathogen-related
EMSA	Electrophoretic mobility shift assay

Supplementary Information

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Supplementary Material 1

Author Contributions

Y.Z. and Y.W.K. designed the research and conducted the experiments; G.Q.W. and W.J.D. performed the pathogen inoculation; Y.X.Z., W.X.W., D.B.C., Y.B.H., L.P.S., X.H.S., X.D.Z., S.H.C. participated in the design of the experiments and critical discussions; Q.E.L., L.L., L.Y.C. supervised the research; Y.Z. wrote the manuscript with contributions from all co-authors.

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Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Competing Interests

The authors declare no competing interests.

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References

Fan J, Bai P, Ning Y, Wang J, Shi X, Xiong Y, Zhang K, He F, Zhang C, Wang R, Meng X, Zhou J, Wang M, Shirsekar G, Park CH, Bellizzi M, Liu W, Jeon J, Xia Y, Shan L, Wang G (2018) The Monocot-Specific Receptor-like kinase SDS2 controls cell death and immunity in rice. Cell Host Microbe 23:498–510e5

Javed T, Gao S (2023) WRKY transcription factors in plant defense. Trends Genet 39:787–801

Liu Q, Li X, Yan S, Yu T, Yang J, Dong J, Zhang S, Zhao J, Yang T, Mao X, Zhu X, Liu B (2018) OsWRKY67 positively regulates blast and bacteria blight resistance by direct activation of PR genes in rice. BMC Plant Biol 18:257–257

Liu D, He J, Li Q, Zhang X, Wang Y, Sun Q, Wang W, Zhang M, Wang Y, Xu H, Fang L, Jiang L, Liu S, Chen L, Tian Y, Liu X, Wang R, Zhang Z, Chern M, Dong X, Wang H, Liu Y, Ronald PC, Wan J (2025) A WRKY transcription factor confers broad-spectrum resistance to biotic stresses and yield stability in rice. Proc Natl Acad Sci U S A 122:e2411164122

Matsushita A, Inoue H, Goto S, Nakayama A, Sugano S, Hayashi N, Takatsuji H (2013) Nuclear ubiquitin proteasome degradation affects WRKY45 function in the rice defense program. Plant J 73:302–313

Shi X, Xiong Y, Zhang K, Zhang Y, Zhang J, Zhang L, Xiao Y, Wang G, Liu W (2023) The ANIP1-OsWRKY62 module regulates both basal defense and Pi9-mediated immunity against Magnaporthe oryzae in rice. Mol Plant 16:739–755

Song H, Cao Y, Zhao L, Zhang J, Li S (2023) Review: WRKY transcription factors: Understanding the functional divergence. Plant Science: Int J Experimental Plant Biology 334:111770

Vij S, Giri J, Dansana PK, Kapoor S, Tyagi AK (2008) The receptor-like cytoplasmic kinase (OsRLCK) gene family in rice: organization, phylogenetic relationship, and expression during development and stress. Mol Plant 1:732–750

Wang C, Wang G, Zhang C, Zhu P, Dai H, Yu N, He Z, Xu L, Wang E (2017) OsCERK1-Mediated Chitin perception and immune signaling requires Receptor-like cytoplasmic kinase 185 to activate an MAPK cascade in rice. Mol Plant 10:619–633

Wang S, Han S, Zhou X, Zhao C, Guo L, Zhang J, Liu F, Huo Q, Zhao W, Guo Z, Chen X (2023) Phosphorylation and ubiquitination of OsWRKY31 are integral to OsMKK10-2-mediated defense responses in rice. Plant Cell 35:2391–2412

Xu X, Wang H, Liu J, Han S, Lin M, Guo Z, Chen X (2022) OsWRKY62 and OsWRKY76 interact with importin alpha1s for negative regulation of defensive responses in rice nucleus. Rice (N Y) 15:12

Yamamoto T, Yoshida Y, Nakajima K, Tominaga M, Gyohda A, Suzuki H, Okamoto T, Nishimura T, Yokotani N, Minami E, Nishizawa Y, Miyamoto K, Yamane H, Okada K, Koshiba T (2018) Expression of RSOsPR10 in rice roots is antagonistically regulated by jasmonate/ethylene and Salicylic acid via the activator OsERF87 and the repressor OsWRKY76, respectively. Plant Direct 2:e00049–e00049

Yokotani N, Sato Y, Tanabe S, Chujo T, Shimizu T, Okada K, Yamane H, Shimono M, Sugano S, Takatsuji H, Kaku H, Minami E, Nishizawa Y (2013) WRKY76 is a rice transcriptional repressor playing opposite roles in blast disease resistance and cold stress tolerance. J Exp Bot 64:5085–5097

Zhang Y, Liu Q, Zhang Y, Chen Y, Yu N, Cao Y, Zhan X, Cheng S, Cao L (2019) LMM24 encodes Receptor-Like cytoplasmic kinase 109, which regulates cell death and defense responses in rice. Int J Mol Sci 20

Zhang M, Zhao R, Huang K, Huang S, Wang H, Wei Z, Li Z, Bian M, Jiang W, Wu T, Du X (2022) The OsWRKY63-OsWRKY76-OsDREB1B module regulates chilling tolerance in rice. Plant J 112:383–398

Zhang M, Zhao R, Huang K, Wei Z, Guo B, Huang S, Li Z, Jiang W, Wu T, Du X (2023) OsWRKY76 positively regulates drought stress via OsbHLH148-mediated jasmonate signaling in rice. Front Plant Sci 14:1168723–1168723

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