

Supplementary Information for

# **A class of independently evolved transcriptional repressors in plant RNA viruses facilitates viral infection and vector feeding**

Lulu Li<sup>1,2#</sup>, Hehong Zhang<sup>1#</sup>, Changhai Chen<sup>1</sup>, Haijian Huang<sup>1</sup>, Xiaoxiang Tan<sup>1</sup>, Zhongyan Wei<sup>1</sup>, Junmin Li<sup>1</sup>, Fei Yan<sup>1</sup>, Chuanxi Zhang<sup>1</sup>, Jianping Chen<sup>1\*</sup> and Zongtao  $Sun^{1*}$ 

Corresponding Author: Zongtao Sun Email: sunzongtao@nbu.edu.cn Corresponding Author: Jianping Chen Email: jpchen2001@126.com

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Supplementary text Figs.S1-S23 Table S1 References for SI reference citations

### **Materials and methods**

#### **Screening the cDNA Library**

Following the protocol of the BD Matchmaker™ Library Construction and Screening Kits User Manual, whole viral proteins of SRBSDV were used as baits to screen a rice cDNA library in the yeast strain AH109, and then the transformants were transferred to SD/-Leu/-Trp/-His selective medium for about 3 days at 30°C. Following harvest of colonies from SD/-Leu/-Trp/-His/-Ade liquid medium, these positive yeast plasmids were extracted by the TIANprep Yeast plasmid DNA kit (TIANGEN, Cat: #DP112-02) and subsequently introduced into *E. coli* DH5α competent cells, to identify the different interacting proteins by sequencing.

#### **Yeast Two-Hybrid Assay**

Specific interactions were verified by the yeast two-hybrid (Y2H) assay. The full-length coding sequence of viral proteins (SRBSDV SP8, RBSDV P8, RSV P2 and RSMV M) and truncated sequences including SP8<sup>NTP</sup> (in which residue of the conserved NTP-binding motif "GNKGVGKS" was substituted with Ala), SP8<sup>N1</sup> (aa 1-337), SP8<sup>N2</sup> (aa 1-396), SP8<sup>C1</sup> (aa 338-592) and SP8<sup>C2</sup> (aa 397-592) were amplified by PCR using the primers listed in Table S1 and inserted into the bait vector pGKBT7. In addition, the full-length coding sequences of the OsMYCs (OsMYC2, OsMYC3 and OsMYC4), OsJAZ genes (OsJAZ1-15, except OsJAZ2) and their different truncated variants including OsMYC3<sup>N</sup> (aa 1-320), OsMYC3TAD<sup>123</sup> (aa 121-320), OsMYC3TAD<sup>12</sup> (aa 121-250), OsMYC3TAD<sup>23</sup> (aa 180-320), OsMYC3<sup>C</sup> (aa 321-474), OsMYC3<sup>jid</sup> (in which the conserved JID motif  $^{82}$ GWGD<sup>85</sup>" was replaced by  $^{82}$ AAAA $^{85}$ ") and OsJAZ-ZIM (in which the conserved ZIM motif "TIFY" was replaced by "AAAA") were amplified using the primers listed in Table S1, and then cloned into the pGADT7 vectors. Various interaction pairs were co-transformed into the yeast strain AH109 according to the manufacturer's instructions (Takara,

Japan), the transformants were cultivated on SD/-Leu/-Trp plates, and positive clones were transferred to and grown on SD/-Leu/-Trp/-His/-Ade plates. Yeast cells were photographed after 3 days at 30°C to record growth. All experiments were repeated three times with similar results.

#### **Agro-infection assays in** *N. benthamiana*

The recombinant binary expression vectors were transformed into *Agrobacterium tumefaciens* strain GV3101 by electroporation and then grown on LB medium containing 50  $\mu$ g.mL $^{-1}$  of kanamycin and rifampicin and 10  $\mu$ g.mL $^{-1}$  of tetracycline for about 2 days at 28°C. The bacterial cultures were collected by centrifuging at 5000 *rpm* for 2 min and suspended in an induction buffer (10 mM  $MgCl<sub>2</sub>$ , 10 mM MES (pH 5.6), 200 µM Acetosyringone) to  $OD_{600}=1.0$  for 2 hours. After mixing equal quantities of the selected combinations, the suspension was infiltrated into approximately 6-week-old *N. benthamiana* leaves.

#### **Bimolecular fluorescence complementation (BiFC)**

For the generation of the BiFC vectors, the full-length cDNAs of viral proteins (SRBSDV SP8, RSV P2 and RSMV M), OsMYC3, OsMED25 and a set of OsJAZs were amplified by PCR (using the primers listed in Table S1), and then introduced into cYFP and nYFP vectors. Specifically, the different constructs used were as follows: SP8-cYFP, SP8-nYFP, P2-cYFP, P2-nYFP, M-cYFP, M-nYFP, OsMYC3-cYFP, OsMYC3-nYFP, OsMED25-cYFP, OsMED25-nYFP, OsJAZ4-cYFP, OsJAZ4-nYFP, OsJAZ5-cYFP, OsJAZ5-nYFP, OsJAZ9-cYFP, OsJAZ9-nYFP, OsJAZ11-cYFP, OsJAZ-nYFP, OsJAZ12-cYFP and OsJAZ12-nYFP. These vector combinations were infiltrated into *N. benthamiana* leaves at 23°C for 48 hours, the YFP fluorescence signal was captured using a Leica TCS SP5 confocal laser scanning microscope. Three biological repeats were conducted for all experiments.

#### **Co-immunoprecipitation (Co-IP)**

For Co-IP assays, the full-length of viral proteins (SP8, P2, M), OsJAZs, OsMYC2, OsMYC3 and OsMED25, as well as partially truncated versions, were amplified by PCR (using the primers listed in Table S1) and cloned into pCV-3×myc-N1, pCV-3×flag-N1 or pCV-GFP-N1 vectors, respectively. The chosen constructs, for example SP8-myc and its derivate SP8<sup>NTP</sup>-myc, generally driven by a CaMV 35S promoter, were co-expressed respectively with OsMYC3-flag or OsJAZs-flag in *N. benthamiana* leaves. After 48 hours, the leaf tissue was harvested and ground into powder with liquid nitrogen. The total proteins were extracted using cold IP buffer (25 mM Tris-HCl (pH=7.4), 150 Mm NaCl, 1% NP-40, 1Mm EDTA and 5% glycerol with 1 mM DTT and 1 mM PMSF) for 10 min on ice and centrifuged at 1000 ×g and 4°C for about 10 min (1), and then the soluble proteins were carefully transferred to a new microcentrifuge tube (100 μl of the supernatant was used as input control) and the others incubated with 15 μl FLAG-trap beads at 4°C for approximately 2 hours. After that, the supernatant was discarded and the precipitated beads were washed at least three times in cold 1xPBS buffer with protease inhibitor cocktail to remove the nonspecifically bound proteins. Finally, the corresponding volume of 5×SDS-PAGE loading buffer was added and boiled for 5 min before immunoblot analysis with anti-myc and anti-flag antibodies.

#### **Luciferase complementation imaging (LCI) assay**

For the LUC complementation assays, the full-length coding sequences of viral proteins (SRBSDV SP8, RSV P2 and RSMV M), OsMYC (OsMYC2 and OsMYC3) and OsMED25 were ligated into *pCAMBIA1300-cLUC* and *pCAMBIA1300-nLUC* vectors, respectively. The recombinant constructs were transformed into *A. tumefaciens* strain GV3101, and infiltrated into different areas of the same *N. benthamiana* leaf with a final concentration of  $OD_{600} = 1.0$ . The 0.2 mM LUC substrate was then infiltrated into the same areas and imaged at 2 dpi using a low-light cooled CDD imaging apparatus (LUMAZONE

SOPHIA2048B, USA) (2). In the competition LUC complementation assay, GV3101 strains harboring OsMED25-Cluc and OsMYC3-Nluc with viral proteins (SP8-myc, P2-myc or M-myc) were co-infiltrated into *N. benthamiana*  leaves, Gus-myc was co-infiltrated as a negative control.

#### **Generation of transgenic plants**

To generate transgenic rice plants constitutively over-expressing SP8 or OsMYC3, the recombinant binary expression vectors SP8-myc and OsMYC3-myc, driven by a CaMV 35S promoter, were introduced into *Agrobacterium tumefaciens* strain EHA105 and used to transform respectively *Nip* or *ZH11* rice background. The relative expression levels in transgenic rice seedlings were verified by qRT-PCR and western blot. In addition, the *Osmyc3* mutants for *OsMYC3* knockout in *ZH11* background were produced using the CRISPR/Cas9 system and the homozygous lines were further selected by sequencing.

#### **Root growth inhibition assay**

Transgenic seeds of each genotype  $(≥ 20$  seeds per line) were sprinkled on floating plates in rice nutrient solution with various concentrations of MeJA (0, 0.1 and 1 μM) and germinated under short day conditions (8 h light, 25°C/ 16 h dark, 30°C). The relative root lengths, a phenotype used to evaluate different sensibilities mediated by JA response, were measured and recorded after a week. Each measurement was determined in biological triplicate.

#### **Dual luciferase transient transcriptional activity assay**

In Dual-LUC assays, the reporter consisted of the promoter of OsJAZ4 fused with the *pGreen*Ⅱ*0800-Luc* vector (*pOsJAZ4::LUC*), which contains the complete renilla (REN) and firefly (LUC) luciferase reporter genes. This was then agroinfiltrated into *N. benthamiana* leaves in combination with one of the effectors, such as OsMYC3-flag, SP8-myc and SP8<sup>NTP</sup>-myc. At 48 hpi, a disc with a diameter of 0.6 cm was removed from the leaves using a cork borer, placed in a new 2.0 ml microcentrifuge tube, shattered using an automatic oscillator (JingXinTissuelyser-96), and 300 μl 1×PLB (Passive Lysis Buffer) added with gentle shaking to promote rapid lysis of leaf tissues. After centrifugation at 10000×g and 4°C for about 30 sec, 20 μl of the cleared lysates were transferred into wells of a 96-well plate with 100 μl substrate LAR II (Luciferase Assay Reagent II) and the firefly luciferase (LUC) luminescence was initially measured using a 96-well microplate reader (BioTek synergy H1). After this, the activity of the firefly luciferase was quenched by injecting 100 μl diluted 1×Stop & Glo® Reagent to each well and the renilla luciferase (REN) luminescence was then automatically measured (Promega) (3). In general, the REN luminescence was considered as an internal control, thus, the relative luciferase activity was ultimately calculated as the ratio LUC/REN. All these measurements were made from three independent replicates.

#### **Virus inoculation assays**

The methods of virus inoculation including virus acquisition and transmission have been described previously (4). RSV was naturally transmitted by the small brown planthopper (SBPH) and SRBSDV by the white-backed planthopper (WBPH). To adequately acquire virus, quantities of virus-free second-instar nymphs of SBPH or WBPH were allowed to feed continuously on RSV- or SRBSDV-infected rice plants in three independent beakers for 3 days, and then transferred onto healthy Wuyujing3 rice seedlings for about 10 days to accomplish the whole viral multiplication and circulation in planthoppers. Next, the transgenic rice seedlings were infested with viruliferous or virus-free (negative control) planthoppers at the third leaf stage for 3 days, after which the insects were removed completely and these rice seedlings were grown in the field for symptom development. The incidence of plants infected with virus was further determined based on results of RT-PCR at 30 dpi.

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#### **RNA extraction and Quantitative RT-PCR**

Total RNA was extracted from five independent biological replicates of healthy and infested rice plants using the TRIzol reagent (Invitrogen). 1 μg of total RNA was pretreated with gDNA wiper mix to eliminate genomic DNA and then reverse transcribed to cDNA using HiScript® III qRT Super Mix (Vazyme). The actin gene *OsUBQ5* was used to normalize the relative expression levels of viral genes which were determined by quantitative RT-PCR (qRT-PCR) with the Hieff qPCR SYBR® Green Master Mix (Yeasen) and analyzed by the 2<sup>-ΔΔCt</sup> method using an ABI7900HT Sequence Detection System (5, 6).

#### **Western blot analysis**

To analyze the accumulation level of viral proteins in different transgenic rice plants, total proteins were extracted from infected rice leaves by 20% SDS lysis buffer (100 mM Tris-HCl (PH=6.8), 20% SDS, 2% β-mercaptoethanol) and separated on 10%-20% SDS-PAGE gels before transfer to the pre-activated PVDF membrane. The monoclonal antibody anti-P10 specifically recognized both RBSDV and SRBSDV while RSV was detected by an anti-CP antibody. After incubating with IgG-HRP antibody, the protein membranes were imaged using ECL substrate and the BIO-RAD ChemiDoc MP Imaging System.

#### **Honeydew measurement**

The production of honeydew was initially determined to the nearest 0.01 mg with a Mettler XS205 balance. In this assay, fifth-instar adult SBPH or WBPH were selected to pre-starve for about 2 hours and then individually caged on a rice seedling in 5 cm diameter clip-cages, lined with laboratory parafilm (Bemis, USA) under long day conditions (16 h light, 30°C / 8 h dark, 25°C) (7, 8). After 36 hours feeding, the accumulation of honeydew was

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calculated from the difference between the total weight of each clip-cage and the corresponding value after honeydew had been removed.

#### **Statistical analysis**

Differences were analyzed using one-way or two-way ANOVA with Fisher's least significant difference tests. A *p*-value ≤ 0.05 was considered statistically significant. All analyses were performed using ORIGIN 8.0 software.

**Accession numbers:** Sequence data from this study can be found in the rice genome annotation project database under the following accession numbers:





### **Fig. S1. SP8 interacts with OsMYC3.**

**(A)** Scheme for co-expression in Luciferase complementation imaging (LCI) assays in leaves of *N. benthamiana*.

**(B)** Results from LCI assays showing the interactions between SP8 and OsMYC2 or OsMYC3. Co-expression of OsMYC2 and OsMYC3 acts as positive control, and the empty cLUC vector acts as negative control.



**Fig. S2. RBSDV P8 interacts with OsMYC3.** Y2H assays illustrating the interaction between RBSDV P8 and OsMYCs family. The different combinations of constructs transformed into yeast cells were grown on SD-L-T-H-Ade plates at 30°C. Photos were taken after 3 days.





**(A)** Interaction patterns of OsMYC2 and OsMYC3 with various OsJAZ family proteins. Multiple OsJAZs proteins fused with BD vector were transformed together with OsMYC2 or OsMYC3 fused with AD vector into yeast strain AH109 and positive transformants were selected on SD-L-T-H-Ade plates at 30°C. Photos were taken after 3 days.

**(B)** BiFC assays confirming the interaction between OsMYC3 and OsJAZ proteins. OsMYC3-nYFP were agro-injected together with OsJAZs-cYFP (OsJAZ5, OsJAZ9 and OsJAZ11) and imaged by confocal microscopy at 48 hpi.

**(C)** Schematic diagrams showing the direct mutagenesis of OsMYC3 and its effect on the interaction with various OsJAZ proteins. The right panel shows that the JID domain of OsMYC3 is required for the interaction. In the Y2H system, OsJAZ proteins were fused with BD while OsMYC3 and its mutant derivatives were fused with AD yeast vectors.

**(D)** Phenotypes of *Osmyc3* mutants grown for 7 days on the rice nutrient solution containing indicated concentrations of MeJA. The root lengths of *Osmyc3* seedlings were mildly inhibited by MeJA compared with the *ZH11*  control.

**(E)** Quantification of the root lengths of *Osmyc3* lines and *ZH11* background. Error bars represent SD. \* indicates a significant difference between samples (at least 15 seedlings) analyzed by one-way ANOVA and evaluated at *p* ≤ 0.05 by Fisher's least significant difference tests.

**(F to K)** Results of QRT-PCR to determine the transcription levels of JA synthesis (*OsLOX1*, *OsLOX2*, *OsAOC*) and signaling related genes (*OsJAmyb*, *OsJAZ8*, *OsJAZ9*) in wild-type *ZH11* rice seedlings and *Osmyc3* mutant lines treated with different concentrations of MeJA. Error bars represent SD. \* indicates a significant difference between samples analyzed by one-way ANOVA and evaluated at *p* ≤ 0.05 by Fisher's least significant difference tests.





**(A)** Sequencing of the mutated sites in homozygous *Osmyc3* lines. Upper panel represents the target gene editing site of *OsMYC3* using the CRISPR/cas9 system.

**(B)** Schematic diagram of OsMYC3 regions with amino acid (aa) positions.

**(C)** OsMYC3 binding sites in the *OsJAZ4* promoter. Black triangles indicate the G-box residues (CACGTG) numbered to show the nucleotide position ahead of the OsJAZ4 protein-coding region.



**Fig. S5. SP8 compromised induction of JA-mediated defense marker genes in** *vivo***.** Results of QRT-PCR to determine the transcription levels of JA synthesis (*OsLOX1*, *OsLOX2*, *OsAOC*) and signaling related genes (*OsJAmyb*, *OsJAZ8*, *OsJAZ9*) in wild-type *Nip* rice seedlings and *SP8-ox* transgenic lines treated with MeJA. Error bars represent SD. \* indicates a significant difference between samples analyzed by one-way ANOVA and evaluated at *p* ≤ 0.05 by Fisher's least significant difference tests.



# **Fig.S6. Mapping the domain of OsMYC3 required for the interaction with OsMED25.**

The conserved TAD motif of OsMYC3 is required for interaction with OsMED25. For schematic diagrams of TAD mutants of OsMYC transcription factors refer to Fig. 2*F*. TAD derivatives and OsMED25 were fused respectively with the AD and BD yeast vectors. Positive transformants were selected on SD-L-T-H-Ade plates and photographed after 3 days.



### **Fig.S7. SP8 disturbs the association between OsMED25 and OsMYC3.**

Quantification of the fluorescence intensity from leaves co-expressing OsMED25-cYFP and OsMYC3-nYFP with or without SP8-myc. Data are means from 30 to 50 transfected cells. Error bars represent SD, \*\*\* indicates a significant difference between samples analyzed by ANOVA at  $p \le 0.01$  by Fisher's least significant difference tests.



### **Fig.S8. Mapping the domain of OsJAZ-interacting with SP8.**

**(A)** Y2H assays to identify the possible domain of OsJAZ4 that can interact with SP8. Upper panel shows schematic diagrams of OsJAZ4 and its derivatives.

**(B)** ZIM site-direct mutagenesis destroyed the interaction between OsJAZs and SP8. Upper panel shows a schematic diagram of OsJAZs-ZIM mutants.



**Fig.S9. RBSDV P8 interacts with multiple OsJAZ proteins.** Y2H assays illustrating the interaction between RBSDV P8 and multiple OsJAZ proteins (OsJAZ1-15, except OsJAZ2). The different combinations of constructs transformed into yeast cells were grown on SD-L-T-H-Ade plates at 30°C. Photos were taken after 3 days.



### **Fig.S10. SP8 does not disturb the OsJAZ-OsMYC3 association.**

BiFC assays confirming that SP8 protein did not disturb the association of OsJAZs proteins and OsMYC3. The samples were imaged by confocal microscopy at 48 hpi.





**(A)** Y2H assays examining the ability of SP8<sup>NTP</sup> to interact with various OsJAZ proteins. The preys indicated (AD-OsJAZs) were co-transformed with BD-SP8 or BD-SP8<sup>NTP</sup> into yeast strain AH109 and grown on SD-L-T-H-Ade selective medium for 3 days.

**(B)** Co-IP assay showing that the SP8<sup>NTP</sup> mutant did not interact with OsJAZs *in vivo*. Total proteins were extracted from *N. benthamiana* leaves expressing SP8<sup>NTP</sup>-myc and OsJAZ11-flag, the supernatant was precipitated with FLAG beads and the associated proteins were verified using anti-myc antibody. The sample expressing SP8<sup>NTP</sup>-myc and OsJAZ11-flag was a positive control while co-expression with SP8<sup>NTP</sup>-myc and flag-GFP served as a negative control.

**(C)** Schematic representation of the deleted variations of SP8 used in the yeast two-hybrid assay.

**(D)** Interactions between different NTP- truncated SP8 mutants with OsJAZ and OsMYC3 proteins. The preys indicated (AD-OsJAZs) were co-transformed with BD-SP8, BD-SP8<sup>N1</sup>, BD-SP8<sup>N2</sup>, BD-SP8<sup>C1</sup> and BD-SP8<sup>C2</sup> into yeast strain AH109 and grown on SD-L-T-H-Ade selective medium for 3 days.



**Fig.S12. The effect of OsJAZ proteins on the stability of SP8 in** *vivo***.** The relative accumulation levels of SP8 protein co-expressed with various OsJAZ proteins or negative control (GFP-flag) in the leaves of *N. benthamiana*  analyzed by immunoblot.



**Fig.S13. The effect of SP8 on the stability of OsJAZ proteins in** *vivo***. (A-D)** The GFP fluorescence of different OsJAZ proteins co-expressed with SP8 or GUS-myc (negative control) in the leaves of *N. benthamiana* under the laser scanning confocal microscope. The samples were observed 36 hours after infiltration.

**(E-H)** The relative accumulation levels of different OsJAZ proteins co-expressed with SP8 protein or negative control (Gus-myc) in the leaves of *N. benthamiana* analyzed by immunoblot.



**Fig.S14. phylogenetic tree of MYC transcription factors.** Homology and phylogenetic analysis showing the genetic relationships between OsMYC transcription factors from different species according to their amino acid sequences. Tree created using MEGA6 software.



# **Fig.S15. Mapping the domain of OsMYC3 that interacts with viral P2 or M proteins.**

The conserved TAD motif of OsMYC3 is required for interaction with RSV P2 and RSMV M protein. For schematic diagrams of TAD mutants of OsMYC transcription factors refer to Fig. 2*F*. TAD derivatives and viral proteins P2/M were respectively fused with the AD and BD yeast vectors. Positive transformants were selected on SD-L-T-H-Ade plates and photographed after 3 days.



# **Fig.S16. RSV P2 suppresses the transcriptional activation of OsMYC dimerization.**

**(A)** BiFC assays confirm the interaction between OsMYC2 and OsMYC3. OsMYC2 and its homologue OsYC3 were respectively cloned into cYFP and nYFP vectors, and then agro-infiltrated together into *N. benthamiana* leaves. The samples were imaged by confocal microscopy at 48 hpi.

**(B)** Co-IP assay to examine the interaction between OsMYC2 and OsMYC3 in *vivo*. Total proteins were extracted from *N. benthamiana* leaves co-expressing OsMYC2-myc and OsMYC3-flag, the supernatant was precipitated with FLAG beads and the associated proteins were verified using anti-myc antibody. The samples expressing OsMYC2-myc or OsMYC3-flag alone served as negative controls.

**(C)** Scheme of LCI assays co-expressed in leaves of *N. benthamiana*.

**(D)** Results from LCI assays showing the interactions between OsMYC2 and OsMYC3. Co-expression of OsMYC2 and SP8 acts as negative control.

**(E)** Schematic diagrams of the reporter and a series of effectors employed in the GD system. The reporter gene *LUC* driven by *5×GAL4* promoter specifically recognized GD effectors driven by the CaMV 35S promoter. REN, renilla luciferase, an internal control; LUC, firefly luciferase.

**(F)** RSV P2 represses the transcription activity of OsMYC2 and OsMYC3 in the GD system. The *5×GAL4*::*LUC* reporter was infiltrated with the GD-OsMYC3 and P2-myc effectors, while P2-myc injection with empty GD effector was the negative control. Data were analyzed by the ratio LUC/REN. Error bars represent SD (n=6); \* indicates a significant difference between samples at *p* ≤ 0.05 by Fisher's least significant difference tests.



# **Fig.S17. Viral proteins RSV P2 and RSMV M disturb the association between OsMED25 and OsMYC3.**

Quantification of the fluorescence intensity from leaves co-expressing OsMED25-cYFP and OsMYC3-nYFP with or without viral proteins P2/M-myc. Data are means from 30 to 50 transfected cells. Error bars represent SD, \*\*\* indicates a significant difference between samples analyzed by ANOVA at p ≤ 0.01 by Fisher's least significant difference tests.



# **Fig.S18. Neither RSV P2 nor RSMV M protein disturbed the OsJAZ-OsMYC3 association.**

**(A)** RSV P2 and RSMV M protein interacted with OsJAZ family proteins in yeast cells. RSV P2 and RSMV M protein fused with BD and OsJAZ proteins fused with AD vector were co-transferred onto SD-L-T-H-Ade medium. Photos were taken after 3 days.

**(B)** BiFC assays confirmed the interaction between RSV P2 and OsJAZ proteins. RSV P2 was fused with the cYFP vector, while different OsJAZ proteins were respectively cloned into the nYFP vector, and then agro-infiltrated together into *N. benthamiana* leaves. The samples were imaged by confocal microscopy at 48 hpi.

**(C)** BiFC assays confirmed that RSV P2 did not disturb the OsJAZ11-OsMYC3 association. The samples were imaged by confocal microscopy at 48 hpi.

**(D)** BiFC assays confirmed that RSMV M protein did not disturb the OsJAZ11-OsMYC3 association. The samples were imaged by confocal microscopy at 48 hpi.

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**Fig.S19. The negligible effect of RSV P2 and RSMV M on the stability of OsJAZ proteins in** *vivo***.**

**(A-D)** The GFP fluorescence of different OsJAZ proteins co-expressed with viral protein (P2 and M) or GUS-myc (negative control) in the leaves of *N. benthamiana* under the laser scanning confocal microscope. The samples were observed 36 hours after infiltration.

**(E and F)** The relative accumulation levels of different OsJAZ proteins co-expressed with viral protein (P2 and M) or negative control (GUS-myc) in the leaves of *N. benthamiana* analyzed by immunoblot.





**(A, B)** Symptoms in OsMYC3 OE lines (*OsMYC3-14* and *OsMYC3-23*) (A) and *Osmyc3* mutants (*Osmyc3-1* and *Osmyc3-12*) (B) 20 days after mock-inoculation or RSV-infection. The diseased rice plants were verified by RT-PCR. Scale bar, 10 cm.

**(C, D)** QRT-PCR results showing the transcription levels of *CP* in RSV-infected OsMYC3 OE lines (C) and *Osmyc3* mutants (D) at 30 dpi, *OsUBQ5* was used as the internal reference gene. Data were compared with *ZH11* background from three biological replicates in a one-way ANOVA and evaluated at *p* ≤ 0.05 by Fisher's least significant difference tests.

**(E)** Western blot to assess the accumulation of RSV CP protein in RSV-infected *Osmyc3* mutants and OsMYC3 OE lines compared with *ZH11* at 30 dpi. Total proteins were extracted from RSV-infected transgenic rice leaves and examined by anti-CP antibody.





**(A)** Phenotypes of *OsMYC3-ox*, *Osmyc3* mutants and *ZH11* plants 7 days after infestation with five adult SBPHs per seedling.

**(B)** Mortality rate of *OsMYC3-ox*, *Osmyc3* mutants and *ZH11* plants infested by adult SBPHs. Approximately 20 seedlings per genotype were infested with SBPH and the mortality was assessed at 5 dpi and 7 dpi. Data were analyzed by one-way ANOVA and evaluated at *p*≤0.05 by Fisher's least significant difference tests.

**(C)** Phenotypes of *Oscoi1-13* mutants and *Nip* plants 7 days after infestation with five adult SBPHs per seedling.

**(D)** Mortality rate of *Oscoi1-13* mutants and *Nip* plants infested by adult SBPHs. Approximately 20 seedlings per genotype were infested with SBPH and the mortality was assessed at 5 dpi and 7 dpi. Data were analyzed by ANOVA and evaluated at *p*≤0.05 by Fisher's least significant difference tests.

**(E)** Total honeydew secreted from each adult SBPH individually fed on *OsMYC3-ox*, *Osmyc3* mutants, *Oscoi1-13* mutants and *Nip* or *ZH11* wild type plants for about 36 hours. Data were analyzed by ANOVA and evaluated at *p*≤0.05 by Fisher's least significant difference tests.

**(F)** Mortality rate of SBPH. Approximately 30 seedlings per genotype were each infested with four virus-free SBPH and the mortality was assessed at 5 dpi and 7 dpi.

**(G)** Total honeydew secreted from each adult SBPH individually fed on *OsRSVP2-ox* transgenic rice plants and *Nip* controls for about 36 hours. Data were analyzed by ANOVA and evaluated at *p*≤0.05 by Fisher's least significant difference tests.

**(H)** Total honeydew secreted from each adult SBPH individually fed on healthy and RSV-infected rice plants for about 36 hours. Data were analyzed by ANOVA and evaluated at *p*≤0.05 by Fisher's least significant difference tests.

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**Fig. S22. A proposed working model describing the role of viral transcriptional repressors in JA-mediated resistance against viruses and vector insects.** In the nucleus, several different types of plant RNA virus are involved in reprogramming the phytohormone network. In the rice–virus interaction, viral proteins target a key component of auxin signaling (OsARF17) to inhibit OsARF17-mediated antiviral response by different strategies. On the other hand, these conserved viral transcriptional repressors restrain the transcription of OsMYC2 and OsMYC3 (or merely OsMYC3) through physical interaction with their TAD domain. Simultaneously, these repressors compete

with MED25 for binding with MYC3, thus disturbing the association of OsMYC3 and OsMED25. At the same time, the repressors cooperate with a series of OsJAZ proteins to form a transcriptional repression complex. To a large degree, OsJAZ proteins directly enhance the activity of viral transcriptional repressors, leading to the aggravated attenuation of the JA response, thereby conferring a benefit both to virus infection and vector feeding.



**Fig. S23. Full scan data of the immunoblots in this work. Bands shown in main figures are indicated by red asterisk.**















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