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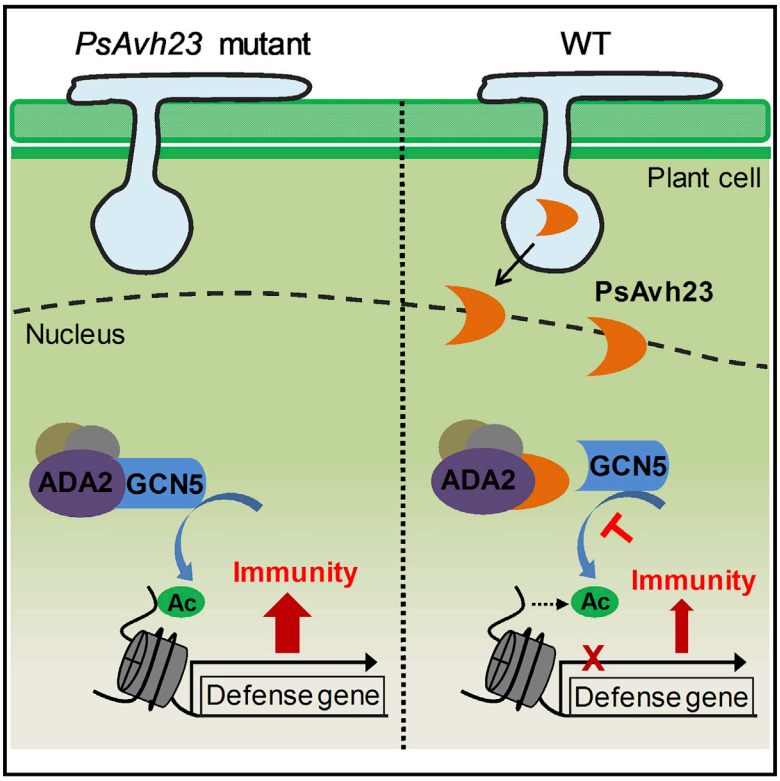
Current Biology

A *Phytophthora* Effector Manipulates Host Histone Acetylation and Reprograms Defense Gene Expression to Promote Infection

Graphical Abstract



Highlights

- PsAvh23 is an essential effector for full virulence of *Phytophthora sojae*
- Binding to ADA2, PsAvh23 disrupts the formation of the ADA2-GCN5 subcomplex
- PsAvh23 suppresses host ADA2/GCN5-mediated H3K9ac levels to enhance susceptibility
- Misregulation of defense genes is most likely due to the decrease of H3K9ac levels

Authors

Liang Kong, Xufang Qiu, Jiangang Kang, ..., Suomeng Dong, Wenbo Ma, Yuanchao Wang

Correspondence

smdong@njau.edu.cn (S.D.), wangyc@njau.edu.cn (Y.W.)

In Brief

Kong et al. find that *Phytophthora* effector PsAvh23 competitively binds to ADA2 to disrupt ADA2-GCN5 subcomplex formation and subsequently represses the expression of defense genes by decreasing GCN5-mediated H3K9ac levels, suggesting that the pathogen manipulates host histone acetylation to gain virulence.

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A *Phytophthora* Effector Manipulates Host Histone Acetylation and Reprograms Defense Gene Expression to Promote Infection

Liang Kong,[1] Xufang Qiu,[1] Jiangang Kang,[1] Yang Wang,[1] Han Chen,[1] Jie Huang,[1] Min Qiu,[1] Yao Zhao,[1,2] Guanghui Kong,[1] Zhenchuan Ma,[1] Yan Wang,[1,3] Wenwu Ye,[1,3] Suomeng Dong,[1,3,*] Wenbo Ma,[2] and Yuanchao Wang[1,3,4,*]
[1]Department of Plant Pathology, Nanjing Agricultural University, Nanjing, Jiangsu 210095, China
[2]Department of Plant Pathology and Microbiology, University of California, Riverside, Riverside, CA 92521, USA
[3]The Key Laboratory of Integrated Management of Crop Diseases and Pests, Ministry of Education, Nanjing, Jiangsu 210095, China
[4]Lead Contact
*Correspondence: smdong@njau.edu.cn (S.D.), wangyc@njau.edu.cn (Y.W.)
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SUMMARY

Immune response during pathogen infection requires extensive transcription reprogramming. A fundamental mechanism of transcriptional regulation is histone acetylation. However, how pathogens interfere with this process to promote disease remains largely unknown. Here we demonstrate that the cytoplasmic effector PsAvh23 produced by the soybean pathogen *Phytophthora sojae* acts as a modulator of histone acetyltransferase (HAT) in plants. PsAvh23 binds to the ADA2 subunit of the HAT complex SAGA and disrupts its assembly by interfering with the association of ADA2 with the catalytic subunit GCN5. As such, PsAvh23 suppresses H3K9 acetylation mediated by the ADA2/GCN5 module and increases plant susceptibility. Expression of *PsAvh23* or silencing of *GmADA2/GmGCN5* resulted in misregulation of defense-related genes, most likely due to decreased H3K9 acetylation levels at the corresponding loci. This study highlights an effective counter-defense mechanism by which a pathogen effector suppresses the activation of defense genes by interfering with the function of the HAT complex during infection.

INTRODUCTION

Animals and plants are engaged in an endless arms race with microbial pathogens. Detection of potential pathogens in the environment leads to the activation of immune responses [1]. In plants, recognition of microbe-associated molecular patterns (MAMPs) activates the mitogen-activated protein kinase (MAPK)-signaling pathway and results in the transcriptional reprogramming of defense-related genes [2]. On the contrary, successful pathogens deliver a multitude of effector proteins into the host cells; collectively, these effectors are indispensable for infection due to their essential role in defeating plant immunity. For example, *Pseudomonas syringae* effector HopM1 induces establishment of the aqueous living space in plants for bacterial reproduction [3]. Understanding effector functions not only provides key insights into microbial pathogenesis, but also reveals regulatory mechanisms of innate immunity [4].

An important pathosystem that has been used as a model to understand effector functions is the interaction between soybean and the stem and root rot pathogen, *Phytophthora sojae* [5]. *P. sojae* is one of the most destructive pathogens of soybean, causing around $1–$2 billion in losses per year worldwide [6]. Like other *Phytophthora* pathogens, *P. sojae* secretes ~400 cytoplasmic effectors, which are delivered into plant cells to directly manipulate host immunity [7]. In *Phytophthora*, many cytoplasmic effectors depend on the RxLR (Arg-any amino acid-Leu-Arg) motif for translocation into host cells [8]. Accumulating evidence demonstrates that RxLR effectors utilize various mechanisms to promote infection [9–11]. In this study, we report a novel mechanism that is utilized by the *P. sojae* effector PsAvh23, which manipulates histone acetylation to counteract host defense.

Transcriptional reprogramming is a central component of plant immunity [12], and histone acetylation has been implicated as an important mechanism to activate immunity genes [13]. Histone acetylation is a highly dynamic process where specific lysine residues on the N-terminal tail of histones are acetylated by histone acetyltransferases (HATs) or deacetylated by histone deacetylases (HDACs) [14]. In rice, the HDAC HDT701 negatively regulates innate immunity by decreasing the acetylation levels of H4K5/16, which leads to reduced expression of defense-related genes [15]. In *Arabidopsis thaliana*, Elongator Protein 3 (ELP3), with HAT activity, positively regulates immune response by enhancing defense gene expression [16]. On the other hand, modulation of histone acetylation by pathogens can impair plant defense. The maize pathogen *Cochliobolus carbonum* produces the HC-toxin as a potent inhibitor of maize HDAC. HC-toxin is required for infection, probably by interfering with the expression of maize genes that are necessary for mounting effective immunity [17, 18]. Although tight/precise control of gene expression is critical for defense [19], how effectors may manipulate host histone acetylation to modulate transcriptional reprogramming during pathogenesis remains unknown.

Our previous research on RxLR effector expression profiling revealed that PsAvh23 is one of the highest expressed effectors during *P. sojae* infection of soybean [20]. Here we show that

PsAvh23 is required for the full virulence of *P. sojae*. By characterizing the host target of PsAvh23, we found that PsAvh23 effectively modulates H3K9 acetylation levels in soybean during infection and interferes with defense gene activation. In particular, PsAvh23 impairs the activity of the HAT General Control Non-depressive 5 (GCN5) by disrupting its association with the regulatory subunit Alteration/Deficiency in Activation 2 (ADA2). GCN5 is the catalytic subunit of the prototypical nucleosome-acetylating modification complex Spt-ADA-Gcn5-Acetyltransferase (SAGA) [21]. The association with ADA2 modulates the HAT activity of GCN5, driving its specificity to the nucleosomal histone H3 tail [22–24]. PsAvh23 is responsible for *P. sojae*-mediated decrease of H3K9 acetylation levels by competitively binding to ADA2 and taking it away from the ADA2-GCN5 subcomplex of the SAGA complex. This study reveals a counter-defense mechanism evolved in a plant pathogen to inactivate host defense response at an epigenetic level.

RESULTS

PsAvh23 Contributes to *P. sojae* Virulence, and the Nuclear Localization Is Required for This Activity

PsAvh23 is a canonical RxLR effector that is induced during infection [20]. To evaluate the contribution of PsAvh23 to the virulence of *P. sojae*, we generated knockout mutants in the strain P6497 (wild-type, WT) by replacing it with an *NPT II* gene (Geneticin-resistance gene) (Figures S1A and S1B) using a CRISPR/Cas9 system [25]. PCR and sequencing of two mutants (T51 and T94) confirmed that *PsAvh23* was successfully replaced (Figures S1C and S1D). We further confirmed that the gene encoding another RxLR effector, PsAvh22, which shares 44% identity in amino acid sequence with PsAvh23, remained intact (Figure S1C). The *PsAvh23* knockout mutants grew at a similar rate as the WT strain and a control strain, which was transformed with the empty vector (EV) (Figure S1E). However, the mutants exhibited significantly reduced virulence on etiolated soybean hypocotyls (Figure 1A), suggesting that PsAvh23 is required for the full virulence of *P. sojae*.

To further investigate the virulence function of PsAvh23, we expressed GFP-tagged PsAvh23 in plants and evaluated their susceptibility to *Phytophthora*. Soybean hairy roots expressing GFP-PsAvh23 or EV control were inoculated with mycelial plugs of *P. sojae* strain P6497 labeled with the red fluorescent protein (RFP) (WT-RFP), and the infection levels were measured by oospore production and *P. sojae* biomass. Our results showed that almost twice as many oospores were formed in *GFP-PsAvh23*-expressing roots compared to EV-transformed hairy roots (Figures 1B and S1F). Consistently, a greater biomass of *P. sojae* was accumulated in *GFP-PsAvh23*-expressing roots (Figure 1C), indicating that PsAvh23 promoted *P. sojae* infection in soybean. We also inoculated the hairy roots with the *PsAvh23* knockout mutant T94. A significantly greater biomass of T94 was found in *GFP-PsAvh23*-expressing roots (Figure 1C), indicating that expression of PsAvh23 in soybean restored the virulence of the *PsAvh23* knockout mutant. In addition, we also expressed GFP-PsAvh23 in *Nicotiana benthamiana* and subsequently inoculated the leaves with mycelial plugs of *Phytophthora capsici* isolate LT263. The lesion diameter on *GFP-PsAvh23*-expressing leaves was significantly larger than that on *GFP*-expressing leaves (Figure 1D, top panels). Taken together, these results provide strong evidence that PsAvh23 enhances plant susceptibility to *Phytophthora*.

We next determined the subcellular localization of PsAvh23 in plant cells by expressing GFP-PsAvh23 in *N. benthamiana*. Confocal imaging showed that the green fluorescence primarily accumulated in the nucleus but was also present in the cytoplasm (Figure 1D). To evaluate whether the nuclear localization of PsAvh23 is required for virulence, we fused a nuclear export signal (NES) or its mutated/nonfunctional form (nes), or a nuclear localization signal (NLS) or its mutated/nonfunctional form (nls), to either the N terminus or C terminus of PsAvh23, respectively. Confocal imaging showed that PsAvh23^{NLS} was exclusively present in nuclei, whereas PsAvh23^{NES} was almost completely excluded from the nuclei. Both PsAvh23^{nes} and PsAvh23^{nls} exhibited the same subcellular localization pattern as PsAvh23 (Figure 1D). When inoculated with *P. capsici*, *N. benthamiana* leaves expressing PsAvh23^{nes}, PsAvh23^{NLS}, or PsAvh23^{nls} promoted infection to a similar extent as WT PsAvh23, whereas PsAvh23^{NES} lost the virulence function (Figures 1D and S1G). Western blot analysis confirmed that the PsAvh23 fusion proteins were all expressed in the leaves (Figure S1H). Therefore, these results suggest that the virulence activity of PsAvh23 requires the nuclear localization in plant cells.

PsAvh23 Interacts with Plant ADA2 Proteins

To better understand the virulence mechanism of PsAvh23, we performed yeast two-hybrid (Y2H) screens using a *P. sojae*-infected soybean cDNA library and investigated proteins potentially associating with PsAvh23 in host cells (Table S1). One gene that was repeatedly identified in four independent screens encodes a protein that is similar to the ADA2 subunit of the prototypical nucleosome-acetylating modification complex SAGA. Three ADA2 homologous genes are present in the soybean genome. A phylogenetic analysis of ADA2 homologs from soybean and other plants suggests that the ADA2 subunit is conserved among different species (Figure S2A), implying that they may have similar functions in histone acetylation. We therefore designated the PsAvh23-interacting protein as GmADA2-1.

The interactions between PsAvh23 and the ADA2 homologs were validated using three independent assays. First, the Y2H assay was conducted using PsAvh23 as the predator and the three ADA2 homologs in soybean (GmADA2-1/2/3) as the prey. PsAvh22 was used as a negative control. Our results showed that PsAvh23 interacted with each of the three GmADA2 proteins (Figures 2A and S2B). Second, we performed an in vitro pull-down assay using proteins produced in *Escherichia coli* and showed that His-PsAvh23 was enriched in glutathione resins bound to GST-GmADA2-1 (Figure 2B). Finally, co-immunoprecipitation (coIP) assay confirmed that PsAvh23 interacted with all three GmADA2 homologs in planta when they were co-expressed in *N. benthamiana* (Figures 2C and S2C). Furthermore, two ADA2 homologs in *N. benthamiana* (hereafter referred to as NbADA2-1/2) also interacted with PsAvh23, using the Y2H and coIP assays (Figures S2B, S2D, and S2E).

The ADA2-Binding Site of PsAvh23 Is Required for Its Virulence Activity

Domain and motif searches using the Simple Modular Architecture Research Tool (SMART) database identified two internal

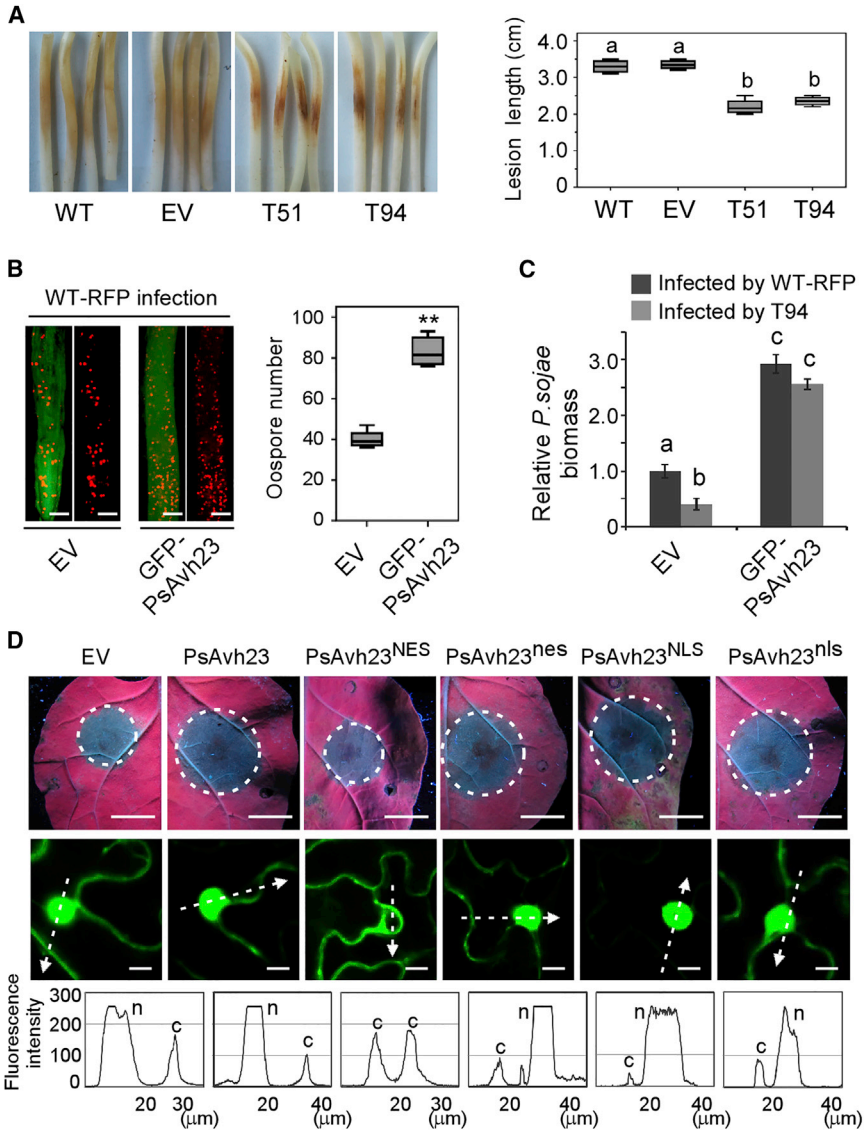

Figure 1. *PsAvh23* Is Required for the Full Virulence of *P. sojae* in Soybean

(A) *P. sojae PsAvh23* knockout mutants exhibited reduced virulence. Etiolated soybean hypocotyls were inoculated with zoospore suspensions from *P. sojae* wild-type strain P6497 (WT), a control strain transformed by the empty vector (EV), or the *PsAvh23* knockout mutants T51 and T94. Disease symptoms (left) were photographed at 2 days post-inoculation (dpi), and the lesion length (right) was measured to evaluate infection severity. The values are means ± SEM of three independent biological replicates. Different letters indicate significant differences ($p < 0.01$, Duncan's multiple range test).

(B) Expression of *PsAvh23* in hairy roots enhances susceptibility to *P. sojae* infection. Soybean hairy roots expressing EV or *GFP-PsAvh23* were inoculated with RFP-labeled *P. sojae* strain P6497 (WT-RFP). Oospore production in the infected roots was observed under a fluorescence microscope (left), and their numbers are presented as means ± SEM (n = 3) (right). Asterisks represent significant differences ($p < 0.01$, Student's t test). Scale bars, 20 μm.

(C) Expression of *PsAvh23* in hairy roots restored the virulence of the *PsAvh23* mutant T94. Relative biomass of *P. sojae* was determined by qPCR at 48 hr post-inoculation (hpi). The values are means ± SEM (n = 3). Different letters indicate significant differences ($p < 0.01$, Duncan's multiple range test).

(D) Nuclear localization of PsAvh23 in plant cells is required for its virulence function. *N. benthamiana* leaves expressing GFP-tagged PsAvh23 derivatives were inoculated with *P. capsici* mycelial plugs 48 hr after *Agrobacterium* infiltration. Top: lesion areas are indicated by white circles (scale bar, 1.5 cm). Middle: confocal images show the subcellular localizations of the PsAvh23 derivatives in plant cells (scale bar, 5 μm). Bottom: GFP intensity profiles were assessed on cytosol-nucleus transects. c, cytosol; n, nucleus; y axis, GFP intensity; x axis, transect length (μm).

See also Figure S1.

repeats (IRs), namely, IR1 and IR2, in the C terminus of PsAvh23 (Figures 2D and S3A). Deletion of both IRs abolished the interaction of PsAvh23 with GmADA2-1, whereas mutants containing any one of the two IRs were still able to interact (Figure S3A), suggesting that at least one IR in PsAvh23 is required for this interaction. Additional mutagenesis experiments revealed that the FVxR (Phe-Val-any-Arg) sequence present at the C-terminal end of both IR1 and IR2 was important for PsAvh23-GmADA2-1 interaction (Figure S3A). A mutant (named PsAvh23M4), where the F, V, and R residues in both IR1 and IR2 were substituted with alanines, was no longer able to interact with GmADA2-1; another mutant, PsAvh23M6, with only IR1 mutated at the FVxR sequence, retained the interaction ability (Figures 2B–2D and S3A). These results suggest that the FVxR sequence at the C-terminal IRs most likely mediates the interaction with GmADA2-1.

To determine whether the interaction with ADA2 is required for the virulence activity of PsAvh23, we expressed the mutants PsAvh23M4 and PsAvh23M6 in soybean hairy roots and *N. benthamiana* leaves and subsequently inoculated the transgenic plants with *Phytophthora*. Results showed that PsAvh23M6, which still interacts with ADA2, promoted *Phytophthora* infection similar to WT PsAvh23 (Figures 2E, 2F, and S3B–S3D). On the contrary, PsAvh23M4 lost the virulence activity, indicating that the interaction with ADA2 is important for PsAvh23 to promote infection.

PsAvh23 Competitively Binds to GmADA2-1 and Affects the Formation of the ADA2-GCN5 Subcomplex

ADA2, together with ADA3 and the SAGA-associated factor 29 (SGF29), is the regulatory subunit of GCN5, which is the catalytic subunit of the SAGA complex. The ADA2-GCN5 subcomplex is the core component required for histone acetylation. In maize and *Arabidopsis*, GCN5 and ADA2 directly interact with each other [26, 27]. A BLASTp search identified two genes homologous to *GCN5* that were present in both soybean (*GmGCN5a/b*) and *N. benthamiana* (*NbGCN5a/b*) (Figure S4A). Y2H and coIP assays showed that GmGCN5a/b interacts with

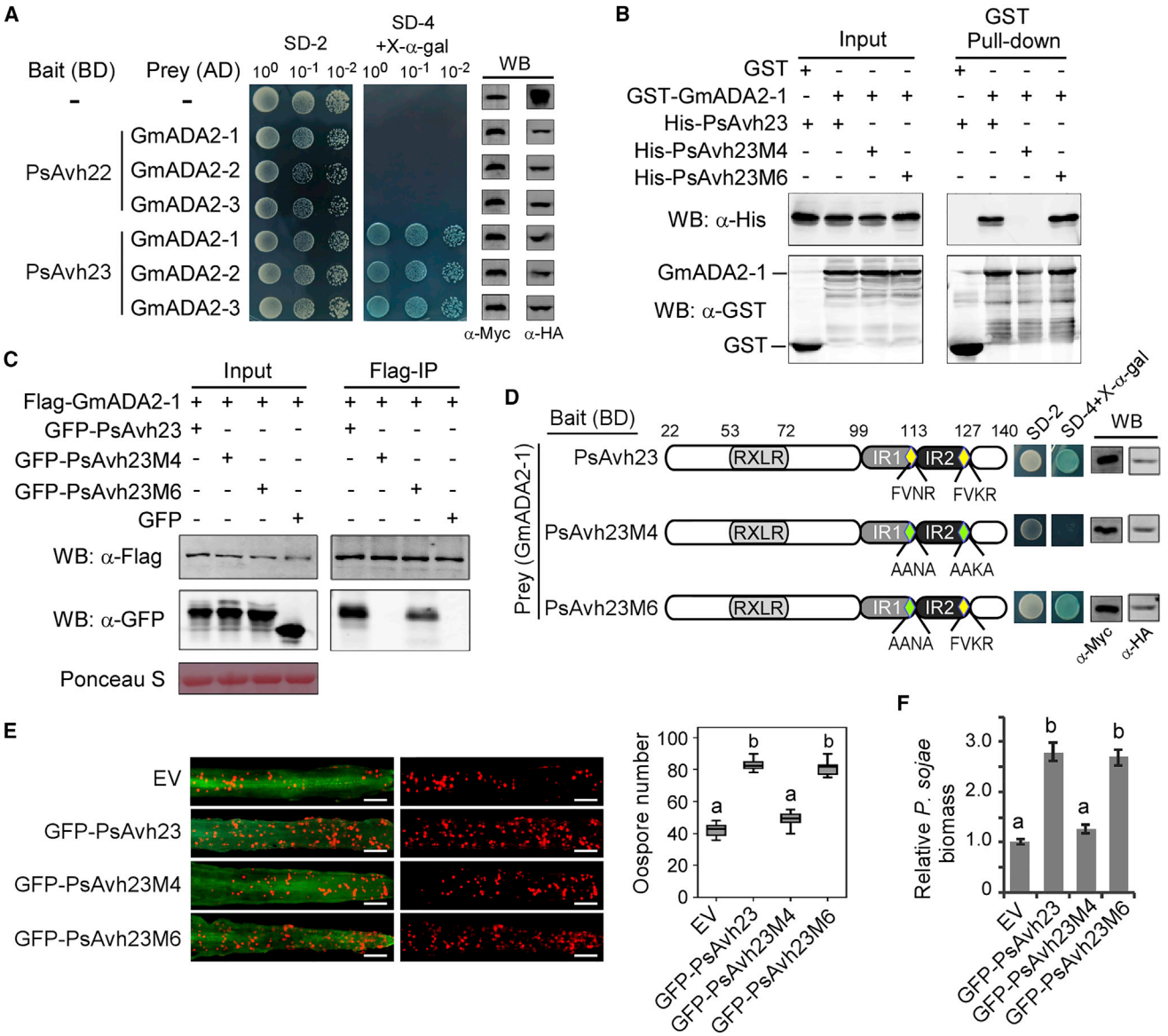

Figure 2. PsAvh23 Interacts with Soybean ADA2 Proteins, and the ADA2-Binding Site Is Required for Virulence Function

(A) PsAvh23 interacts with GmADA2 homologs (GmADA2-1/2/3) in yeast. Another *P. sojae* effector, PsAvh22, was used as a negative control. Yeast transformants were grown on non-selective SD/-Trp/-Leu (SD-2) medium or the selective medium SD/-Trp/-Leu/-His/-Ade (SD-4) supplemented with X-α-gal (80 mg/L). Plates were photographed at 3 days after inoculation. Protein expression in yeast cells was examined by western blot (WB) using anti-Myc and anti-HA antibodies. The minus sign represents EV.

(B) In vitro pull-down of His-PsAvh23 derivatives with GST-GmADA2-1. His-PsAvh23 derivatives and GST-GmADA2-1 were expressed in *E. coli*. Co-precipitation of GmADA2-1 with PsAvh23 was examined by GST pull-down.

(C) In vivo coIP of GFP-PsAvh23 derivatives with FLAG-GmADA2-1. Total proteins were extracted from *N. benthamiana* leaves expressing GmADA2-1 together with WT or mutant forms of PsAvh23. The immune complexes were pulled down using anti-FLAG agarose beads.

(D) Schematic representation of WT and mutant PsAvh23 and their interaction with GmADA2-1. PsAvh23 contains two internal repeats (IR1 and IR2). A conserved FVxR sequence (shown in yellow) is present in each of the repeats at the C termini. The FVxR residues are substituted with alanines (shown in green) in the mutants PsAvh23M4 and PsAvh23M6.

(E and F) The ADA2-binding site of PsAvh23 is required for its virulence function. Soybean hairy roots expressing EV or *GFP-PsAvh23* derivatives were inoculated with WT *P. sojae* (WT-RFP). Oospore production was observed and numerated at 48 hpi (E). Relative biomass of *P. sojae* was determined by qPCR at 48 hpi and was presented as means ± SEM (n = 3) (F). Different letters indicate statistically significant differences ($p < 0.01$, Duncan's multiple range test). Scale bars, 20 μm. These experiments were repeated three times with similar results.

See also Figures S2 and S3 and Table S1.

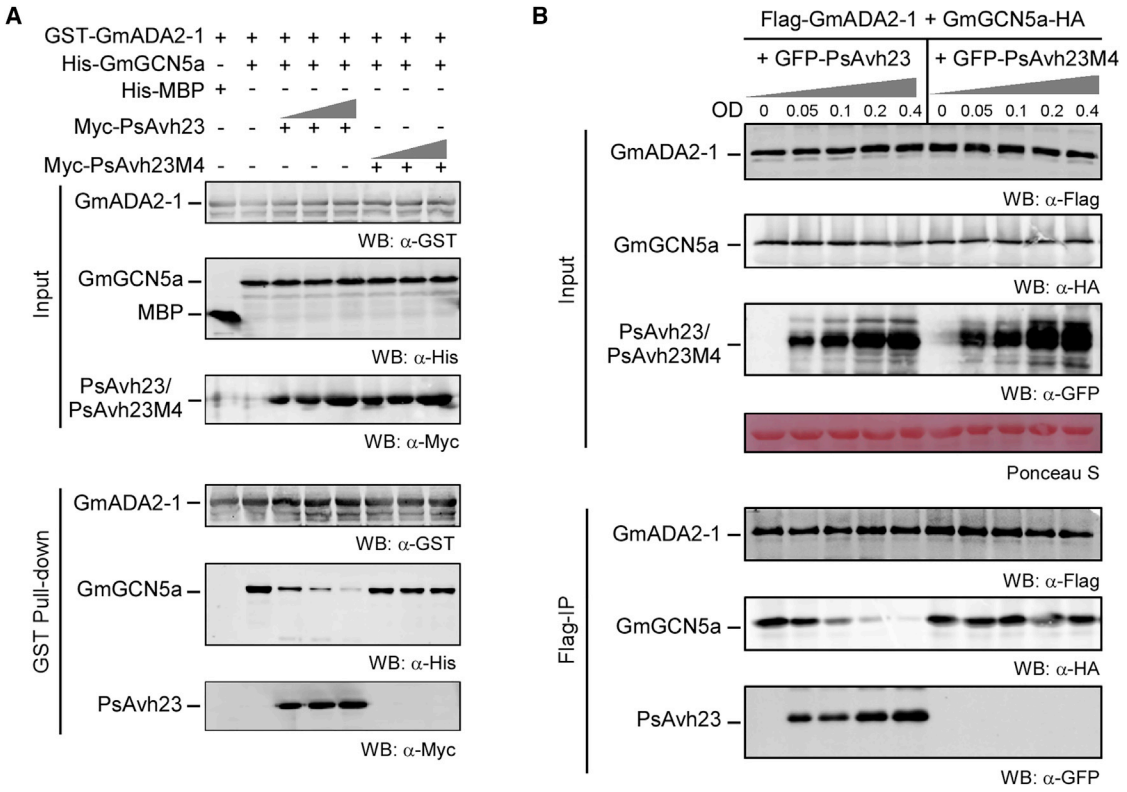

Figure 3. PsAvh23 Takes GmADA2-1 away from the ADA2-GCN5 Subcomplex

(A) PsAvh23 competes with GmGCN5a to bind GmADA2-1 in vitro in a dose-dependent manner. Co-precipitation of GmADA2-1 with GmGCN5a in the presence of PsAvh23 or PsAvh23M4 was examined by western blotting before (input) and after affinity purification (GST pull-down). Different gradient dilutions (1×, 2×, and 4×) of Myc-PsAvh23 were added to the mix using total protein extracts from *E. coli*.

(B) PsAvh23 interferes with the association of GmGCN5a with GmADA2-1 in plant cells. FLAG-GmADA2-1 and GmGCN5a-HA were co-expressed in *N. benthamiana* in the presence or absence of GFP-PsAvh23 or GFP-PsAvh23M4 though *Agro*-infiltration. The concentrations of *Agrobacteria* carrying different constructs are optical density 600 (OD_{600}) = 0.2 (GmADA2-1 and GmGCN5a) and OD_{600} = 0–0.4 (PsAvh23 or PsAvh23M4). The immune complexes were pulled down by using anti-FLAG agarose beads. These experiments were repeated three times with similar results.

See also Figure S4.

GmADA2-1 and NbGCN5a/b interacts with NbADA2-1/2 (Figures S4B–S4E), suggesting that ADA2 and GCN5 form a conserved subcomplex in plants.

Because ADA2 interacts with both PsAvh23 and GCN5, we examined whether the interaction with PsAvh23 would affect ADA2 interaction with GCN5. First, we constructed four GmADA2-1 mutants and determined the domain requirement of GmADA2-1 for its association with PsAvh23 or GmGCN5a (Figure S4F). Y2H results showed that the middle region (268–418 amino acids) of GmADA2-1 was required and sufficient for interaction with both PsAvh23 and GmGCN5a (Figure S4G). This observation suggests that the same region in GmADA2-1 mediates association with both proteins, leading to our hypothesis that PsAvh23 could affect the formation of the ADA2-GCN5 subcomplex.

To test this hypothesis, we conducted an in vitro pull-down assay of His-GmGCN5a and GST-GmADA2-1 in the presence of different concentrations of Myc-PsAvh23. Interestingly, with the increasing concentration of PsAvh23 added to the system, the enrichment of GmGCN5a in the GmADA2-1-bound resins gradually decreased, suggesting a competition between PsAvh23 and GmGCN5a in binding to GmADA2-1 (Figure 3A). This was confirmed by in vivo coIP assays between FLAG-GmADA2-1 and HA-GmGCN5a in the presence of GFP-PsAvh23. The increasing amount of GFP-PsAvh23 expressed in *N. benthamiana* was accomplished by using an increasing concentration of *Agrobacterium* cell suspension for infiltration. Again, expression of PsAvh23 in plant cells reduced the association of GmGCN5a with GmADA2-1 in a dose-dependent manner (Figures 3B and S4H). These findings demonstrate that PsAvh23 competes with GmGCN5a to bind to GmADA2-1, thus disrupting the ADA2-GCN5 subcomplex formation. In both experiments, the mutant PsAvh23M4 was used as a control, because it lost the ability to interact with GmADA2; consistently, the addition of PsAvh23M4 did not affect the association of GmGCN5a with GmADA2-1.

ADA2 and *GCN5* Regulate Plant Defense against *Phytophthora* Infection

To investigate the potential role of *ADA2* and *GCN5* in plant immunity, we silenced the three *GmADA2* and the two *GmGCN5* genes in soybean hairy roots, where *GmADA2* and *GmGCN5* were strongly reduced at mRNA levels (Figure 4A). After inoculation with *P. sojae* strain P6497-RFP, ~2.5-fold more oospores

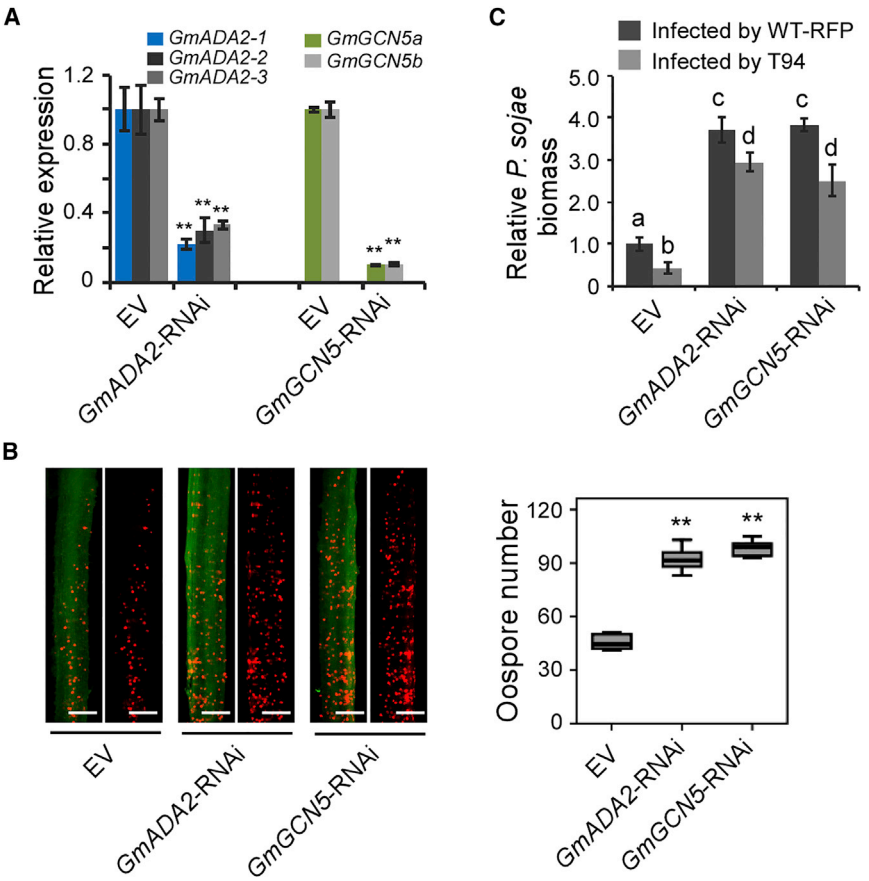

Figure 4. *GmADA2* and *GmGCN5* Contribute to Soybean Defense against *P. sojae*

(A) Silencing of *GmADA2* or *GmGCN5* in soybean hairy roots was confirmed by qRT-PCR. Transcript abundances were normalized with CYP2 as the internal standard. The values are means ± SEM (n = 3). Asterisks represent significant differences ($p < 0.01$, Student's t test).

(B) Silencing of *GmADA2* or *GmGCN5* in soybean led to hypersusceptibility to *P. sojae*. Oospore production in *GmADA2*- or *GmGCN5*-silenced roots infected by WT *P. sojae* (WT-RFP) was observed (left) and numerated (right) at 48 hpi. The values are means ± SEM (n = 3). Asterisks represent significant differences ($p < 0.01$, Student's t test). Scale bars, 20 μm.

(C) Silencing of *GmADA2* or *GmGCN5* in soybean partially restored the virulence of *P. sojae PsAvh23* knockout mutant. Relative biomass of *P. sojae* was determined by qPCR at 48 hpi and presented as means ± SEM (n = 3). Different letters indicate significant differences ($p < 0.01$, Duncan's test). These experiments were repeated three times with similar results.

See also Figures S5A–S5C.

were produced in the *GmADA2*- or *GmGCN5*-silenced roots compared to hairy roots expressing EV (Figure 4B). qPCR also showed an ~4-fold increase in the accumulation of *P. sojae* biomass in the *GmADA2*- or *GmGCN5*-silenced roots (Figure 4C), suggesting that *GmADA2* and *GmGCN5* are required for soybean defense against *P. sojae* infection. Interestingly, when the *PsAvh23* knockout mutant T94 was used to inoculate the *GmADA2*- or *GmGCN5*-silenced roots, the relative *P. sojae* biomass was largely increased compared to the biomass of T94 in WT plants (Figure 4C). These results indicate that silencing of *GmADA2* or *GmGCN5* in soybean partially restored the reduced virulence of T94, supporting the hypothesis that PsAvh23 promotes *Phytophthora* infection by affecting the function of the ADA2-GCN5 subcomplex. Notably, *P. sojae* biomass in *GmADA2*- or *GmGCN5*-silenced roots infected with T94 was still lower than those inoculated with WT strain (Figure 4C). This may be due to additional functions of PsAvh23 besides its interference with the ADA2-GCN5 subcomplex.

To further support a role of the ADA2-GCN5 subcomplex in plant defense, we silenced the *NbADA2* and *NbGCN5* genes in *N. benthamiana* using virus-induced gene silencing (VIGS). qPCR analysis confirmed the 80%–90% silencing efficiency of both constructs (Figure S5A). Consistent with our observation in soybean, *NbADA2*- and *NbGCN5*-silenced plants were also more susceptible to *P. capsici* (Figures S5B and S5C). Taken together, these results suggest that *ADA2* and *GCN5* contribute to plant immunity during *Phytophthora* infection.

PsAvh23 Reduces H3K9ac Levels in the Soybean during *P. sojae* Infection

Association with ADA2 modulates the HAT activity of GCN5 on nucleosomal histones. On its own, GCN5 acetylates free histones, but not nucleosomes [26, 28]; association with ADA2 drives the enzymatic activity of GCN5 to the nucleosomal histone H3 tail [29]. Given that PsAvh23 interferes with ADA2-GCN5 subcomplex formation, we hypothesized that PsAvh23 may inhibit the HAT activity of GCN5 on nucleosomal histones. To test this hypothesis, we measured the HAT activity of GST-GmGCN5a using an in vitro assay in the presence of GST-GmADA2-1 purified from *E. coli* (Figure S5D). In *Arabidopsis*, GCN5 was shown to acetylate H3K9 [30]. Therefore, we used histone H3 or nucleosomes as the substrates, and we evaluated the HAT activity by immunoblotting using H3ac and H3K9ac antibodies. The p300-CBP-associated factor (PCAF) was used as a positive control [31]. Our results show that GmGCN5a exhibited clear acetylation activity, using histone H3 as the substrate, and this activity was inhibited by C646, a chemical inhibitor of HAT; on the contrary, GmGCN5a alone only had a weak activity on nucleosomal H3 (Figure 5A, lanes 2–4). The addition of GmADA2-1 into the reactions enhanced the HAT activity of GmGCN5a on both histone H3 and nucleosomal H3, but this enhancement was much more significant on the nucleosomal H3 (Figure 5A, lane 5). Importantly, although PsAvh23 was unable to affect the activity of GmGCN5a when it was added to the reactions alone (Figure 5A, lanes 6 and 7), it inhibited GmADA2-1-mediated enhancement of the HAT activity in a dose-dependent manner (Figure 5A, lanes 8–10). In contrast, PsAvh23M4 did not affect the activity of GmGCN5a (Figure 5A, lanes 11–13), suggesting that PsAvh23 impaired

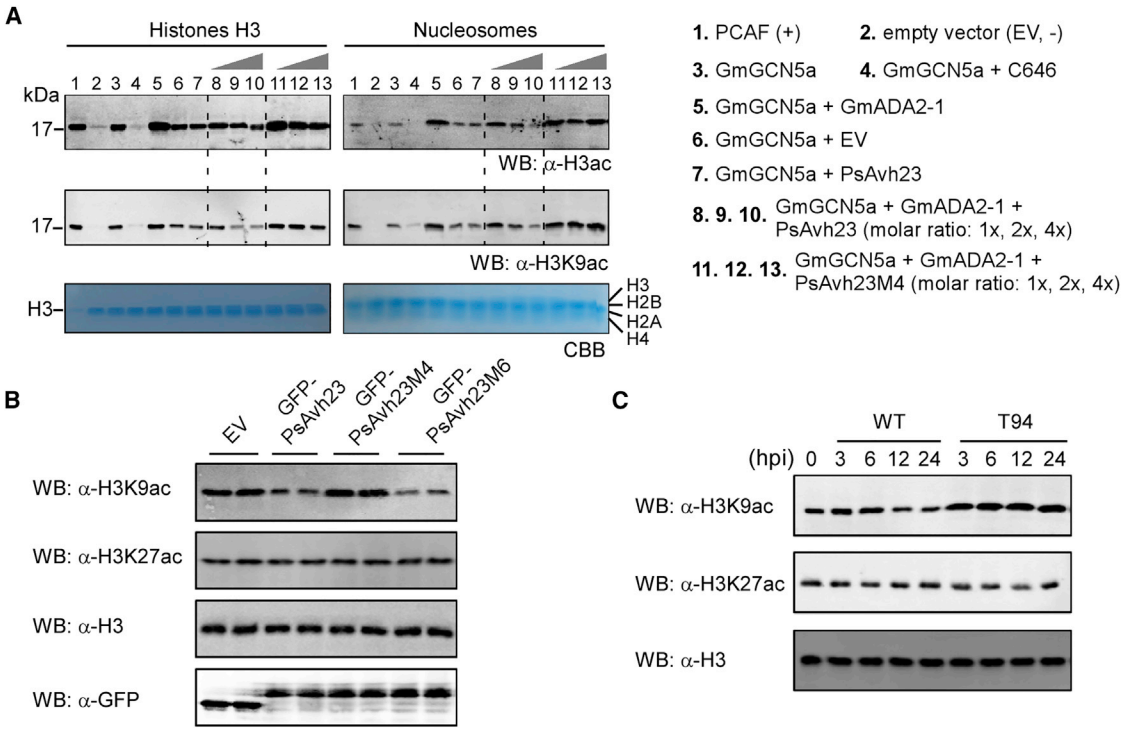

Figure 5. PsAvh23 Decreases the H3K9ac Levels in Soybean during *P. sojae* Infection

(A) In vitro assay evaluating the HAT activity of GmGCN5a in the presence or absence of GmADA2-1 and PsAvh23. HAT activity on histone H3 or nucleosomes was determined by western blotting using anti-H3ac and anti-H3K9ac antibodies. Protein mixture containing GmADA2-1 and GmGCN5a was incubated with PsAvh23 or PsAvh23M4 at different molar ratios (1 × −4 × in relative to GmADA2-1). p300-CBP-associated factor (PCAF) was used as a positive control with known HAT activity. The chemical inhibitor of HAT, C646, was used as an additional control.

(B) Expression of *PsAvh23* in soybean hairy roots led to reduced acetylation levels of H3K9. The nuclear fraction was separated from hairy roots expressing EV or *GFP-PsAvh23*. Duplicated samples were examined in each treatment.

(C) H3K9ac levels were decreased in soybean hypocotyls infected by *P. sojae*. Soybean tissues inoculated with WT *P. sojae* or the *PsAvh23* mutant T94 were collected at different time points. The nuclear fraction was separated and the levels of H3K9ac and H3K27ac were determined using antibodies. The experiments were repeated three times with similar results.

See also Figures S5D–S5G.

histone acetylation by the ADA2-GCN5 subcomplex, probably by competitively binding to GmADA2-1.

To confirm that PsAvh23 affects the acetylation levels of H3K9 (H3K9ac) in soybean and this inhibitory effect is dependent on its interaction with GmADA2, we expressed PsAvh23 and its mutant forms PsAvh23M4 and PsAvh23M6 in hairy roots. Because histone acetylation mainly occurs on nucleosomal histones of nuclear chromatin, we isolated the nuclear fraction (Figure S5E) from soybean hairy roots and detected the levels of H3K9ac. As shown in Figure 5B, reduced levels of H3K9ac, but not the acetylation levels of H3K27 (H3K27ac), were observed in hairy roots expressing PsAvh23 or PsAvh23M6. Expression of PsAvh23M4 did not alter H3K9ac levels, suggesting that the interaction with GmADA2 is required for the inhibitory effect of PsAvh23 on GCN5-mediaed H3K9ac. Similarly, expression of PsAvh23 in hairy roots also decreased the H3K9ac levels at 6 and 12 hr post-inoculation (hpi) during *P. sojae* infection (Figure S5F). Furthermore, silencing of *GmADA2* or *GmGCN5* also led to decreased levels of H3K9ac, but not H3K27ac (Figure S5G), consistent with the notion that PsAvh23 influences H3K9ac levels through interference with the ADA2-GCN5 subcomplex.

We next examined the inhibitory effect of PsAvh23 on H3 acetylation during *P. sojae* infection of soybean. Nuclear fraction of infected tissues was isolated from soybean hypocotyls at 0, 3, 6, 12, and 24 hpi, and the levels of H3K9ac and H3K27ac were detected. A clear decrease in H3K9ac, but not H3K27ac, was observed in soybean infected by WT *P. sojae*, especially at 12 and 24 hpi (Figure 5C). However, this decrease was abolished in samples inoculated with the *PsAvh23* knockout mutant T94 (Figure 5C). Interestingly, this pattern is consistent with the expression profile of PsAvh23, which is induced at 3 hpi and reaches the maximal level at 12 hpi [20].

PsAvh23 Represses the Expression of Defense-Related Genes by Modulating GmGCN5-Mediated Histone Acetylation

PsAvh23-expressing or *GmGCN5*-silenced soybean roots exhibited decreased H3K9ac levels and increased susceptibility to *P. sojae*. Since the H3K9ac is correlated with transcriptional activation, we investigated genes that are regulated by PsAvh23 and GmGCN5 using RNA sequencing (RNA-seq). Soybean root tissues expressing *PsAvh23* or transformed with *Agrobacterium* carrying EV were inoculated with *P. sojae* strain P6497, and the

transcriptomes were analyzed at 6 hpi due to H3K9ac levels being decreased at this stage (Figure S5F). Comparison of the differentially expressed gene (DEG) profiles revealed that expression of PsAvh23 or silencing of *GmGCN5* in soybean mainly caused decreases in gene expression (Figure S6A; Tables S2 and S3). For example, 484 genes showed increased expression in *PsAvh23*-expressing roots, whereas the expression of 1,712 genes was decreased (Figure S6A; Table S2). qRT-PCR on 19 randomly selected genes showed a strong positive correlation with the RNA-seq data, validating the RNA-seq results (Figure S6B). Importantly, the DEG profiles of *PsAvh23*-expressing and *GmGCN5*-silenced roots exhibited a positive Pearson correlation coefficient ($R^2 = 0.73$) (Figure S6C). In particular, 1,338 genes that showed reduced expression in *PsAvh23*-expressing roots were also downregulated in *GmGCN5*-silenced roots (Figure S6A; Table S4). These results suggest that PsAvh23 represses gene expression in soybean, most likely through its inhibition of GmGCN5-mediated histone acetylation.

Among 1,338 genes that were downregulated in both *PsAvh23*-expressing and *GmGCN5*-silenced roots, 141 genes are most likely involved in plant defense against *Phytophthora* infection (Table S5); these include genes encoding polygalacturonase-inhibiting protein (*PGIP1-1*) [32], heat shock proteins (*HSP20-1*, *HSP20-2*, and *HSP90*) [33], WRKY transcription factors (*WRKY33* and *WRKY41*) [34, 35], NAC transcription factors (*NAC-1* and *NAC-2*) [36], and MAP kinases (*MAPKKK14-2* and *MAPKKK18*) [37] (Figure S6D; Table S5). Using qRT-PCR, we confirmed that the expression of these genes was significantly decreased in soybean roots expressing *PsAvh23* or with *GmGCN5* or *GmADA2* silenced (Figure 6A). We further determined the impact of PsAvh23, GmGCN5, and GmADA2 on the H3K9ac levels at the promoter regions of these defense-related genes using chromatin immunoprecipitation (ChIP) followed by qPCR (ChIP-qPCR). The results showed that promoters of these defense-related genes exhibited markedly lower H3K9ac levels in *PsAvh23*-expressing, *GmGCN5*-silenced, or *GmADA2*-silenced roots infected with *P. sojae* compared to roots expressing EV (Figure 6B). As a control, the expression of *Ubiquitin5* (*UBQ5*), whose expression is not affected by H3K9ac [38], was not altered in these roots. Furthermore, the expression of these genes was significantly higher in soybean inoculated with the *PsAvh23* knockout mutant T94 in comparison to that in soybean inoculated with WT *P. sojae* (Figure 6C). Taken together, our data support a model in which PsAvh23 disrupts the formation of the ADA2-GCN5 subcomplex, suppresses the activation of defense-related genes by reducing H3K9ac levels, and, consequently, compromises plant immunity (Figure 6D).

DISCUSSION

Plants and animals have evolved robust innate immune systems, preventing infection from a large majority of potential pathogens in the environment. To cause diseases, microbial pathogens utilize various strategies to defeat host immunity. In this study, we report a previously unknown mechanism employed by the *P. sojae* effector PsAvh23, which plays an essential role in soybean infection. Our study shows that PsAvh23 disrupts the formation of the ADA2-GCN5 histone acetylation module in the SAGA complex, leading to reduced HAT activity of GCN5. As such, PsAvh23 inhibits H3K9 acetylation in the host plants and impairs the activation of defense-related genes. Previously, we showed that PsAvh23 suppressed cell death triggered by the mammalian pro-apoptosis factor BAX as well as other *P. sojae* effectors [20]. This activity could be explained by some of the downregulated genes, which may be involved in programmed cell death. Furthermore, we observed that the nuclear localization of PsAvh23 was required for its virulence function, consistent with its influence on histone acetylation in the nuclei of plant cells.

Immune responses require extensive transcriptional reprogramming; histone acetylation is an essential epigenetic marker that is linked to transcription regulation [39]. GCN5-mediated histone acetylation was shown to play an essential role in plant development and responses to environmental stresses [40]. We demonstrate that GCN5 homologs in soybean and *N. benthamiana* also contribute to plant immunity. In soybean, silencing of *GmGCN5* led to decreased expression of defense-related genes. Similar to GmGCN5, GmADA2 also acts as a positive regulator of H3K9 acetylation and soybean defense against *P. sojae* infection. AtADA2b has been shown to associate with AtGCN5 and to enhance its H3K9 acetylation activity on nucleosomal histone [26, 41]. It is likely that GmADA2 also influences the HAT activity of GmGCN5 in a similar manner. Since PsAvh23 also interacts with GmADA2, our results support a model in which PsAvh23 takes GmADA2 away from the GmADA2-GmGCN5 subcomplex, thereby dampening plant defense response.

PsAvh23 does not share sequence homology with GCN5; the key amino acid residues (FVxR) in PsAvh23 that are required for ADA2 interaction are also absent in GCN5. Therefore, although the same sequence fragment, i.e., the middle region of ADA2, seems to mediate its interaction with both PsAvh23 and GCN5, molecular details on how PsAvh23 competes with GCN5 for ADA2 binding remain unknown. Further investigations on the structural features of protein complexes containing PsAvh23, ADA2, and/or GCN5 will help explain how PsAvh23 affects the formation of the ADA2-GCN5 subcomplex.

Accumulating evidence from a large variety of pathogens supports that defeating host immunity through manipulating epigenetic regulation is an important virulence strategy. Suppressors of small RNA silencing are commonly encoded in RNA viruses [42], but they have also been reported in bacteria [43] and *Phytophthora* pathogens [44–46]. In addition, some pathogens inhibit the activation of host defense genes by manipulating histone methylation. For example, two plant geminiviruses encode the protein TrAP, which inhibits H3K9 methylation in *Arabidopsis* to counter host defense [47]. The human bacteria pathogen *Legionella pneumophila* secretes the effector RomA, which acts as a histone methyltransferase to directly methylate histones and repress immune gene expression [48]. On the contrary, examples of pathogen factors targeting histone acetylation are rare. The animal parasite *Toxoplasma* produces an effector TgIST to manipulate histone deacetylase complex Mi-2/NuRD function, which is often linked to transcription repression [49]. TgIST recruits Mi-2/NuRD complex to specific promoter regions of immune genes and blocks their expression [50]. Another example is the HC-toxin produced by the fungal pathogen *C. carbonum*. HC-toxin promotes infection as an inhibitor of histone

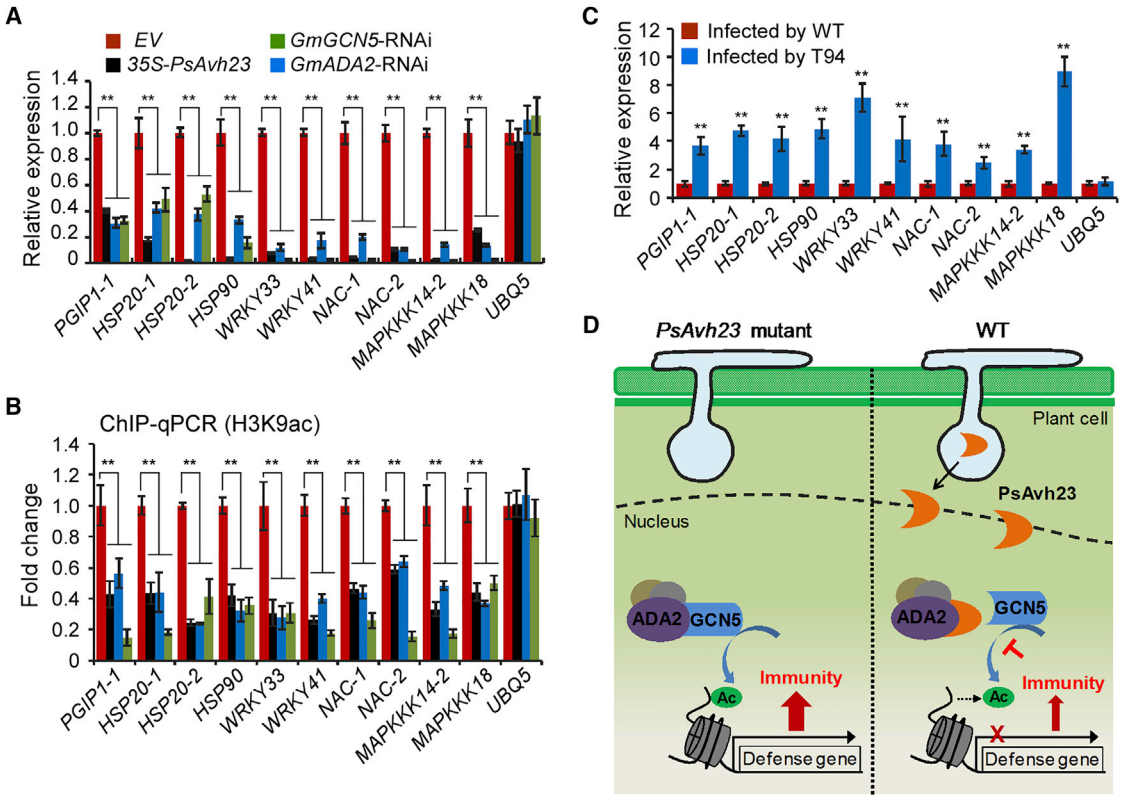

Figure 6. PsAvh23 Regulates Defense-Related Gene Expression

(A) Expression levels of selected defense-related genes in *PsAvh23*-expressing, *GmADA2*-silenced, or *GmGCN5*-silenced roots after infection with WT *P. sojae* strain P6497. Infected tissues were collected at 6 hpi and analyzed by qRT-PCR. Relative expression levels were normalized with *CYP2* as an internal standard and presented as means ± SEM (n = 3). Asterisks represent significant differences ($p < 0.01$, Student's t test). Ubiquitin5 (UBQ5) served as the negative control. See also Figure S6 and Tables S2, S3, S4, and S5.

(B) H3K9ac levels at the promoter regions of selected defense-related genes were analyzed by ChIP-qPCR. Immunoprecipitation of acetylated H3K9 was performed on *PsAvh23*-expressing, *GmADA2*-silenced, or *GmGCN5*-silenced hairy roots infected by *P. sojae* strain P6497 at 6 hpi, and the H3K9 acetylation levels were quantified by qPCR using gene-specific primers. Values for the ChIP samples were first normalized to the input control and then divided by the EV control to obtain the fold enrichment values. The values are the means ± SEM of three independent biological replicates. Asterisks represent significant differences ($p < 0.01$, compared with EV control, Student's t test).

(C) Expression levels of selected defense-related genes in soybean roots inoculated with *P. sojae* strain P6497 (WT) or the *PsAvh23* mutant T94. Relative expression levels were normalized with *CYP2* as the internal standard and presented as means ± SEM (n = 3). Asterisks represent significant differences ($p < 0.01$, Student's t test). These experiments were repeated three times with similar results.

(D) A working model illustrating how PsAvh23 manipulates the ADA2-GCN5 subcomplex to suppress the activation of defense-related genes during *P. sojae* infection. GCN5 and ADA2 subunits are key components of the HAT complex SAGA, which acetylates nucleosomal histones and activates the expression of a subset of defense-related genes in response to pathogen infection. As a counter-defense strategy, *P. sojae* secretes the effector PsAvh23 that competitively binds to ADA2 and inhibits GCN5-mediated histone acetylation. As a result, PsAvh23 suppresses the expression of the defense genes, promoting *P. sojae* infection.

deacetylases in maize [17, 18]; however, whether HC-toxin directly affects defense gene expression remains unknown.

Here we show that PsAvh23 suppresses defense-related gene expression by manipulating the HAT complex SAGA-mediated histone acetylation. This is a novel virulence strategy employed by microbial pathogens during the arms race with hosts to counteract defense response. Interestingly, both the *Toxoplasma* effector TgIST and the *Phytophthora* effector PsAvh23 repress the expression of immune/defense genes, although using distinctive mechanisms. Whether other animal or plant pathogens have also evolved strategies to regulate plant immunity through manipulation of epigenetic regulation is an appealing question to explore in the future.

EXPERIMENTAL PROCEDURES

Phytophthora Strains

The strains used in this study include *P. sojae* isolate P6497 (WT), RFP-labeled *P. sojae* isolate P6497 (WT-RFP), the *PsAvh23* knockout mutants of *P. sojae* T51 and T94, and *P. capsici* isolate LT263. Also see the Supplemental Experimental Procedures.

PsAvh23/GmGCN5a Competition Assays for GmADA2-1 Binding

The competition assays were performed in vitro and in vivo. Also see the Supplemental Experimental Procedures.

HAT Activity Assays

The HAT activity assays were done as previously described [51]. Also see the Supplemental Experimental Procedures.

Soybean Cotyledon Transformation and *P. sojae* Infection Assays

These assays were done as previously described [52]. Also see the Supplemental Experimental Procedures.

RNA-Seq Analysis and ChIP-qPCR Assay

See the Supplemental Experimental Procedures.

ACCESSION NUMBERS

The accession number for the RNA-seq data reported in this paper is NCBI SRA: PRJNA376551.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, six figures, and five tables and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2017.02.044.

AUTHOR CONTRIBUTIONS

Yuanchao Wang, W.M., S.D., and L.K. designed the research. L.K., X.Q., J.K., H.C., J.H., M.Q., and Y.Z. performed the experiments. Yan Wang, W.Y., Yang Wang, G.K., and Z.M. contributed new tools and analyzed the data. L.K., S.D., W.M., and Yuanchao Wang wrote the paper. All authors commented on the manuscript.

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