APIP5 functions as a transcription factor and an RNA-binding protein to modulate cell death and immunity in rice

Fan Zhang1, Hong Fang1, Min Wang1, Feng He1, Hui Tao1, Ruyi Wang1, Jiawei Long1, Jiyang Wang1, Guo-Liang Wang2,* and Yuese Ning1,*

1State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China and 2Department of Plant Pathology, The Ohio State University, Columbus, OH 43210, USA

Received December 30, 2021; Revised April 08, 2022; Editorial Decision April 19, 2022; Accepted April 20, 2022

ABSTRACT

Many transcription factors (TFs) in animals bind to both DNA and mRNA, regulating transcription and mRNA turnover. However, whether plant TFs function at both the transcriptional and post-transcriptional levels remains unknown. The rice (Oryza sativa) bZIP TF AVRPIZ-T-INTERACTING PROTEIN 5 (APIP5) negatively regulates programmed cell death and blast resistance and is targeted by the effector AvrPiz-t of the blast fungus Magnaporthe oryzae. We demonstrate that the nuclear localization signal of APIP5 is essential for APIP5-mediated suppression of cell death and blast resistance. APIP5 directly targets two genes that positively regulate blast resistance: the cell wall-associated kinase gene OsWAK5 and the cytochrome P450 gene CYP72A1. APIP5 inhibits OsWAK5 expression and thus limits lignin accumulation; moreover, APIP5 inhibits CYP72A1 expression and thus limits reactive oxygen species production and defense compounds accumulation. Remarkably, APIP5 acts as an RNA-binding protein to regulate mRNA turnover of the cell death- and defense-related genes OsLSD1 and OsRac1. Therefore, APIP5 plays dual roles, acting as TF to regulate gene expression in the nucleus and as an RNA-binding protein to regulate mRNA turnover in the cytoplasm, a previously unidentified regulatory mechanism of plant TFs at the transcriptional and post-transcriptional levels.

INTRODUCTION

Programmed cell death (PCD) is a ubiquitous genetically regulated process in prokaryotes and eukaryotes. In plants, PCD occurs during germination, growth, development and senescence, or under abiotic or biotic stresses (1). For example, PCD has important functions in disease resistance: the hypersensitive response (HR), a plant-specific form of PCD, occurs during effector-triggered immunity (ETI) (2) and pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) (3,4). The PCD and plant immunity pathways are intertwined and defense signal transduction cascades depend on several layers of gene regulation at the transcriptional, translational and post-translational levels (5,6).

Transcription factors (TFs) play pivotal roles in regulating the transcriptional reprogramming of plant immune responses and the coordination of defense signaling networks with other stress and growth pathways (7). Most TFs localize to the nucleus where they bind to DNA and selectively activate or suppress defense gene expression (7). The cytoplasmic-nuclear trafficking of activated TFs is also essential for their specific roles in different cellular compartments (8,9). For example, the NAC TFs NAC Targeted by Phytophthora (NTP)1 and NTP2 were released from the endoplasmic reticulum membrane into the nucleus in potato (Solanum tuberosum) following treatment with P. infestans culture filtrate, while the RXLR effector Pi03192 directly interacted with and inhibited their re-localization to prevent defense activation (10). Similarly, the membrane-tethered NAC TF BnaNAC60 from oilseed rape (Brassica napus) was translocated into the nucleus in response to oxidative stress, inducing reactive oxygen species (ROS) accumulation and HR-like cell death, in transgenic tobacco (Nicotiana tabacum) (11).

Some TFs are re-translocated into the cytoplasm to regulate PCD or other cellular processes during pathogen infection. For example, when the basic leucine zipper (bZIP) TF bZIP10 was co-expressed with its interactor LESIONS SIMULATING DISEASE 1 (LSD1) in Arabidopsis thaliana, its binding activity was weakened and most of the bZIP10 was translocated into the cyto-
plasm, thereby enhancing LSD1-mediated cell death and antagonistically modulating RECOGNITION OF PEROSONOSPORA PARASITICA 2 (RPP2)-mediated resistance to *Hyaloperonospora parasitica* (12). However, the molecular mechanisms underlying the dynamic homeostasis of TFs in different subcellular compartments in PCD and plant immunity, and the effects of changes in TF localization, are largely unknown.

The bZIP TF family is one of the largest TF families in plants. Most bZIP TFs investigated to date localize to the nucleus, preferentially bind to *cis* elements with an ACGT core sequence in their target genes via their basic region, and form dimers via their leucine zipper region (13). In *Arabidopsis*, several bZIP TFs are known to be involved in plant immunity such as the TGACG-Binding (TGA) subfamily members in NON-EXPRESSION OF PATHOGENESIS-RELATED GENES 1 (NPR1)-mediated resistance (14) and ELONGATED HYPOCHOTYL 5 (HYS) in ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1)-dependent ROS and salicylic acid signaling (15) and defense to *Hyaloperonospora arabidopsidis* (16). Among the 89 bZIP TFs in rice (*Oryza sativa*), several are known to regulate PCD and plant immunity, but the target genes of only a few TFs have been identified. For instance, rTGGA2.1/OsbZIP63 coordinates with rice NPR1 HOMOLOG 1 (NH1) to activate the expression of CYSTEINE RECEPTOR KINASE 6 (CRK6) and CRK10 during NH1-mediated immunity against Xanthomonas oryzae pv. oryzae (Xoo) (17). OsTGA2/OsbZIP28 selectively binds to TGACGT sequences in the promoters of defense-related genes and overexpressing OsTGA2/OsbZIP28 significantly increased resistance to *Xoo* (18).

In animal systems, a significant number of TFs bind to mRNAs to regulate their turnover (19,20). These DNA and RNA binding proteins allow multiple signals to be integrated into cellular signaling networks for the fine-tuning of target gene expression and the modulation of cellular metabolism during stress. To date, no plant TF has been shown to bind to mRNA to regulate target gene expression at the post-transcriptional level. Nevertheless, a few RNA-binding proteins exhibit DNA-binding activity in plants. For example, the tandem zinc finger domain-containing CCCH proteins TZF1, C3H14 and C3H15 in *Arabidopsis* show both RNA- and DNA-binding capacity and shuttle between cytoplasmic foci and the nucleus (21–23).

In rice, the CCCH-type zinc finger protein OsLIC binds to both double-stranded DNA and single-stranded poly(A), (G) and (U) (24). However, the target genes of these CCCH proteins remain to be identified. Another example is the RNA recognition motif (RRM)-containing protein PIBP1 in rice, which not only has RNA-binding capacity but can also bind to the OsWAK14 and OsPAL1 promoters to modulate resistance to *Magnaporthe oryzae* (25).

We previously demonstrated that the rice bZIP TF AVRPIZ-T-INTERACTING PROTEIN 5 (APIP5) negatively regulates PCD and immunity against *M. oryzae*, and is directly suppressed by the *M. oryzae* effector AvrPiz-t (26). APIP5 contains a nuclear localization signal (NLS) motif and a nuclear export signal (NES) motif and mainly localizes to the cytoplasm. In the current study, we discovered that the NLS of APIP5 is essential for APIP5-mediated repression of age-dependent cell death and resistance to *M. oryzae*. Upon pathogen infection, APIP5 translocates from the cytoplasm to the nucleus and directly suppresses the expression of the defense-related genes WALL ASSOCIATED KINASE 5 (*OsWAK5*) and CYTOCHROME P450 72A1 (*CYP72A1*), thereby weakening lignin and ROS accumulation. Intriguingly, APIP5 also functions as an unconventional RNA-binding protein that directly targets and regulates the mRNA turnover of the cell death- and defense-related genes *OsLSD1* (a functional homolog of LSD1 in *Arabidopsis*) and RHO-LIKE GTPASE GENE 1 (*OsRac1*) to regulate PCD and plant immunity against *M. oryzae*. Therefore, APIP5 plays dual roles as a TF and an RNA-binding protein in cell death and defense pathways.

**MATERIALS AND METHODS**

**Plant materials and growth conditions**

All transgenic rice lines used in this study are in the Nipponbare (NPB) background. Rice plants were grown in a greenhouse for breeding and phenotypic observation. Plants used for *M. oryzae* inoculation were grown in growth chambers (26/20°C day/night, 70% humidity with a 12-h light/dark photoperiod). *Nicotiana benthamiana* plants used for transient expression were grown in a phytotron at 22°C under a 16-h light/8-h dark photoperiod.

**Blast fungus inoculation and disease resistance assay**

*Magnaporthe oryzae* isolate was cultured on oatmeal medium in the dark for 7 days at 26°C and transferred to the light for sporulation for 7–10 days before use. The spore concentration in the suspension was adjusted to approximately 5 × 10⁵ conidia/ml for punch inoculation. Six-week-old rice plants were subjected to punch inoculation as previously described (27). At 14 days after inoculation, the inoculated leaves were photographed, and relative fungal biomass was determined by DNA-based quantitative PCR as previously reported (27). All inoculation experiments were repeated at least three times independently. The primers used for PCR analysis are listed in Supplementary Table S1.

**Nuclear and cytoplasmic protein extraction**

Cell fractionation assays were performed as described previously (28). Briefly, 0.5 g samples of *GFP-APIP5* and *GFP-APIP5* plants were homogenized in liquid nitrogen and mixed with Honda buffer (25 mM Tris-HCl, pH 7.4, 10 mM MgCl₂, 0.4 M sucrose, 2.5% Ficoll 400, 5% dextran, 10 mM β-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride (PMSF), 0.5% Triton X-100 with 5 mM DTT and 1 × protease inhibitor cocktail [Roche, Cat. No. 04693132001] added before use). Each mixture was vortexed, incubated on ice for 30 min and filtered through Miracloth (Millipore, 475855). The flow-through was centrifuged at 1500 g for 5 min at 4°C, and the supernatants were collected as the cytoplasmic fraction. The pellets were washed with Honda buffer, centrifuged at 1000 g for 5 min at 4°C to pellet starch and cellular debris, and re-centrifuged at 1800 g for 5 min at
4°C to pellet the nuclei. The pellets were washed eight times with nuclear re-suspension buffer (20 mM Tris-HCl, pH 7.5, 2.5 mM MgCl₂, 25% glycerol, 0.2% Triton X-100, 5 mM β-mercaptoethanol and 1 mM PMSF, with 1× protease inhibitor cocktail [Roche, Cat. No. 0469312001] added before use). The pellets were resuspended in denatured extraction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 4 M urea and 1 mM PMSF), vortexed and centrifuged at 16,000 g at 4°C. The supernatants were collected as the nuclear fractions. Both the cytoplasmic and nuclear fractions were mixed with 4× SDS loading buffer and boiled for the immunoblot assays. GFP-APIP5 and GFP-APIP5nls were detected using anti-GFP antibody (Roche, Cat. No. 11814460001). HSP82 proteins were detected using anti-HSP antibody (Beijing Protein Innovation, AbM51099-31-PU) as a cytoplasmic marker, and histone H3 proteins were detected using anti-Histone H3 antibody (TransGen, HL102) as a nuclear marker.

Electrophoretic mobility shift assay (EMSA)

EMSA was performed as described previously (29). The recombinant proteins MBP, MBP-APIP5, MBP-APIP5nls and MBP-APIP5nes were expressed in Escherichia coli Rosetta (DE3) and purified with amylose resin (NEB, E8201) according to the manufacturer’s instructions. DNA fragments were 5′-end-labeled with 5-carboxyfluorescein (FAM). The labeled DNA probes were incubated with purified MBP, MBP-APIP5, MBP-APIP5nls and MBP-APIP5nes in a 20 μl binding reaction system (50 mM Tris-HCl, pH 7.5, 5 mM EDTA, 10 mM MgCl₂, 0.5 M NaCl, 5 mM DTT, 0.05 mg/ml poly [di-dC] and 40% glycerol) at 25°C for 30 min. For the competition assays, non-labeled competitor DNA probe was also added to the reaction. The reaction mixtures were electrophoresed at 4°C on a 6% native polyacrylamide gel in 0.5 × Tris-borate-EDTA (TBE) buffer for 60-90 min and imaged on a Typhoon 9400 imager (GE Healthcare).

For RNA EMSA, synthesized RNA fragments were 5′-end-labeled with FAM and incubated with MBP, MBP-APIP5, MBP-APIP5nls and MBP-APIP5nes. For the competition assay, unlabeled RNA fragments were added to the reactions. The reaction mixtures were also electrophoresed at 4°C on a 6% native polyacrylamide gel in 0.5 × TBE buffer and imaged on a Typhoon 9400 imager (GE Healthcare). EMSA experiments were repeated three times.

Dual-luciferase reporter assay

The ~2000-bp OsWAK5 and CYP72A1 promoter sequences were amplified from NPB genomic DNA and inserted into pGreenII 0800-LUC vector through HindIII + BamHI and SalI + SmaI to generate the reporter constructs, respectively. The Renilla luciferase (REN) gene under the control of the 35S promoter in the pGreenII 0800-LUC vector was used as the internal control, and the 35SPro::GFP, 35SPro::GFP-APIP5nls and 35SPro::GFP-APIP5nes constructs were used as effectors. The plasmids were infiltrated into N. benthamiana leaves. At 48-70 h after infiltration, leaf discs were collected and examined for LUC activity using the Dual-Luciferase Reporter Assay System (Promega, E2920) with a GLOMAX 96 microplate luminometer (Promega).

Chromatin immunoprecipitation (ChIP) assay

The ChIP assay was performed as previously reported (30). Briefly, the precipitated DNA fragments were recovered with cetyltrimethyl ammonium bromide (CATB) and dissolved in sterile water for constructing the sequencing library. The libraries were constructed according to Illumina’s instructions, and then sequenced on the Illumina HiSeq X10. The high-quality reads were mapped to the MSU Rice Genome Annotation Project database using Bowtie2 (31,32). The enriched peaks were identified using MACS (version 2.0.0) software and annotated using ChIPseeker (33,34), which was followed by MEME Suite (MEME and DREME tools for motif discovery, and TOMTOM searches for similar motifs in databases of known motifs) to generate the APIP5-binding motif (35). The raw sequence data reported in this paper were deposited in Gene Expression Omnibus (GEO), under accession number GSE198135.

For ChIP-qPCR assay, N. benthamiana leaves co-infiltrated with GFP, GFP-APIP5nls or GFP-APIP5nes and reporter plasmids (0800-OsWAK5Pro or 0800-CYP72A1Pro) or the leaves of GFP-APIP5, GFP-APIP5nls and GFP-APIP5nes rice plants at the tillering stage were ground into a powder in liquid nitrogen, and the extracted protein was cross-linked in cross-linking buffer with 1% (v/v) formaldehyde via vacuum infiltration for 15 min. Glycine (0.25 M) was added to quench the cross-linking reaction. The chromatin complexes were isolated, sonicated and immunoprecipitated with anti-GFP antibody (Roche, Cat. No. 11814460001). Immunoprecipitated proteins were captured by Protein G magnetic beads (Invitrogen, 88847) and thoroughly washed to remove non-specific bound DNA fragments. The precipitated and input DNA fragments were recovered with CTAB and dissolved in sterile water. ChIP-qPCR results were calculated as the percentage of input DNA. Independent ChIP-qPCR experiments were performed three times with similar results. All primers used for ChIP-qPCR are listed in Supplementary Table S1.

In vitro and in vivo RNA-immunoprecipitation (RIP) assays

In vitro RIP assays were performed as previously described (36). Briefly, total RNA was extracted from rice plants and divided into two equal fractions for incubation with MBP-APIP5 or MBP. MBP and MBP-APIP5 were purified with amylose resins (NEB, E8201) and washed five times with binding/washing buffer (150 mM NaCl, 20 mM Tris-HCl, pH 8.0, 2 mM EDTA, 1% Triton X-100, 0.1% SDS and 20 units/ml RNase inhibitor [Invitrogen, N8080119]). Following incubation with total RNA for 3 h at 4°C, RNA/MBP and RNA/MBP-APIP5 resin complexes were thoroughly washed eight times with binding/washing buffer and combined with TRIzol reagent (Invitrogen, 15596018) to extract RNA. The cDNA synthesized from the eluted RNA was used for qRT-PCR. Each RIP value was normalized to its respective input RNA value.
For the in vivo RIP assay, 2-week-old GFP-APIP5 seedlings were homogenized in liquid nitrogen, cross-linked in cross-linking buffer with 1% (v/v) formaldehyde, and suspended in extraction buffer (100 mM KCl, 2.5 mM MgCl2, 10 mM HEPES pH 7.5, 10% glycerol, 0.5% NP40, 1 mM DTT, 100 U/ml RNasin RNase inhibitor [Invitrogen, N8080119], 25 mM Mg132 and EDTA-free protease inhibitor cocktail [Roche, Cat. No. 0469312001]). Following two rounds of centrifugation at 16 000 g for 10 min at 4°C to remove the insoluble material, the supernatants were divided into two fractions and incubated with or without anti-GFP antibodies (Roche, Cat. No. 11814460001) for 2 h at 4°C before adding Protein G magnetic beads (Invitrogen, 88847). The bead mixtures were washed ten times with washing buffer (100 mM KCl, 2.5 mM MgCl2, 10 mM HEPES pH 7.5, 10% glycerol, 0.5% NP40, 40 U/ml RNasin RNase inhibitor [Invitrogen, N8080119]) and eluted with TRizol reagent (Invitrogen, 15596018). The resulting RNA was analyzed by qRT-PCR.

Analysis of cellulose and lignin content

To analyze cellulose and lignin contents, 6- to 8-week-old OsWAK5-overexpression and NPB plants were harvested individually and dried thoroughly at 60°C. Dry leaf tissue (0.3 g) was ground in liquid nitrogen. The cellulose content was measured according to the method described previously (37).

To analyze lignin content, after adding 7 ml of 50 mM potassium phosphate buffer (pH 7.0), the mixtures were incubated at 4°C overnight and centrifuged at 1400 g for 10 min. The pellets were extracted with the following solvents: 7 ml PBS (pH 7.0; repeated twice), 7 ml PBS containing 1% (v/v) Triton X-100 (repeated three times), 7 ml PBS containing 1 M NaCl (repeated twice), 7 ml distilled water (repeated twice) and 5 ml acetone (repeated twice). The pellets were dried at 60°C for 24 h to obtain protein-free cell wall samples. The acetyl bromide method was used to quantify lignin content as described previously (38).

Measuring Cytochrome c Oxidase (COX) complex activity and oxidative burst

COX IV activity in CYP72A1-overexpression and NPB plants was measured using a Mitochondrial Complex IV Assay Kit (FHTD-2-Y, Suzhou Keming Biotechnology) according to the manufacturer’s protocol.

Oxidative burst detection in CYP72A1-OE, Cas9-cyp72a1 and NPB plants was detected as described previously (39).

Statistical analysis

All data for quantification analyses are presented as mean ± standard error (SE). Statistical analysis was performed by two-tailed Student’s t-test (*P < 0.05, **P < 0.01).

Accession numbers

The accession numbers of major genes mentioned in this study are as follows: APIP5 (LOC_Os01g26174), CYP72A1 (LOC_Os03g25500), OsLSD1 (LOC_Os08g06280) and OsRac1 (LOC_Os01g12900).

RESULTS

APIP5 regulation of age-dependent cell death and plant immunity requires its NLS

We previously demonstrated that APIP5 contains an NLS in its DNA-binding domain and an NES in its C terminus (26). To further explore the functions of these domains in APIP5, we mutated the hydrophilic amino acids in the NLS and NES to alanine (Ala), producing APIP5nls and APIP5nes, respectively (Supplementary Figure S1A). We then generated N-terminal green fluorescent protein (GFP)-labeled fusion proteins with APIP5, APIP5nls and APIP5nes. When the constructs encoding these proteins were expressed in rice protoplasts, GFP-APIP5 and GFP-APIP5nls primarily localized to the cytoplasm, whereas GFP-APIP5nes mainly localized to the nucleus (Supplementary Figure S1B). Meanwhile, APIP5nls and APIP5nes had similar self-transactivation activities in yeast and formed homodimers in N. benthamiana and rice protoplasts, like APIP5 (Supplementary Figure S1C–G). These results suggest that the mutations in the NLS and NES do not affect the self-transactivation activity or homodimerization of APIP5. Furthermore, APIP5nls formed heterodimers with APIP5 in yeast-two hybrid (Y2H), luciferase complementation (LCI), and glutathione S-transferase (GST) pull-down assays (Supplementary Figure S1H–J).

To investigate the biological functions of the APIP5 NLS and NES in APIP5-mediated cell death and blast resistance, we cloned APIP5, APIP5nls and APIP5nes into the transformation vector pRHvGFP under the control of the maize UBIQUITIN promoter. The resulting plasmids containing GFP-APIP5, GFP-APIP5nls and GFP-APIP5nes were transformed into the japonica rice cultivar Nipponbare (NPB). Because the dimerization of GFP-APIP5, APIP5nls and APIP5nes with the intrinsic APIP5 may interfere with the ectopic expression of the transgenes, we checked the transcript and protein levels of GFP-APIP5, APIP5nls and APIP5nes in the transgenic plants by quantitative reverse-transcription PCR (qRT-PCR) and immunoblot analysis. The results showed that the transcript and protein levels of GFP-APIP5, APIP5nls and APIP5nes were much higher than the intrinsic APIP5 (Supplementary Figure S2A–F). We also used CRISPR/Cas9 to generate Cas9-apip5 mutants, and APIP5 was significantly down-regulated in the mutants than in NPB plants (Supplementary Figure S2G and H).

At the seedling stage, GFP-APIP5, GFP-APIP5nls and GFP-APIP5nes transgenic plants and Cas9-apip5 mutants grew normally and accumulated similar levels of H2O2 compared to NPB, as detected by 3,3′-diaminobenzidine (DAB) staining (Supplementary Figure S3). However, at the tillering stage, both GFP-APIP5nls and Cas9-apip5 mutant plants displayed cell death-like lesions and greater H2O2 accumulation on the older leaves (Figure 1A and B; Supplementary Figure S4A). However, the growth of GFP-APIP5 and GFP-APIP5nes transgenic plants was similar to NPB at this stage (Supplementary Figure S4C, D, F).
Figure 1. The nuclear localization signal is required for avoiding APIP5-mediated age-dependent cell death and resistance to *M. oryzae*. (A) Cell death phenotypes of *GFP-APIP5nls* transgenic plants growing in a greenhouse. (B) Cell death phenotypes in the leaves of *GFP-APIP5nls* transgenic plants (left panel) and ROS levels detected by DAB staining (right panel). (C) Expression levels of cell death-related gene *OsMT2b* in *GFP-APIP5nls* transgenic plants detected by qRT-PCR. *UBIQUITIN* (*UBI*) was used as an internal control. Values are means ± SE (*n* = 3, technical repeats). The mean and SE values were obtained from three independent experiments (one leaf disc each). (D and E) Disease symptoms and relative fungal biomass of representative leaves of *GFP-APIP5nls* and NPB plants after punch inoculation with *M. oryzae* isolate RO1-1. The images were taken at 14 d after inoculation. Similar results were obtained from three independent experiments (biological replicates), and the representative data from one replicate are shown. Values are means ± SE (*n* = 3, technical repeats). The mean and SE values were obtained from three biological samples (one leaf disc each). (F) Expression levels of defense-related gene *OsCHITINASE3* in *GFP-APIP5nls* transgenic plants detected by qRT-PCR. *UBI* was used as an internal control. Values are means ± SE (*n* = 3, technical repeats). The mean and SE values were obtained from five samples. (G and H) Disease symptoms and relative fungal biomass of representative leaves of *GFP-APIP5* and NPB plants after punch inoculation with *M. oryzae* isolate RO1-1. The images were taken at 14 d after inoculation. Similar results were obtained from three independent experiments (biological replicates), and the representative data from one replicate are shown. Values are means ± SE (*n* = 3, technical repeats). The mean and SE values were obtained from five samples. (I) Expression levels of defense-related gene *OsCHITINASE3* in *GFP-APIP5nles* transgenic plants detected by qRT-PCR. *UBI* was used as an internal control. Values are means ± SE (*n* = 3, technical repeats). The mean and SE values were obtained from three biological samples (one leaf disc each). Asterisks represent significant difference determined by Student’s *t*-test (*P* < 0.05, **P** < 0.01) compared to NPB.
and G). Consistent with this phenotype, ORYZA SATIVA METHALLOTHIONINE2b (OsMT2b), encoding a negative regulator of cell death, was significantly downregulated in both GFP-APIP5nls and Cas9-apip5 plants compared with NPB (Figure 1C and Supplementary Figure S4B). By contrast, the expression of OsMT2b was not discernibly affected in GFP-APIP5 or GFP-APIP5nes plants (Supplementary Figure S4E and H). Additionally, to confirm GFP-APIP5 is functional, we transformed the Cas9-apip5 construct into the calli of GFP-APIP5 overexpression seeds and obtained transgenic plants with deletions in the endogenous APIP5 gene and the intact GFP-APIP5 transgene. The Cas9-apip5/GFP-APIP5 transgenic plants did not show any cell death and excessive accumulation of H$_2$O$_2$, which was similar with that of NPB and GFP-APIP5 plants (Supplementary Figure S4I and J). These results indicate that GFP-APIP5 can functionally complement Cas9-apip5 and the NLS in APIP5 is essential for the negative regulation of age-dependent cell death in rice.

Next, we punch-inoculated GFP-APIP5, GFP-APIP5nls and GFP-APIP5nes plants with the compatible M. oryzae isolate RO1-1 before visible cell death lesions appeared on GFP-APIP5nls plants. Compared to NPB plants, GFP-APIP5nls plants exhibited elevated resistance level to RO1-1 with reduced fungal biomass (Figure 1D and E), while GFP-APIP5 and GFP-APIP5nes plants displayed decreased resistance, with elevated fungal biomass (Figure 1G, H, J and K). Additionally, GFP-APIP5 and GFP-APIP5nes transgenic plants were also more susceptible to both RB22 (without AvrPiz-t) and RB22-AvrPiz-t isolates than NPB plants (Supplementary Figure S4K, L, O and P). In contrary, GFP-APIP5nls transgenic plants exhibited more resistance to RB22 and RB22-AvrPiz-t (Supplementary Figure S4M and N). As expected, the defense-associated gene OsCHITINASE3 was significantly upregulated in GFP-APIP5nls plants but downregulated in GFP-APIP5 and GFP-APIP5nes plants compared to NPB (Figure 1F, I and L). Taken together, these results indicate that the NLS in APIP5 also plays an important role in the defense response to M. oryzae.

APIP5 accumulation in the nucleus is associated with plant development and blast infection

The importance of the NLS in APIP5 suggested that APIP5 might undergo nucleocytoplasmic trafficking during plant development. To examine this, we analyzed GFP-APIP5 protein abundance in the nucleus and cytoplasm in GFP-APIP5 plants at the seedling and tillering stages. Immunoblot of the nuclei-enriched fraction and confocal image analyses revealed that GFP-APIP5 mainly accumulated in the nucleus at the tillering stage but not the seedling stage (Figure 2A and Supplementary Figure S5A). By contrast, GFP-APIP5nls mainly localized to the cytoplasm at both the seedling and tillering stages (Figure 2B and Supplementary Figure S5B). We transiently expressed APIP5 fused with an N-terminal GFP tag under the control of the 35S promoter in 1- and 2-month-old N. benthamiana. Both confocal image analysis and western blotting consistently showed that GFP-APIP5 was predominantly expressed in the cytoplasm in 1-month-old leaves, whereas a portion of GFP-APIP5 translocated into the nucleus in 2-month-old leaves (Figure 2C and D).

Given that AvrPiz-t targets APIP5 during blast infection (26), we reasoned that activated APIP5 might translocate into the nucleus to act on defense activation. To examine this possibility, we inoculated GFP-APIP5 plants with M. oryzae strain RO1-1 and investigated the nucleocytoplasmic distribution of APIP5 during blast infection. More GFP-APIP5 accumulated in the nuclear pools at 1, 2, and 3 day after inoculation (DAI) compared to the control, but GFP-APIP5 decreased to basal levels at 4 DAI (Figure 2E). Interestingly, APIP5 accumulated rapidly in the cytoplasm at 1 DAI but reduced at 5 DAI (Figure 2E). These results suggest that plant development and blast infection induce the trafficking of APIP5 from the cytoplasm into the nucleus during the early stage of infection.

APIP5 directly targets the cell wall-associated defense gene OsWAK5

To identify the target genes regulated by APIP5, we performed an in vitro chromatin immunoprecipitation-sequencing (ChIP-seq) experiment using anti-GST antibody against GST-APIP5 (30). In total, we identified 4810 GST-APIP5 binding peaks (corresponding to 880 genes) and 84 GST binding peaks (corresponding to 4 genes), respectively (Supplementary Figure S6A). Genome-wide distribution analysis revealed that the APIP5 binding sites were highly enriched in the promoter region (3 kb upstream of the transcription start site), which accounted for about 64% of all peaks (Supplementary Figure S6B). Gene Ontology analysis further revealed that the specific APIP5 targeting genes were enriched in several biological pathways such Catabolic process, DNA metabolic process and RNA binding (Supplementary Figure S6C). KEGG pathway enrichment showed that APIP5 specific targeted genes were associated with 2-oxocarboxylic acid metabolism, RNA degradation and biosynthesis of amino acids (Supplementary Figure S6D). De novo motif analyses revealed that YYTYYYY is the best enriched putative transcription factor binding elements under APIP5 binding peaks (Supplementary Figure S6E). Then we used electrophoretic mobility shift assay (EMSA) to confirm the binding by APIP5 (Supplementary Figure S6E). Among the identified candidate genes, we selected OsWAK5 for further analysis because the YYTYYYY motif was identified in the promoter of OsWAK5, and WAK proteins play critical roles in rice immunity (40,41).

To confirm that APIP5 targets the OsWAK5 promoter (OsWAK5Pro), we designed P1 and P2 probes based on the predicted promoter sequence of OsWAK5 for an EMSA. MBP-APIP5 specifically bound to P1 and P2, but not MBP (Figure 3A and Supplementary Figure S7A). Interestingly, MBP-APIP5nls showed very little binding to P1 and P2, and the binding activities of MBP-APIP5nes to P1 and P2 were comparable to that of MBP-APIP5 (Supplementary Figure S7B and C). To confirm the in vitro data, we performed ChIP-qPCR assays using APIP5 transgenic plants. The OsWAK5Pro region was markedly enriched in GFP-APIP5nls and slightly in GFP-APIP5nes and in GFP-APIP5 plants at the tillering stage but not in GFP-APIP5nls plants (Figure 3B). These
Figure 2. Developmental- and pathogen-dependent nuclear accumulation of GFP-APIP5. (A) GFP-APIP5 abundance in cytosolic- and nuclei-enriched fractions from GFP-APIP5 transgenic plants at the seedling and tillering stages. S represents the seedling stage; T represents the tillering stage. Histone H3 served as a nuclear marker and HSP as a cytosolic marker. The experiment was repeated twice (biological replicates) with similar results, and the representative data from one replicate are shown. (B) GFP-APIP5nls abundance in cytosolic- and nuclei-enriched fractions from GFP-APIP5nls transgenic plants at the seedling and tillering stages. S represents the seedling stage; T represents the tillering stage. Histone H3 served as a nuclear marker and HSP as a cytosolic marker. The experiment was repeated twice (biological replicates) with similar results, and the representative data from one replicate are shown. (C) Confocal images showing the subcellular localization of GFP-APIP5 transiently expressed in the leaves of 1- and 2-month-old N. benthamiana plants. mCherry was used as a whole-cell localization marker. Scale bars represent 20 μm. (D) GFP-APIP5 abundance in cytosolic- and nuclei-enriched fractions from 1- to 2-month-old N. benthamiana plants. Histone H3 served as a nuclear marker and Actin as a cytosolic marker. The experiment was repeated twice (biological replicates) with similar results, and the representative data from one replicate are shown. (E) GFP-APIP5 abundance in cytosolic- and nuclei-enriched fractions from 3-week-old GFP-APIP5 transgenic plants after inoculation with RO1-1. DAI represents day after inoculation. The experiment was repeated twice (biological replicates) with similar results, and the representative data from one replicate are shown.
Figure 3. Targeting and suppression of OsWAK5 by APIP5 and the disease phenotypes of OsWAK5 overexpression and knock out plants. (A) Binding of MBP-APIP5 to the P1 and P2 probes in the OsWAK5 promoter in an EMSA. MBP alone served as a control. The experiment was repeated three times (biological replicates) with similar results, and the representative data from one replicate are shown. (B) ChIP-qPCR of the binding of GFP-APIP5 and GFP-APIP5nes to the OsWAK5 promoter in transgenic plants at the tillering stage. Similar results were obtained from three independent experiments (biological replicates), and the representative data from one replicate are shown. Values are means ± SE (n = 3, technical repeats). (C) Luciferase reporter assays of APIP5nes-induced suppression of OsWAK5 pro-LUC in N. benthamiana. LUC activity was measured by normalizing to REN signal. Similar results were obtained from three independent experiments (biological replicates) with similar results, and the representative data from one replicate are shown. Values are means ± SE (n = 3, technical repeats). (D) qRT-qPCR analysis of OsWAK5 expression in GFP-APIP5 and GFP-APIP5nes and plants. UBIQUITIN (UBI) was used as an internal control. Values are means ± SE (n = 3, technical repeats). The mean and SE values were obtained from three biological samples (one leaf disc each). (E and F) Disease symptoms and relative fungal biomass of representative leaves of OsWAK5-overexpression and NPB plants after punch inoculation with M. oryzae isolate RO1-1. The images were taken at 14 d after inoculation. Similar results were obtained from three independent experiments (biological replicates), and the representative data from one replicate are shown. Values are means ± SE (n = 3, technical repeats). (G and H) Disease symptoms and relative fungal biomass of representative leaves of Cas9-oswak5 and NPB plants after punch inoculation with M. oryzae isolate RO1-1. The images were taken at 14 d after inoculation. Similar results were obtained from three independent experiments (biological replicates), and the representative data from one replicate are shown. Values are means ± SE (n = 3, technical repeats). The mean and SE values were obtained from five samples. OsWAK5-OE plants represent OsWAK5-overexpression plants. (G and H) Disease symptoms and relative fungal biomass of representative leaves of Cas9-oswak5 and NPB plants after punch inoculation with M. oryzae isolate RO1-1. The images were taken at 14 d after inoculation. Similar results were obtained from three independent experiments (biological replicates), and the representative data from one replicate are shown. Values are means ± SE (n = 3, technical repeats). The mean and SE values were obtained from five samples. (I) Lignin contents of OsWAK5-overexpression and NPB plants. Values are means ± SE (n = 3, technical repeats). The mean and SE values were obtained from more than five biological samples. (J and K) Disease symptoms and relative fungal biomass of representative leaves of GFP-APIP5, OsWAK5-OE, GFP-APIP5/OsWAK5-OE and NPB plants after punch inoculation with M. oryzae isolate RO1-1. Asterisks represent significance levels determined by Student’s t-test (***p < 0.01) compared to the negative controls.
APIP5 directly targets the cytochrome P450 defense gene CYP72A1

Cytochrome P450 enzymes (CYPs) participate in the biosynthesis and catabolism of phytohormones, antioxidants and defense compounds (43,44). CYP72s comprise one of the largest subgroups of CYPs (45). We identified CYP72A1 in the in vitro ChiP-seq assay described above and CYP72A1 contains a YTTYYTT motif in its promoter. Therefore, in addition to OsWAK5, we focused on CYP72A1 as a potential target gene. To assess whether APIP5 binds to the CYP72A1 promoter (CYP72A1-Pro), we performed EMSA. Both MBP-APIP5 and MBP-APIP5nes specifically bound to P1 and P2 in the CYP72A1 promoter, whereas MBP-APIP5nes showed little binding to P1 and P2 (Figure 4A and Supplementary Figure S8A–C). ChiP-qPCR assays indicated that the CYP72A1-Pro region was significantly enriched in GFP-APIP5nes plants and slightly in GFP-APIP5 plants at the tillering stage but not in GFP-APIP5nes plants (Figure 4B). These results indicate that the nls mutation in APIP5 may affect its nuclear localization and DNA binding activity to the CYP72A1 promoter.

Because of the localization of GFP-APIP5 mainly in cytoplasm and slightly enrichment in OsWAK5 expression, we co-expressed the OsWAK5::LUC reporter with GFP, GFP-APIP5nls or GFP-APIP5nes in N. benthamiana plants (Figure 3C). Compared to GFP, the luciferase-to-Renilla (LUC/REN) ratio decreased in the presence of GFP-APIP5nes, whereas there were no significant differences in the presence of GFP and GFP-APIP5nls (Figure 3C). Consistent with the LUC/REN data, ChiP-qPCR assays showed that GFP-APIP5nes was enriched in the OsWAK5-Pro region in N. benthamiana plants, whereas GFP-APIP5nls were not (Supplementary Figure S7D). We then evaluated the expression of OsWAK5 in GFP-APIP5nls and GFP-APIP5nes plants at the tillering stage. OsWAK5 was significantly downregulated in GFP-APIP5nes plants but not in GFP-APIP5nls plants compared to NPB (Figure 3D). Collectively, these data suggest that APIP5 targets OsWAK5 to negatively regulate its expression in the nucleus.

To validate the role of OsWAK5 in blast resistance, we generated OsWAK5-overexpression lines (OsWAK5-OE, independent lines #38 and #42) and CRISPR/Cas9 mutants (Cas9-oswak5, independent lines #1 and #5) (Supplementary Figure S7E and F) and evaluated their resistance to M. oryzae. Compared with NPB, OsWAK5-OE plants showed elevated resistance to M. oryzae isolate RO1-1, with smaller lesions and reduced fungal biomass (Figure 3E and F), whereas Cas9-oswak5 mutants displayed reduced resistance to RO1-1, with larger lesions and greater fungal biomass (Figure 3G and H). Moreover, the defense-associated gene OsCHITINASE3 was significantly upregulated in OsWAK5-OE plants but downregulated in Cas9-oswak5 plants compared to NPB (Supplementary Figure S7G and H).

Cellulose and lignin are the main components of cell walls and involved in the resistance to rice pathogens. For example, the resistance gene Xa4 encodes a WAK protein that promotes cellulose synthesis to enhance resistance against Xoo (40) and OsMYB30 positively regulates the resistance to M. oryzae by enhancing the accumulations of lignin (42). These findings prompted us to analyze the cellulose and lignin contents in the OsWAK5-OE plants. The cellulose contents were comparable in OsWAK5-OE and NPB plants (Supplementary Figure S7I), while the lignin contents were much higher in the OsWAK5-OE plants than in NPB (Figure 3I). To further confirm the genetic relationship between OsWAK5 and APIP5, we generated the GFP-APIP5/OsWAK5-OE hybrid plants by genetic crossing of the GFP-APIP5 plants with OsWAK5-OE plants (Supplementary Figure S7J). Similar with the OsWAK5-OE plants, GFP-APIP5/OsWAK5-OE plants displayed more resistance to isolate RO1-1, with smaller lesions and reduced fungal biomass (Figure 3J and K). Together, these results demonstrate that APIP5 suppresses the expression of OsWAK5, which positively regulates resistance to M. oryzae by inducing defense gene expression and promoting lignin biosynthesis.

results indicate that the nls mutation in APIP5 may affect its nuclear localization and DNA binding activity to the Os-WAK5 promoter.

Next, we performed a dual-luciferase reporter assay to dissect the role of APIP5 in regulating OsWAK5 expression. Because of the localization of GFP-APIP5 mainly in cytoplasm and slightly enrichment in OsWAK5 expression, we co-expressed the OsWAK5::LUC reporter with GFP, GFP-APIP5nls or GFP-APIP5nes in N. benthamiana plants (Figure 3C). Compared to GFP, the luciferase-to-Renilla (LUC/REN) ratio decreased in the presence of GFP-APIP5nes, whereas there were no significant differences in the presence of GFP and GFP-APIP5nls (Figure 3C). Consistent with the LUC/REN data, ChiP-qPCR assays showed that GFP-APIP5nes was enriched in the OsWAK5-Pro region in N. benthamiana plants, whereas GFP-APIP5nls were not (Supplementary Figure S7D). We then evaluated the expression of OsWAK5 in GFP-APIP5nls and GFP-APIP5nes plants at the tillering stage. OsWAK5 was significantly downregulated in GFP-APIP5nes plants but not in GFP-APIP5nls plants compared to NPB (Figure 3D). Collectively, these data suggest that APIP5 targets OsWAK5 to negatively regulate its expression in the nucleus.

To validate the role of OsWAK5 in blast resistance, we generated OsWAK5-overexpression lines (OsWAK5-OE, independent lines #38 and #42) and CRISPR/Cas9 mutants (Cas9-oswak5, independent lines #1 and #5) (Supplementary Figure S7E and F) and evaluated their resistance to M. oryzae. Compared with NPB, OsWAK5-OE plants showed elevated resistance to M. oryzae isolate RO1-1, with smaller lesions and reduced fungal biomass (Figure 3E and F), whereas Cas9-oswak5 mutants displayed reduced resistance to RO1-1, with larger lesions and greater fungal biomass (Figure 3G and H). Moreover, the defense-associated gene OsCHITINASE3 was significantly upregulated in OsWAK5-OE plants but downregulated in Cas9-oswak5 plants compared to NPB (Supplementary Figure S7G and H).

Cellulose and lignin are the main components of cell walls and involved in the resistance to rice pathogens. For example, the resistance gene Xa4 encodes a WAK protein that promotes cellulose synthesis to enhance resistance against Xoo (40) and OsMYB30 positively regulates the resistance to M. oryzae by enhancing the accumulations of lignin (42). These findings prompted us to analyze the cellulose and lignin contents in the OsWAK5-OE plants. The cellulose contents were comparable in OsWAK5-OE and NPB plants (Supplementary Figure S7I), while the lignin contents were much higher in the OsWAK5-OE plants than in NPB (Figure 3I). To further confirm the genetic relationship between OsWAK5 and APIP5, we generated the GFP-APIP5/OsWAK5-OE hybrid plants by genetic crossing of the GFP-APIP5 plants with OsWAK5-OE plants (Supplementary Figure S7J). Similar with the OsWAK5-OE plants, GFP-APIP5/OsWAK5-OE plants displayed more resistance to isolate RO1-1, with smaller lesions and reduced fungal biomass (Figure 3J and K). Together, these results demonstrate that APIP5 suppresses the expression of OsWAK5, which positively regulates resistance to M. oryzae by inducing defense gene expression and promoting lignin biosynthesis.

APIP5 directly targets the cytochrome P450 defense gene CYP72A1

Cytochrome P450 enzymes (CYPs) participate in the biosynthesis and catabolism of phytohormones, antioxidants and defense compounds (43,44). CYP72s comprise one of the largest subgroups of CYPs (45). We identified CYP72A1 in the in vitro ChiP-seq assay described above and CYP72A1 contains a YTTYYTT motif in its promoter. Therefore, in addition to OsWAK5, we focused on CYP72A1 as a potential target gene. To assess whether APIP5 binds to the CYP72A1 promoter (CYP72A1-Pro), we performed EMSA. Both MBP-APIP5 and MBP-APIP5nes specifically bound to P1 and P2 in the CYP72A1 promoter, whereas MBP-APIP5nes showed little binding to P1 and P2 (Figure 4A and Supplementary Figure S8A–C). ChiP-qPCR assays indicated that the CYP72A1-Pro region was significantly enriched in GFP-APIP5nes plants and slightly in GFP-APIP5 plants at the tillering stage but not in GFP-APIP5nes plants (Figure 4B). These results indicate that the nls mutation in APIP5 may affect its nuclear localization and DNA binding activity to the CYP72A1 promoter.

Because of the localization of GFP-APIP5 mainly in cytoplasm and slightly enrichment in CYP72A1-Pro, we co-expressed the CYP72A1-Pro::LUC reporter with GFP, GFP-APIP5nls or GFP-APIP5nes in N. benthamiana plants, the results showed that GFP-APIP5nes repressed the LUC activity of CYP72A1-Pro, whereas GFP and GFP-APIP5nls did not (Figure 4C). Indeed, ChiP-qPCR revealed that GFP-APIP5nes was enriched at CYP72A1-Pro in N. benthamiana plants (Supplementary Figure S8D). We analyzed the expression levels of CYP72A1 in GFP-APIP5nes and GFP-APIP5nls plants at the tillering stage. CYP72A1 was significantly downregulated in GFP-APIP5nes plants, but there were no significant differences in CYP72A1 expression between GFP-APIP5nls and NPB plants (Figure 4D). These results indicate that APIP5 binds to the CYP72A1 promoter to negatively regulate its expression following M. oryzae infection.

To determine the role of CYP72A1 in blast resistance, we generated CYP72A1-overexpression lines (CYP72A1-OE, independent lines #8 and #9) and CRISPR/Cas9 mutants (Cas9-cyp72a1, independent lines #1 and #2) (Supplementary Figure S8E and F) and evaluated their resistance to M. oryzae. Compared to NPB, CYP72A1-OE plants were more resistant to RO1-1, with smaller disease lesions and less fungal biomass (Figure 4E and F), whereas Cas9-cyp72a1 plants were more susceptible to this pathogen, with larger disease lesions and greater fungal biomass (Figure 4G and H). The defense-associated gene OsCHITINASE3 was significantly upregulated in CYP72A1-OE plants but downregulated in Cas9-cyp72a1 plants compared to NPB (Supplementary Figure S8G and H).

CYPs are heme-containing enzymes involved in various oxidation-reduction reactions (46); therefore, we analyzed whether CYP72A1 affects cytochrome c metabolism in rice. COX is the last electron acceptor of the mitochondrial respiratory chain, which is associated with ROS generation (47,48). We found that COX IV activity was much lower in CYP72A1-OE plants than in NPB (Figure 4I). Moreover, the ROS burst in CYP72A1-OE plants were more ro-
Figure 4. Targeting and suppression of CYP72A1 by APiP5 and the disease phenotypes of CYP72A1 overexpression and knockout plants. (A) MBP-APiP5 binding to P1 and P2 probes of the CYP72A1 promoter in an EMSA. MBP alone served as a control. The experiment was repeated three times (biological replicates) with similar results, and the representative data from one replicate are shown. (B) ChIP-qPCR analysis of GFP-APiP5 and GFP-APiP5nes binding to the CYP72A1 promoter in transgenic plants at the tillering stage. Similar results were obtained from three independent experiments (biological replicates), and the representative data from one replicate are shown. Values are means ± SE (n = 3, technical repeats). (C) Luciferase reporter assay of APiP5nes-induced suppression of CYP72A1 expression in N. benthamiana. LUC activity was measured by normalizing to the REN signal. Similar results were obtained from three independent experiments (biological replicates) with similar results, and the representative data from one replicate are shown. Values are means ± SE (n = 3, technical repeats). (D) qRT-PCR analysis of CYP72A1 expression in GFP-APiP5nes and GFP-APiP5nes plants. UBQUITIN (UBI) was used as an internal control. Values are means ± SE (n = 3, technical repeats). (E and F) Disease symptoms and relative fungal biomass of representative leaves of CYP72A1-overexpression and NPB plants after punch inoculation with M. oryzae isolate RO1-1. The images were taken at 14 d after inoculation. Similar results were obtained from three independent experiments (biological replicates), and the representative data from one replicate are shown. Values are means ± SE (n = 3, technical repeats). (G and H) Disease symptoms and relative fungal biomass of representative leaves of Cas9-cyp72a1 and NPB plants after punch inoculation with M. oryzae isolate RO1-1. The images were taken at 14 d after inoculation. Similar results were obtained from three independent experiments (biological replicates), and the representative data from one replicate are shown. Values are means ± SE (n = 3, technical repeats). (I) Cytochrome c oxidase activity in CYP72A1-overexpression and NPB plants. Values are means ± SE (n = 3, technical repeats). (J and K) Disease symptoms and relative fungal biomass of representative leaves of GFP-APiP5, CYP72A1-OE, GFP-APiP5/CYP72A1-OE and NPB plants after punch inoculation with M. oryzae isolate RO1-1. Asterisks represent significance levels determined by Student’s t-test (*P < 0.05, **P < 0.01) compared to the negative controls.
bust than that in NPB after treatment with chitin, while ROS burst in Cas9-cyp72A1 mutants were much weaker than that in NPB (Figure 4J and K). CYPs were reported in the biosynthesis and catabolism of antioxidants and defense compounds (43,49). We therefore analyzed the non-targeted metabolite profiling in CYP72A1-OE plants. The result showed that some defense compounds were significantly increased in CYP72A1-OE plants than in NPB plants, such as Oryzalexin S, Momilactone B, Oryzalexin C and so on (Supplementary Figure S8I). Meanwhile, other compounds, like Oryzalexin F(17)-H2O, Diosgenin and Manglieside E, were significantly decreased in CYP72A1-OE plants than in NPB plants (Supplementary Figure S8I).

To confirm the genetic relationship between CYP72A1 and APIP5, we crossed GFP-APIP5 plants with CYP72A1-OE plants to generate GFP-APIP5/CYP72A1-OE plants (Supplementary Figure S8J). Similar with CYP72A1-OE plants, GFP-APIP5/CYP72A1-OE plants displayed more resistance to isolate RO1-1, with smaller lesions and reduced fungal biomass (Figure 4L and M). Collectively, these results suggest that APIP5 directly suppresses the expression of CYP72A1 and thus regulates ROS and defense compounds accumulations in rice during M. oryzae infection.

APIP5 functions as a RNA-binding protein to regulate the cell death and defense response genes OsLSD1 and OsRac1

When the GFP-APIP5 plasmids were transiently expressed in rice protoplasts, we observed a few bright spots in the cytoplasm (Figure 5A). These bright spots co-localized with the processing body (P-body) markers NbDCP1 and NbDCP2, which recognize some RNAs and target them for turnover (50,51) (Figure 5A). Strikingly, the P-body localization in GFP-APIP5 was significantly increased in GFP-APIP5 sheath epidermal cells at 48 h after infection with RO1-1 (Supplementary Figure S9A). These findings suggest that APIP5 may selectively associate with some RNA transcripts in the cytoplasm.

To assess whether APIP5 binds to RNA, we performed in vitro RNA binding assays using recombinant MBP-APIP5 fusion proteins and homopolymers of A, U and C, as a control, we used OsTZF1, a CCCH-type zinc finger protein that directly binds to U-rich regions in the 3’ untranslated regions (UTRs) of mRNAs (52). An RNA EMSA (REMSA) showed that APIP5 strongly bound to poly(U) and weakly bound to poly(C) (Figure 5B; Supplementary Figure S9B and C), like the control OsTZF1. The binding activity of MBP-APIP5 to poly(U) probe was out-competed by unlabeled poly(U) probe (Figure 5C and Supplementary Figure S9D), and this activity was partially competed by unlabeled DNA motif (YTTYTTY) probe (Supplementary Figure S9E), indicating that there may be some cross-talk between RNA binding and DNA binding of APIP5. Interestingly, MBP-APIP5ns and MBP-APIP5nes showed almost no binding activity with poly(U) (Figure 5D and E, Supplementary Figure S9F and G). These results suggest that APIP5 functions as an unconventional RNA-binding protein selectively with some RNA transcripts in cytoplasmic foci, which may be influenced by some amino acids in the NLS and NES motifs.

To identify mRNAs that are regulated by APIP5, we searched for poly(U)-enriched mRNA transcripts of genes known to be involved in defense responses. The 3’UTRs of OsLSD1 and OsRac1 were enriched with poly(U) (Supplementary Figure S10). OsLSD1 negatively regulates PCD and is involved in the defense response to M. oryzae (53). OsRac1, a small GTPase, is associated with ROS production, PCD and plant immunity (54,55). To determine whether APIP5 binds to the 3’UTRs of OsLSD1 and OsRac1, we performed REMSAs and confirmed that biotinylated OsLSD1 and OsRac1 3’UTRs were able to pull down MBP-APIP5 but not MBP (Figure 6A and B). In in vitro RIP assays, MBP-APIP5 was substantially enriched in OsLSD1 and OsRac1 mRNA, whereas MBP was not (Figure 6C). We then performed an in vivo RIP assay using GFP-APIP5 transgenic plants. We designed primers for qPCR in the A and C regions of OsLSD1 and OsRac1, which are poor in poly(U), as negative controls (Figure 6D). GFP-APIP5 was specifically enriched with OsLSD1 and OsRac1 mRNA, especially in the 3’UTRs (Figure 6D). These results demonstrate that APIP5 directly binds to OsLSD1 and OsRac1 mRNA in vitro and in vivo.

Finally, we analyzed the mRNA turnover rates of OsLSD1 and OsRac1 in Cas9-api5 and GFP-APIP5 plants. We designed primers for qPCR to detect the 5’- and 3’-ends of OsLSD1 and OsRac1 mRNA, respectively. The ratio of 5’- to 3’-ends of OsLSD1 was significantly lower in Cas9-api5 and much higher in GFP-APIP5 plants compared to NPB, as determined by qPCR (Figure 6E). Similarly, the ratio of 5’- to 3’-ends of OsRac1 was lower in Cas9-api5 and slightly higher in GFP-APIP5 plants than in NPB (Figure 6F). In addition, the ratio of 5’- to 3’-ends of OsLSD1 rapidly increased in NPB after inoculation by M. oryzae at 1 d, but not in the Cas9-api5 mutant (Supplementary Figure S11A), and the ratio of 5’- to 3’-ends of OsRac1 reached its highest level at 5 d after M. oryzae inoculation in NPB plants, while no changes in the Cas9-api5 mutant (Supplementary Figure S11B). Together, these results demonstrate that APIP5 functions as an RNA-binding protein to regulate the expression of the defense-related genes OsLSD1 and OsRac1 at the post-transcriptional level.

DISCUSSION

Emerging evidence in animal systems shows that, in addition to their roles in regulating gene expression, TFs bind to different types of RNA and regulate RNA turnover (19,20). However, whether plant TFs have similar functions is still unknown. In the current study, we discovered that APIP5, a negative regulator of plant defense responses, translocates from the cytoplasm to the nucleus to directly target OsWAK5 and CYP72A1 and suppress their expression, thereby reducing lignin contents and ROS accumulation during M. oryzae infection. Strikingly, in the cytoplasm, APIP5 functions as an unconventional RNA-binding protein that directly binds to OsLSD1 and OsRac1 mRNAs and regulates their turnover in response to M. oryzae. Our study thus uncovered the first plant bZIP type TF with dual functions in transcriptional regulation and RNA turnover during plant PCD and immunity responses. These findings open an avenue to exploring the molecular mechanisms un-
derlying how plant TFs partition between the nucleus and cytoplasm and how the plant cell coordinates these two activities during defense activation.

WAKs comprise a unique group of receptor like kinases (RLKs) that likely associate with pectin in the cell wall, transduce signals from the cell wall and apoplast to the cytoplasm, and contribute to cell expansion during development and plant responses to pathogens (56,57). The rice genome contains 125 genes encoding WAK-like proteins, including 67 encoding WAK-RLKs. The disease resistance gene Xa4 encodes a WAK that promotes cellulose synthesis and strengthens the plant cell wall, thus enhancing plant resistance against Xoo infection and lodging (40). In the current study, we demonstrated that OsWAK5 is a target of APIP5 and that overexpressing OsWAK5 enhances blast resistance and promotes lignin synthesis. Upon pathogen infection, some cytoplasmic APIP5 protein translocates into the nucleus where it might bind to the promoter of OsWAK5 to suppress its expression. GhWAK7A functions in the resistance of cotton (Gossypium hirsutum) to Fusarium oxysporum f. sp. vasinfecum and Verticillium dahliae by interacting with both GhCERK1 and GhLYK5 and phosphorylating GhLYK5 to induce chitin-induced responses (58).

In tomato (Solanum lycopersicum), StWak1 acts as an important positive regulator during the late stages of flagellin-mediated PTI responses in the apoplast and associates with

---

**Figure 5.** APIP5 functions as a novel RNA-binding protein. (A) Co-localization of GFP-APIP5 with CFP-NbDCP1 and mCherry-NbDCP2 in NPB protoplasts. Scale bars represent 10 μm. (B) Binding affinity of MBP-APIP5 toward poly(A), poly(U) and poly(C). The experiment was repeated three times (biological replicates) with similar results, and the representative data from one replicate are shown. (C) Unlabeled probe competed with the binding activity of MBP-APIP5 with poly(U). Binding activity of MBP-APIP5nls (D) and MBP-APIP5nes (E) with poly(U).
Figure 6. APIP5 binds to OsLSD1 and OsRac1 mRNAs and regulates their turnover. (A) Binding activity of MBP-APIP5 with OsLSD1-3′UTR in a REMSA. The experiment was repeated three times (biological replicates) with similar results, and the representative data from one replicate are shown. (B) Binding activity of MBP-APIP5 with OsRac1-3′UTR in a REMSA. The experiment was repeated three times (biological replicates) with similar results, and the representative data from one replicate are shown. (C) qRT-PCR analysis of OsLSD1 and OsRac1 mRNA enrichment in vitro RIP experiments. Relative enrichment was calculated as the fold change of RNA abundance following pull-down by MBP-APIP5 vs. MBP. The experiment was repeated twice (biological replicates) with similar results, and the representative data from one replicate are shown. Values are means ± SE (n = 3, technical repeats). (D) In vivo RIP assays of GFP-APIP5 binding to OsLSD1 and OsRac1 mRNAs in GFP-APIP5 transgenic plants. The experiment was repeated twice (biological replicates) with similar results, and the representative data from one replicate are shown. Values are means ± SE (n = 3, technical repeats). In the diagram of OsLSD1 and OsRac1 mRNA structures, the line represents the untranslated region (UTR), and the black box represents the coding sequence (CDS) region. A, B, C and D are the sequence regions used for the RIP assays. (E) OsLSD1 mRNA turnover rates in Cas9-api5, GFP-APIP5 and NPB plants. Values are means ± SE (n = 3, technical repeats). The mean and SE values were obtained from three biological samples (one leaf disc each). (F) OsRac1 mRNA turnover rates in Cas9-api5, GFP-APIP5 and NPB plants. Values are means ± SE (n = 3, technical repeats). The mean and SE values were obtained from three biological samples (one leaf disc each). (G) A working model illustrating the molecular mechanism of APIP5-mediated cell death and immunity to M. oryzae in rice. See details in the Discussion section. Asterisks represent significance levels determined by Student’s t-test (**P < 0.01) compared to the negative controls or NPB.
FLAGELLIN SENSITIVE2 (FLS2) and FLS3 to trigger immune signaling (59). Thus, OsWAK5 may act as a coreceptor with OsCERK1 and OsLYP4/6 to perceive chitin (60,61), which leads to the delivery of signals to downstream defense pathways and enhances lignin biosynthesis.

CYPs function in secondary metabolism, development and plant responses to pathogens (45,49). The CYP72A subgroup is one of the largest groups of P450s, which are recruited for the biosynthesis of monoterpene indole alkaloids and triterpenoids (46). Suppressing the expression of TaCYP72A in wheat (Triticum aestivum) via gene silencing significantly reduced plant tolerance to mycotoxin deoxynivalenol (62). In barberry (Berberis vulgaris), BvCYP72A552 catalyzes the formation of hederagenin-based saponins to mediate plant defense responses against herbivores (63). In Catharanthus roseus, CYP72A1 catalyzes the early step of the indole alkaloid biosynthesis pathway (64). In rice, CYP72A18, CYP72A19, CYP72A22 and CYP72A23 are differentially regulated during the incompatible and compatible interactions between rice and M. oryzae (65). However, the biological functions of CYP72As are poorly understood, especially their roles in coordination with secondary metabolism and plant immunity and their regulatory mechanisms. In the current study, we demonstrated that CYP72A1 is a positive regulator of blast resistance and that APIP5 directly targets CYP72A1 to inhibit its expression, thereby comprising plant defense. Intriguingly, CYP72A1-overexpression plants had higher COX IV activity, stronger ROS burst and accumulated more defense-related secondary metabolism than NBP, suggesting that CYP72A1 may promote ROS burst through defense compound accumulation in rice immunity.

Proteins containing CCCH motif-, RRM- or TRP-containing domains are associated with RNA processing (66). The roles of a few such proteins in plant immunity have been elucidated, such as AtTZF9, Bsr-k1, and PIBP1 (25,36,67). In the current study, we demonstrated that APIP5 co-localizes with NbDCP1 and NbDCP2 and has RNA-binding activity, preferentially for poly(U). Notably, APIP5 co-localizes with NbDCP1 and NbDCP2 and has sequences, especially in their 3′ UTRs. In addition, APIP5 directly binds to OsLSD1 and OsRac1 mRNAs and regulates their turnover rates during M. oryzae infection. In Arabidopsis, LSD1 negatively regulates PCD and directly interacts with catalases (CAT1, CAT2 and CAT3) to regulate light-dependent runaway cell death and pathogen-induced cell death (68). OsLSD1 also plays a negative role in PCD and regulates resistance to M. oryzae in rice, but the underlying molecular mechanisms are still unknown (53). OsRac1 is involved in PTI and Pib-mediated resistance against M. oryzae (69,70). The constitutively active form of OsRac1 increases ROS production and cell death (71) and directly interacts with the N-terminal region of the NADPH oxidase Oryza sativa respiratory burst oxidase homolog B (OsRbohB) (55). The mRNA turnover rates of OsLSD1 and OsRac1 were much lower in Cas9-apip5 mutants and significantly higher in GFP-APIP5 overexpression plants compared to NPB. Therefore, it is likely that APIP5 functions in the cytoplasm to modulate the RNA processing of OsLSD1 and OsRac1, which is essential for the production and metabolism of ROS. Further studies are needed to decipher how APIP5 interacts with and regulates the mRNAs of these two defense genes during immunity responses in rice.

In summary, we characterized the dual roles of APIP5 in regulating PCD and defense responses in rice by functioning as a TF in the nucleus and as a novel RNA-binding protein in the cytoplasm. We propose a working model for the dual roles of APIP5 in PCD and plant immunity (Figure 6G). APIP5 displays development- and pathogen-dependent nucleocytoplasmic shuttling, which may be also regulated by other factors such as light, temperature, humidity and phytohormone. Under normal conditions, APIP5 is predominantly localized to the cytoplasm to control the turnover of OsLSD1 and CYP72A1 and suppress their expression. These two functions are essential to avoid auto-immunity and promote plant growth in the absence of M. oryzae. Upon infection with M. oryzae, APIP5 is induced and some of the APIP5 protein in the cytoplasm is translocated into the nucleus during the early stages of infection (Figure 2E). The increased amount of APIP5 in both subcellular compartments leads to the suppression of defense gene transcription and the rapid turnover of defense-related mRNAs, which is beneficial for biotrophic invasion by M. oryzae at this stage. However, when the fungal hyphae grow rapidly inside the rice cells, AvrPiz-t and other effectors are secreted into the cells to suppress the transcriptional activity and accumulation of APIP5 (26). During the late stage of infection (approximately 3 days after inoculation), APIP5 levels gradually decrease in the nucleus, and this protein may be also degraded in the cytoplasm during the infection stage (Figure 2E), which leads to increased gene activation, the accumulation of defense-related transcripts and ultimately the inhibition of fungal infection. Nevertheless, the intrinsic regulatory mechanisms underlying the nucleocytoplasmic partitioning of APIP5 and the APIP5-mediated suppression of OsWAK5 and CYP72A1 expression and OsLSD1 and OsRac1 mRNA turnover warrant further investigation.

DATA AVAILABILITY
The accession number for the data reported in this study is GEO: GSE198135.

SUPPLEMENTARY DATA
Supplementary Data are available at NAR Online.

ACKNOWLEDGEMENTS
We thank Dr Fangfang Li from Institute of Plant Protection, Chinese Academy of Agricultural Sciences for providing CFP-NbDCP1 and mCherry-NbDCP2 plasmids.

FUNDING
National Natural Science Foundation of China [32161143009 to Y.N.; 31801692 to F.Z.]. Funding for open access charge: National Natural Science Foundation of China [32161143009].
Conflict of interest statement. None declared.

REFERENCES

1. Love,A.J., Milner,J.J. and Sadanandom,A. (2008) Timing is everything: regulatory overlap in plant cell death. *Trends Plant Sci.*, 13, 589–595.

2. Mukhtar,M.S., McCormack,M.E., Argueso,C.T. and Pajerowska-Mukhtar,K.M. (2016) Pathogen tactics to manipulate plant cell death. *Curr. Biol.*, 26, R608–R619.

3. Gao,M., Wang,X., Wang,D., Xu,F., Ding,X., Zhang,Z., Bi,D., Cheng,Y.T., Chen,S., Li,X. et al. (2009) Regulation of cell death and innate immunity by two receptor-like kinases in Arabidopsis. *Cell Host Microbe.*, 6, 34–44.

4. de Oliveira,M.V., Xu,G., Li,B., de Souza Vespoli,L., Meng,X., Chen,X., Yu,X., de Souza,S.A., Intorre,A.C., de,A.M.A.M. et al. (2016) Specific control of Arabidopsis BAK1/SERK4-regulated cell death by protein glycosylation. *Nat. Plants.*, 2, 15218.

5. Zhou,J.M. and Zhang,Y.L. (2020) Plant immunity: danger perception and signaling. *Cell*, 181, 978–989.

6. Withers,J. and Dong,X.N. (2017) Post-translational regulation of plant immunity. *Plant Biol.*, 38, 124–132.

7. Burke,R., Schwarze,J., Sherwood,O.L., Jnaid,Y., McCabe,P.F. and Kapczynski,J. (2020) Stressed to death: the role of transcription factors and signaling in cell death. *Cell*, 11, 1235.

8. Meier,I. and Somers,D.E. (2011) Regulation of nucleocytoplasmic trafficking in plants. *Curr. Opin. Plant Biol.*, 14, 538–546.

9. Wang,W.M., Liu,P.Q., Xu,Y.J. and Xiao,S.Y. (2016) Protein trafficking during plant innate immunity. *Int. J. Plant Biol.*, 58, 284–298.

10. McLellan,H., Boevink,P.C., Armstrong,M.R., Pritchard,L., Gomez,S., Morales,J., Whisson,S.C., Beynon,J.L. and Birch,P.R. (2013) An RxLR effector from Phytophthora infestans prevents Canlas,P.E., Jain,R., Chen,X.W. and Ronald,P.C. (2016) A genetic screen identifies a requirement for cysteine-rich-receptor-like kinases in Arabidopsis. *Curr. Opin. Plant Biol.*, 38, 114–126.

11. Yan,J.L., Chen,Q.Q., Cui,X., Zhao,P.Y., Yang,B., Liu,J.X., Kacprzyk,J. and Chong,K. (2008) OsLIC, a novel CCCH-Type zinc finger protein with transcription activation, mediates rice architecture via brassinosteroids signaling. *PLoS ONE*, 3, e3521.

12. Zhai,K.R., Deng,W.Y., Liang,D., Tang,J., Liu,J., Yan,B.X., Yin,X., Lin,H., Chen,F.D., Yang,D.Y. et al. (2019) RRM transcription factors interact with NLRs and regulate broad-spectrum blast resistance in *rice*. *Mol. Cell.*, 74, 996–1009.

13. Wang,R.Y., Ying,Y.S., Shi,X.T., He,F., Zhang,C.Y. and Fan,J.B. (2016) Immunity to rice blast disease by suppression of effector-triggered necrosis. *Curr. Biol.*, 26, 2399–2411.

14. Zhang,C.Y., Fang,H., Shi,X.T., He,F., Wang,R.Y., Fan,J.B., Bai,P.F., Wang,J.Y., Park,C.H., Bellizzi,M. et al. (2020) A fungal effector and a rice NLR protein have antagonistic effects on a Bowman-Birk trypsin inhibitor. *Plant Biotechnol. J.*, 18, 2354–2363.

15. Lee,H.J., Park,J.Y., Seo,P.J., Kim,J.H., Kim,S.G. and Park,C.M. (2015) Systemic immunity requires SnRK2.8-mediated nuclear import of NPR1 in Arabidopsis. *Plant Cell*, 27, 3425–3438.

16. Fang,H., Shen,S.Q., Wang,D., Zhang,Z., Zhang,C.Y., Wang,Z.X., Zhou,Q.Q., Wang,R.Y., Tao,H., He,F. et al. (2021) A monocot-specific hydroxycinnamoylputrescine gene cluster contributes to immunity and cell death in rice. *Sci. Bull.*, 66, 2381–2393.

17. Wi,T., Zhu,Z.W., Chen,M., Yin,J.J., Yang,C., Ran,L., Cheng,M.P., He,M., Wang,K. and Jiang,Y. et al. (2017) A natural allele of a transcription factor in rice confers broad-spectrum blast resistance. *Cell*, 170, 114–126.

18. Kawahara,Y., de la Bastide,M., Hamilton,J.P., Kamamori,H., McComb,W.R., Ouyang,S., Schwartz,D.C., Tanaka,T., Wu,J.Z., Zhou,S.G. et al. (2013) Improvement of the *Oryza sativa* Nipponbare genome using next generation sequence and optical map data. *Rice*, 6, 4.

19. Langmead,B. and Salzberg,S.L. (2012) Fast gapped-read alignment with Bowtie 2. *Nat. Methods*, 9, 357–359.

20. Li,Y.G., Wang,L.G. and He,Q.Y. (2015) ChlPeeker: an R/Bioconductor package for ChlPeak annotation, comparison and visualization. *Bioinformatics*, 31, 2382–2383.

21. Zhang,Y., Liu,T., Meyer,C.A., Eeckhoute,J., Johnson,D.S., Bernstein,B.E., Nussbaum,C., Myers,R.M., Brown,M., Li,W. et al. (2008) Model-based analysis of ChIP-Seq (MACS). *Genome Biol.*, 9, R137.

22. Bailey,T.L., Boden,M., Buske,F.A., Frith,M., Grant,C.E., Clementi,L., Ren,J.Y., Li,W.W. and Noble,W.S. (2009) MEME SUITE: tools for motif discovery and searching. *Nucleic Acids Res.*, 37, W202–W208.

23. Zhou,X.G., Liao,H.C., Chen,M., Yin,J.J., Chen,Y.F., Wang,J.P., Zhu,X., Chen,Z.X., Yuan,C., Zhao,W. et al. (2018) Loss of function of a rice TPR-domain RNA-binding protein confers broad-spectrum disease resistance. *Proc. Natl. Acad. Sci. U.S.A.*, 115, 3174–3179.

24. He,J., Liu,Y.Q., Yuan,D.Y., Duan,M.J., Liu,Y.L., Shen,Z.J., Yang,C.Y., Qiu,Z.Y., Liu,D.M., Wen,P.Z. et al. (2020) An R2R3 MYB transcription factor confers brown plant hopper resistance by regulating the phenylalanine ammonia-lyase pathway in *rice*. *Proc. Natl. Acad. Sci. U.S.A.*, 117, 271–277.

25. Fukushima,R.S. and Hatfield,R.D. (2001) Extraction and isolation of lignin for utilization as a standard to determine lignin concentration.
using the acetyl bromide spectrophotometric method. J. Agric. Food Chem., 49, 3133–3139.
39. Shi,X.T., Long,Y., He,F., Zhang,C.Y., Wang,R.Y., Zhang,T., Wu,W., Hao,Z.Y., Wang,Y., Wang,G.L. et al. (2018) The fungal pathogen Magnaporthe oryzae suppresses innate immunity by modulating a host potassium channel. PLoS Pathog., 14, e1006878.
40. Hu,K.M., Cao,J.B., Zhang,J., Xia,F., Ke,Y.G., Zhang,H.T., Xie,W.Y., Liu,H.B., Cui,Y., Cao,Y.L. et al. (2017) Improvement of multiple agronomic traits by a disease resistance gene via cell wall rearrangement. Nat. Plants, 3, 17009.
41. Malukani,K.K., Ranjan,A., Hota,S.J., Patel,H.K. and Sonti,R.V. (2011) A P450-centric view of plant evolution. Phytochemistry, 62, 149–162.
42. Mizo, I. M. and Sato, F. (2011) Unusual P450 reactions in plant secondary metabolism. Arch. Biochem. Biophys., 507, 194–203.
43. Nelson,D. and Werck-Reichhart,D. (2011) A P450-centric view of plant evolution. Plant J., 66, 194–211.
44. Hamberger,B. and Bak,S. (2013) Plant P450s as versatile drivers for evolution of species-specific chemical diversity. Philos. Trans. R. Soc. Lond., B. Biol. Sci., 368, 20120426.
45. Jacobsy,R.P., Li,L., Huang,S.B., Pong Lee,C., Millar,A.H. and Taylor,N.L. (2012) Mitochondrial composition, function and stress response in plants. J. Integr. Plant Biol., 54, 887–906.
46. Timon-Gomez,A., Nyvltova,E., Abriata,L.A., Vila,A.J., Hosler,J. and Barrientos,A. (2018) Mitochondrial cytochrome c oxidase biogenesis: recent developments. Semin. Cell Dev. Biol., 76, 163–178.
47. Miziutani,M. and Ohta,D. (2010) Diversification of P450 genes during land plant evolution. Annu. Rev. Plant Biol., 61, 291–315.
48. Li,F.F. and Wang,A.M. (2018) RNA decay is an antiviral defense in plants that is counteracted by viral RNA silencing suppressors. PLoS Pathog., 14, e1007228.
49. Xu,J., Yang,J.Y., Niu,Q.W. and Chu,H.N. (2006) Arabidopsis DCP2, DCP1, and VARICOSE form a decapping complex required for postembryonic development. Plant Cell, 18, 3386–3398.
50. Jan,A., Maruyama,K., Todaka,D., Kidokoro,S., Abo,M., Yoshimura,E., Shinozaki,K., Nakashima,K. and Yamaguchi-Shinozaki,K. (2013) OsTZF1, a CCCH-Tandem zinc finger protein, confers delayed senescence and stress tolerance in rice by regulating stress-related genes. Plant Physiol., 161, 1201–1216.
51. Wang,L.J., Pei,Z.Y., Tian,Y.C. and He,C.Z. (2005) OsLSL1, a rice finger protein, regulates programmed cell death and callus differentiation. Mol. Plant Microbe Interact., 18, 375–384.
52. Liu,J.L., Park,C.H., He,F., Nagano,M., Wang,M., Bellizzi,M., Zhang,K., Zeng,X.S., Liu,W.D., Ning,Y.S. et al. (2015) The RhOGAP SPIN6 associates with SPL11 and OsRac1 and negatively regulates programmed cell death and innate immunity in rice. PLoS Pathog., 11, e1004629.
53. Wong,H.L., Pinontono,R., Hayashi,K., Tabata,R., Yaeno,T., Hasegawa,K., Kojima,C., Yoshioka,H., Iba,K., Kawasaki,T. et al. (2007) Regulation of rice NADPH oxidase by binding of Rac GT-Pase to its N-terminal extension. Plant Cell, 19, 4022–4034.
54. Amsbury,S. (2020) Sensing attack: the role of wall-associated kinases in plant pathogen responses. Plant Physiol., 183, 1420–1421.
55. Kohorn,B.D. and Kohorn,S.L. (2012) The cell wall-associated kinases, WAKs, as pectin receptors. Front. Plant Sci., 3, 88.