BcSas2-Mediated Histone H4K16 Acetylation Is Critical for Virulence and Oxidative Stress Response of *Botrytis cinerea*

Guangyuan Wang,¹ Limin Song,² Tingting Bai,² and Wenxing Liang^{2,†}

¹ College of Life Sciences, Shandong Province Key Laboratory of Applied Mycology, Qingdao Agricultural University, Qingdao 266109, China

² College of Plant Health and Medicine, the Key Laboratory of Integrated Crop Pest Management of Shandong Province, Qingdao Agricultural University, Qingdao 266109, China

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Histone acetyltransferase plays a critical role in transcriptional regulation by increasing accessibility of target genes to transcriptional activators. *Botrytis cinerea* is an important necrotrophic fungal pathogen with worldwide distribution and a very wide host range, but little is known of how the fungus regulates the transition from saprophytic growth to infectious growth. Here, the function of BcSas2, a histone acetyltransferase of *B. cinerea*, was investigated. Deletion of the *BcSAS2* gene resulted in significantly reduced acetylation levels of histone H4, particularly of H4K16ac. The deletion mutant Δ BcSas2.1 was not only less pathogenic but also more sensitive to oxidative stress than the wild-type strain. RNA-Seq analysis revealed that a total of 13 *B. cinerea* genes associated with pathogenicity were down-regulated in the Δ BcSas2.1 mutant. Independent knock-outs of two of these genes, *BcXYGA* (xyloglucanase) and *BcCAT* (catalase), led to dramatically decreased virulence and hypersensitivity to oxidative stress, respectively. Chromatin immunoprecipitation followed by quantitative PCR confirmed that BcSas2 bound directly to the promoter regions of both these pathogenicity-related genes. These observations indicated that BcSas2 regulated the transcription of pathogenicity-related genes by controlling the acetylation level of H4K16, thereby affecting the virulence and oxidative sensitivity of *B. cinerea*.

Keywords: BcSas2, *Botrytis cinerea*, histone H4K16ac, oxidative stress, RNA-Seq, virulence

Guangyuan Wang and Limin Song contributed equally to this work.

[†]Corresponding author: W. Liang; wliang1@qau.edu.cn

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Botrytis cinerea, an important necrotrophic fungal pathogen, causes a wide range of plant gray mold diseases and enormous economic losses worldwide. It infects over 200 plant species, including several economically important plants, such as strawberry, tomato, cucumber, and grape vine. Various organs in plants, including flowers, root tubers, bulbs, fruits, and leaves, are susceptible to *B. cinerea* infection (Yang et al. 2018b). It has been well-documented that the gray mold infection process mainly includes two typical stages, early local lesions and late, rapidly spreading lesions (Williamson et al. 2007). To successfully colonize the host, *B. cinerea* must control gene expression through a variety of transcriptional regulatory mechanisms to cope with changing environmental conditions, such as osmotic and oxidative stresses.

Previous studies have shown that chromatin histone acetylation is closely associated with transcriptional activation (Wolffe and Pruss 1996). Histone acetylation, a posttranslational modification (PTM), alters chromatin structure by neutralizing positively charged nucleosomes, thereby increasing the accessibility of transcription factors to target genes (Grunstein 1997; Lee et al. 2014). Various modifications of histone N-terminal tails, including acetylation, methylation, phosphorylation, and ubiquitination, impact many intracellular biological processes by genetically regulating gene expression (Su et al. 2016). Current evidence has indicated that global histone acetylation in cells plays a critical role in the regulation of chromatin structure and transcriptional activation (Grunstein 1997; Kouzarides 2007; Wolffe and Pruss 1996). Histone acetylation is a dynamic and reversible PTM that is dynamically controlled by histone acetyltransferases (HATs) and histone deacetylases (HDACs). The acetyl groups of acetyl-CoA are transferred to histone lysine residues by HATs, whereas the acetylated histones are deacetylated by HDACs (Kuo and Allis 1998). The MYST (MOZ, YBF2/SAS3, SAS2, TIP60) proteins are a conserved family of HATs, and histone acetylation by MYST proteins is involved in multiple nuclear processes, such as transcription, DNA repair, and DNA replication (Sapountzi and Côté 2011).

Sas2, a founding member of the MYST family of HATs, is the catalytic subunit of the SAS complex (Heise et al. 2012; Sutton et al. 2003). In *Saccharomyces cerevisiae*, Sas2 forms the SAS complex with Sas4 and Sas5, which are required for Sas2 HAT activity (Meijsing and Ehrenhofer-Murray 2001; Sutton et al. 2003). Sas2 was found to interact with Cac1 (the largest subunit of chromatin assembly factor I [Caf-I]) and nucleosome assembly factor Asf1, and deletions of *cac1*, *asf1* and *sas2* had similar effects on gene silencing (Meijsing and

Ehrenhofer-Murray 2001). Previous evidence has indicated that the acetylation of lysine 16 of histone H4 (H4K16ac) is catalyzed by Sas2 (Sutton et al. 2003). The acetylation of lysine residues on histone H4 can loosen chromatin structure and promote the interaction of transcription factors and coactivators with DNA (Grunstein 1997; Lee et al. 2014). In yeast, high levels of H4K16ac due to Sas2 HAT activity inhibit the formation of telomeric heterochromatin by blocking the telomeric gene silencing-associated protein SIR (Suka et al. 2002). At a genome-wide level, the deacetylation of H4K16 is associated with downregulation of autophagy-related genes (Füllgrabe et al. 2013). In *Drosophila* spp., H4K16ac counteracts chromatin compaction mediated by chromatin-remodeling ATPase ISWI, resulting in upregulated transcription of the X chromosome (Corona et al. 2002). In mammals, high levels of H4K16ac is also associated with the upregulation of male X chromosomal transcription initiation (Deng et al. 2013). Acetylome bioinformatic analyses revealed a large number of acetylated proteins, including H4K16ac, that are involved in many metabolic processes in the filamentous fungi *B. cinerea* (Lv et al. 2016) and *Fusarium graminearum* (Zhou et al. 2016). These findings suggest that MYST-mediated H4K16ac plays an important role in transcriptional regulation in eukaryotes. However, its function remained unknown in *B. cinerea*.

In this study, the roles of BcSas2 in *B. cinerea*, an ortholog of *S. cerevisiae* Sas2, were investigated. The *B. cinerea* BcSAS2 deletion mutant showed defects in virulence, oxidative stress responses, sporulation, and sclerotia. The purposes of this study were to investigate the roles of the acetyltransferase BcSas2 in *B. cinerea* and to identify the genes regulated by BcSas2.

RESULTS

Effect of BcSAS2 gene deletion on H4K16 acetylation.

Gene BcSAS2 (National Center for Biotechnology Information [NCBI] BC1G_05395) encodes a 721-amino acid ortholog of yeast Sas2 with a DNA sequence of 3,018 bp with two introns. A phylogenetic tree of Sas2 orthologs was constructed (Fig. 1A). Orthologs of Sas2 are found among necrotrophic fungi (*B. cinerea*, *Colletotrichum incanum*), biotrophic fungi (*Sporisorium reilianum*, *Melampsora larici-populina*, *Blumeria graminis* f. sp. *hordei*), and yeasts (*Saccharomyces cerevisiae*, *Zygosaccharomyces mellis*, *Kluyveromyces marxianus*, *Cyberlindnera jadinii*). These orthologs clustered into three distinct groups: Pezizomycotina, Saccharomycotina, and Basidiomycota. All orthologs contained a HAT acetyltransferase and a C₂HC-type zinc finger domain (Fig. 1B).

To elucidate the functions of BcSas2 in *B. cinerea*, we deleted the BcSAS2 gene in the wild-type strain B05.10 via

Fig. 1. Identification of BcSas2 and Western blotting. **A**, Phylogenetic tree of BcSas2 based on a neighbor-joining analysis using MEGA 5.0. The orthologs are from *Saccharomyces cerevisiae* Sas2 (National Center for Biotechnology Information [NCBI] KZV08970), *Zygosaccharomyces mellis* Sas2 (NCBI GCE98240.1), *Kluyveromyces marxianus* Sas2 (NCBI XP_022675924), *Cyberlindnera jadinii* Sas2 (NCBI CEP21246), *Colletotrichum incanum* MOZ/Sas (NCBI KZL80431), *Cordyceps confragosa* MYST (NCBI OAA77306.1), *Coleophoma cylindrospora* MOZ/Sas (NCBI RDW68033.1), *Coleophoma cylindrospora* Moz/Sas (NCBI RDW68033.1), *Blumeria graminis* f. sp. *hordei* DH14 Sas2 (NCBI CCU76060.1), and *Sporisorium reilianum* SRZ2 ESA1/Sas2 (NCBI CBQ72444.1). **B**, Functional domains of the Sas2 protein. HAT = catalytic acetyltransferase domain, C₂HC = C₂HC-type zinc finger. **C**, Mycelia images for subcellular localization of the green fluorescent protein (GFP)-tagged BcSas2. **D**, Western blotting for acetylation levels of histone H4 and H4K16. The nucleoproteins were extracted from the 3-day-old YPD (1% yeast extract, 2% peptone, 2% glucose) cultures of ΔBcSas2.1, B05.10, and ΔBcSas2-C strains. A total of 50 μg of total protein was loaded for Western blotting analysis.

homologous recombination (Supplementary Fig. S1). The open reading frame (ORF) of *BcSas2* in the wild-type strain B05.10 was replaced with the hygromycin B phosphotransferase (*HPH*) gene as previously described (Yang et al. 2018a). After the deletion cassette was transformed into the wild-type strain, a total of 57 transformants were obtained. One of the transformants, $\Delta BcSas2.1$, was characterized and exhibited physical evidence of *BcSas2* gene deletion and exchange with *HPH* (Supplementary Fig. S1). The $\Delta BcSas2.1$ strain was complemented using the *BcSas2* gene cloned into plasmid pBS-neo as previously described (Yang et al. 2018a). The complemented strain $\Delta BcSas2-C$ was validated by PCR (Supplementary Fig. S2).

Since the SAS complex catalyzes the acetylation of histone H4K16 in yeast (Sutton et al. 2003), we speculated that BcSas2 was located in the nucleus in *B. cinerea*. To test this hypothesis, we constructed the fusion protein BcSas2-GFP and expressed it in the *BcSas2* deletion mutant strain. Fluorescence microscopy was used to observe the transformed mycelia after growth on potato dextrose agar (PDA) plates for 2 days. Consistent with this hypothesis, the green signal of the green fluorescent protein (GFP)-tagged BcSas2 overlapped with nuclei stained with Hoechst 33258 fluorescent dye (Fig. 1C). Since Sas2 orthologs reportedly target the acetylation of histone H4 at the lysine-16 site in yeast (Suka et al. 2002; Sutton et al. 2003), the acetylation levels of H4K16 in different *B. cinerea* strains were investigated by Western blotting analysis using antihistone H4,

antiacetyl-histone H4, and antiacetyl-histone H4K16 antibodies. As shown in Figure 1D, after strains were grown on PDA plants for 3 days, the acetylation of histone H4K16 in $\Delta BcSas2.1$ was almost undetectable compared with that in the wild-type strain B05.10 and the complemented strain $\Delta BcSas2-C$. Furthermore, the acetylation of histone H4 in the $\Delta BcSas2.1$ mutant was attenuated by 90% compared with that in the wild type and complemented strains (Fig. 1D). These results indicated that the *BcSas2* deletion significantly reduced the acetylation levels of histone H4, particularly H4K16ac.

Impact of *BcSas2* deletion on radial growth, sporulation, and sclerotia development.

The impact of the *BcSas2* deletion on mycelial growth, sporulation, and sclerotia development were investigated. Strain $\Delta BcSas2.1$ exhibited slightly decreased radial growth as compared with the wild type and complemented strains (Fig. 2A and B). In addition, deletion of *BcSas2* significantly reduced conidium formation after these strains were incubated on PDA plates for 10 days (Fig. 2C). The number of conidia produced by wild-type strain B05.10 and the complemented strain $\Delta BcSas2-C$ were 4.7×10^8 conidia per plate and 4.5×10^8 conidia per plate, respectively, after being grown on PDA plates for 10 days (Fig. 2D). However, only 3.9×10^7 conidia per plate were produced by strain $\Delta BcSas2.1$ on PDA plates under the same conditions (Fig. 2D). These data indicated an essential role for BcSas2 in sporulation development in *B. cinerea*. Since

Fig. 2. Effects of *BcSas2* deletion on mycelial growth, sporulation, and sclerotia development. Data are given as the mean $\pm$ standard deviation, $n = 3$. Significant difference compared with B05.10: asterisks (**) indicate $P < 0.01$. **A**, Mycelial growth of B05.10, $\Delta BcSas2.1$ and $\Delta BcSas2-C$ on potato dextrose agar (PDA) plates after 3 days of cultivation. **B**, Colony diameter of the strains grown on PDA for 3 days. **C**, Conidia development of different strains on PDA plates after 10 days of cultivation. **D**, Number of conidia produced by different strains on PDA plates (diameter, 6 cm). **E**, Sclerotium formation of B05.10, $\Delta BcSas2.1$, and $\Delta BcSas2-C$ on PDA medium after 5 weeks of incubation in darkness. **F**, Number of sclerotia produced by various strains.

sclerotial development is a critical survival mechanism in *B. cinerea* (Williamson et al. 2007; Yang et al. 2018a), the number of sclerotia produced by the three strains was observed. After 5 weeks of incubation in the dark, the *BcSAS2* deletion mutant produced fewer sclerotia than the wild-type strain B05.10 and the complementation strain Δ BcSas2-C (Fig. 2E and F), indicating BcSas2 also plays a role in sclerotia formation in *B. cinerea*.

***BcSAS2* deletion affects virulence and stress response of *B. cinerea*.**

To investigate the function of BcSas2 in *B. cinerea* pathogenicity, an infection assay on tomato leaves was performed. Three days after inoculation with Δ BcSas2.1, primary lesions but no spreading lesions were observed (Fig. 3A), while spreading lesions were induced by infection with both the wild-type strain B05.10 and the complemented strain Δ BcSas2-C after day 1. These results demonstrated that BcSas2 was required for host infection in *B. cinerea* strain B05.10.

An onion penetration assay with these strains was further performed, revealing that the deletion of *BcSAS2* resulted in a severe penetration defect (Fig. 3B). No penetration was observed with the deletion mutant. After 16 h of incubation, the germings of Δ BcSas2.1 could not penetrate onion epidermis cells (Fig. 3B), demonstrating an important role of BcSas2 in the penetration efficiency of *B. cinerea*.

Since sensitivity to oxidative stress is associated with a loss of pathogenicity of *B. cinerea* (Yan et al. 2011; Yang et al. 2015, 2018a), the sensitivity of each strain to oxidative stress was examined. Compared with the wild-type strain B05.10 and the complementation strain Δ BcSas2-C, colony diameters achieved by Δ BcSas2.1 were more than 60% reduced after growth on PDA plates supplemented with 0.2% hydrogen peroxide (H_2O_2) for 3 days, reflecting increased sensitivity to H_2O_2 (oxidant) (Fig. 3C and D).

***BcSAS2* inactivation downregulated the expression of 13 genes involved in pathogenicity.**

Previous studies have demonstrated that H4K16ac is closely associated with transcriptional activation in eukaryotes (Grunstein 1997; Lee et al. 2014; Wolffe and Pruss 1996). As shown in Figure 1D, *BcSAS2* deletion significantly reduced the acetylation level of H4K16ac. Therefore, three biological replicates were subjected to RNA-Seq analyses to detect genes that might exhibit changes in regulation affected by BcSas2 in *B. cinerea*. A total of 1,118 genes were differentially expressed in the *BcSAS2* deletion mutant compared with the wild-type strain B05.10 (Supplementary Table S1), which accounted for 9.56% (1,118 of 11,698) of the annotated *B. cinerea* genes. Deletion of the *BcSAS2* gene resulted in the upregulation of the expression of 351 genes and the downregulation of the expression of 767 genes (Supplementary Fig. S3A and B). From the RNA-Seq data, it appeared that H4K16ac may interact directly with BcSas2 in *B. cinerea*.

Cell wall-degrading enzymes and oxidative stress sensitivity are well-established factors affecting pathogenicity of gray mold (Brito et al. 2006; Noda et al. 2010; Yang et al. 2015; Zhu et al. 2017). The genome of *B. cinerea* contains 118 genes related to plant cell-wall degradation (Amselem et al. 2011; Hahn et al. 2014) and 33 genes involved in oxidative stress response. We chose to further investigate all the genes associated with cell-wall degradation and oxidative stress identified in the RNA-Seq data. Twelve of 118 (10%) of the genes encoding cell wall-degrading enzymes were differentially expressed when the *BCSAS2* gene was deleted. Among the 12 differentially expressed genes, 11 were down-regulated and only one (BC1G_07822, cellulose hydrolase) was up-regulated. We speculated that the 11 downregulated genes might lead to the reduced virulence of the mutant and these genes were therefore studied further. None of the oxidative stress-related genes were up-regulated in the *BCSAS2* deletion mutant. Two

Fig. 3. Impact of *BcSAS2* deletion on virulence and stress responses. **A**, Pathogenicity on tomato leaves 3 days after inoculation. **B**, Onion epidermis penetration caused by different strains 16 h after inoculation. The hyphae growing inside the onion cells were not stained, and the penetrated sites are indicated by red arrows. **C**, Sensitivity of the strains after growth on potato dextrose agar (PDA) supplemented with 0.2% H_2O_2 for 3 days. **D**, Colony diameter of the strains grown on PDA supplemented with H_2O_2 . Data are given as the mean $\pm$ standard deviation, $n = 3$. Asterisks (**) indicate a significant difference compared with B05.10, $P < 0.01$.

of 33 (6%) genes encoding oxidative stress–response enzymes were also down-regulated in Δ BcSas2.1. These two down-regulated oxidative-stress genes together with the 11 down-regulated cell wall–degrading genes were subsequently confirmed using reverse transcription-quantitative PCR (RT-qPCR). Figure 4A shows 11 genes (xyloglucan-specific endo-beta-1,4-glucanase A, endo-beta-1,4-glucanase D, endoglucanase H, exoglucanase 1, endo-1,3(4)-beta-glucanase xgeA, endoglucanase type K, endo-beta-1,4-glucanase B, and endoglucanase-4) related to cell-wall degradation and two genes (*BcCAT* and *BcPRX3*) associated with oxidative stress were all down-regulated in the *BcSAS2* deletion mutant. The reduced transcript levels of these genes were also confirmed by RT-qPCR (Fig. 4B).

BcSas2 and H4K16ac bind to the promoters of virulence and oxidative stress–related genes.

BcSas2 significantly affected the acetylation level of H4K16ac (Fig. 1D), and deletion of *BcSAS2* significantly reduced the expression levels of virulence and oxidative stress–related genes (Fig. 4B). BcSas2 contains a zinc-finger structure, which is an important domain for protein-DNA interaction. Therefore, we speculated that BcSas2 interacted with these genes. To test this hypothesis, a selective chromatin immunoprecipitation (ChIP)-qPCR assay (Luo et al. 2012; Zhang et al. 2014; Liu et al. 2019) was used to cross-link promoter target fragments that bind BcSas2 in vivo. The relative availability of target promoter regions of 13 genes in the Δ BcSas2.1 mutant versus wild type was assessed after crosslinking. BcSas2 and GFP were fused and were transferred into

A

	Gene	Gene tag	Annotation
Cell wall degradation	<i>BcXYGA</i>	BC1G_04913	Xyloglucan-specific endo-beta-1,4-glucanase A
	<i>BcGLUD1</i>	BC1G_03038	Endo-beta-1,4-glucanase D
	<i>BcGLUH</i>	BC1G_02011	Endoglucanase H
	<i>BcGLU1</i>	BC1G_14702	Exoglucanase 1
	<i>BcGLUA1</i>	BC1G_09829	Endo-1,3(4)-beta-glucanase xgeA
	<i>BcGLUK</i>	BC1G_13862	Endoglucanase type K
	<i>BcGLUA2</i>	BC1G_09991	Endo-1,3(4)-beta-glucanase xgeA
	<i>BcGLUD2</i>	BC1G_13139	Endo-beta-1,4-glucanase D
	<i>BcGLUB</i>	BC1G_00642	Endo-beta-1,4-glucanase B
	<i>BcGLU4a</i>	BC1G_07653	Endoglucanase-4
	<i>BcGLU4b</i>	BC1G_07658	Endoglucanase-4
Oxidative stress	<i>BcPRX3</i>	BC1G_06262	Peroxidase
	<i>BcCAT</i>	BC1G_12146	Catalase

B

Fig. 4. Downregulation of genes involved in virulence and stress responses. **A**, Gene tags and annotations. **B**, Real-time reverse transcription quantitative PCR (qPCR) validation of RNA-Seq data of selected downregulated genes. The beta-tubulin gene was used as the reference gene to normalize the gene expression for qPCR. Data are given as the mean $\pm$ standard deviation, $n = 3$.

Δ BcSas2.1. The wild-type strain containing GFP alone was used as the negative control. As shown in Figure 5A, the promoter regions of 13 genes involved in virulence and oxidative stress were highly enriched by BcSas2-GFP compared with the negative control strain. *ACTIN* was used as a negative reference gene, and the *ACTIN* sequence was not enriched by ChIP (Fig. 5A).

Previous studies indicated that a 12-nucleotide G-rich sequence motif was found in H4K16ac-enriched DNA fragments, and a CCCTC-binding motif was also identified in enriched fragments (Horikoshi et al. 2013). We searched the promoter regions of the 13 genes confirmed by ChIP as interactive with BcSas2 and found that the promoters of these genes contain G-rich sequences (Supplementary Table S2) and CCCTC-binding motifs (Supplementary Table S3). Thus, we hypothesized that H4K16ac could interact directly with these DNA fragments. To test whether these promoter regions were H4K16ac locations in genomic DNA, we further performed ChIP using an anti-H4K16ac antibody followed by qPCR. The results showed that these regions were also highly enriched by H4K16ac in the wild-type strain compared with the *BcSas2* deletion mutant (Fig. 5B). As expected, the promoter sequence of *ACTIN* could not be enriched in any of the strains (Fig. 5B). These observations demonstrated that BcSas2 and H4K16ac could enrich the promoter regions of virulence and oxidative stress-related genes, indicating that BcSas2 and H4K16ac participated in gene transcriptional regulation in *B. cinerea*.

Further confirmation of two key genes in the pathogenicity of *B. cinerea*.

Deletion of *BcSas2* significantly reduced the expression levels of specific genes related to cell-wall degradation and

Fig. 5. Relative enrichment levels of immunoprecipitated promoter regions of virulence and oxidative stress-related genes. The amount of DNA in chromatin immunoprecipitation quantitative PCR was normalized to an internal control (*TUB-p*). Data are given as the mean $\pm$ standard deviation, $n = 3$. **A**, Relative levels in the immunoprecipitated chromatin from the BcSas2-GFP strain and the negative control. Relative enrichments were calculated by dividing the amount of DNA immunoprecipitated with the anti-GFP (green fluorescent protein) antibody from the BcSas2-GFP strain by that from the negative control strain and were compared with the input DNA. **B**, Relative levels in the immunoprecipitated DNA from wild type and Δ BcSas2.1. Relative enrichments were calculated by dividing the amount of DNA immunoprecipitated with the anti-H4K16ac antibody from wild type by that from Δ BcSas2.1 and were compared with input DNA.

oxidative stress (Fig. 4B). We randomly selected two of these genes for targeted disruption. *BcXygA*, encoding a xyloglucanase, and *BcCat*, encoding a catalase, were separately deleted in wild-type strain B05.10, resulting in two gene deletion mutants, Δ BcXygA.3 and Δ BcCat.5, respectively. The *BcXYGA* gene and the *BcXYGA* gene were cloned and used for complementation of Δ BcXygA.3 and Δ BcCat.5, resulting in two complementation strains, Δ BcXygA-C and Δ BcCat-C, respectively. Infection assays of tomato leaves were performed using mutant, complemented, and wild-type strains, revealing that deletion of *BcXYGA* and *BcCAT* both greatly reduced the pathogenicity of *B. cinerea* on tomato leaves (Fig. 6A). Furthermore, after 16 h of incubation on onion, the germings of both Δ BcXygA.3 and Δ BcCat.5 did not penetrate the epidermal cells (Fig. 6B). However, deletion of either *BcXYGA* or *BcCAT* had little effect on sporulation and sclerotia development (data not shown). The cell sensitivity of each strain to H_2O_2 was also assayed. As expected, when compared with the wild-type strain B05.10, only the *BcCAT* deletion strain exhibited significantly decreased tolerance to the oxidant agent H_2O_2 (Fig. 6C and D). These data demonstrated that *BcXYGA* and *BcCAT* are both involved in the pathogenicity of *B. cinerea*, but only the catalase is involved in oxidative stress tolerance.

DISCUSSION

As a highly conserved PTM, lysine acetylation has been extensively studied in both eukaryotes and prokaryotes

Fig. 6. Impact of *BcXYGA* and *BcCAT* deletions on H_2O_2 response and virulence. **A**, Pathogenicity of different strains on tomato leaves were measured after 3 days inoculation. **B**, The pictures of onion epidermis penetration were taken 16 h after inoculation with conidia of different strains and the penetrated hyphae are indicated by red arrows. **C**, Strains were grown on potato dextrose agar (PDA) plates supplemented with 0.2% H_2O_2 for 3 days for determining hydrogen peroxide (H_2O_2) sensitivity. **D**, Colonies of the strains grown on PDA supplemented with H_2O_2 . Data are given as the mean $\pm$ standard deviation, $n = 3$. Significant difference compared with B05.10: one asterisk (*) indicates $P < 0.05$ and two (**) $P < 0.01$.

(Choudhary et al. 2009). The proteins that were first discovered to be modified by acetylation were histones (Allfrey et al. 1964; Phillips 1963). Histone acetylation levels are dynamic and controlled by two enzymes, HATs and HDACs (Lee and Workman 2007; Yang and Seto 2008). In this study, we investigated the roles of BcSas2, which is a HAT in *B. cinerea*. HATs can transfer acetyl groups to histones and improve the acetylation level of histones. As shown in Figure 1D, the acetylation level of histone H4 was significantly reduced in the deletion mutant Δ BcSas2.1. Our results also showed that the target for acetylation by BcSas2 was the lysine-16 site of histone H4 (Fig. 1D). Consistent with the results in yeast, the acetylation modification of H4K16 is also related to the catalysis of Sas2 in *S. cerevisiae* (Suka et al. 2002; Sutton et al. 2003).

Sas2 is a protein in the MYST superfamily, and it has been well-documented that MYST proteins have significant roles in multiple nuclear processes (Sapountzi and Côté 2011). Previous studies have shown that acetylation levels of histones are involved in gene transcriptional regulation and that high acetylation levels of histones can affect gene transcription (Corona et al. 2002; Deng et al. 2013). The transcriptional expression of 1,118 genes (351 upregulated genes, 767 downregulated genes) was changed significantly in the Δ BcSas2.1 mutant compared with the wild-type strain (Supplementary Table S1), indicating that BcSas2 strongly affected transcriptional regulation in *B. cinerea*, including genes involved in DNA binding, replication, regulation of transcription, DNA topoisomerase, RNA-dependent DNA polymerase. Among the significantly downregulated genes were 13 genes annotated as cell wall-degrading or involved in oxidative stress regulation and therefore likely involved in pathogenicity (Fig. 4B). Based on the results of ChIP-qPCR, BcSas2 bound to the promoters of downregulated virulence and oxidative stress-related genes (Fig. 5A), indicating that these downregulated genes were directly regulated by BcSas2 in *B. cinerea*.

B. cinerea is a necrotrophic pathogen that causes gray mold diseases on many important agricultural crops. Cell wall-degrading enzymes play an important role in the pathogenesis of necrotrophic pathogens (Zhu et al. 2017). These enzymes degrade plant cell-wall polysaccharides, thereby reducing the complex polymers of the cell wall and increasing the effect of pathogen infection on plants (Cantarel et al. 2009; Kubicek et al. 2014). We found that knocking out the *BcSAS2* gene significantly reduced the pathogenicity (Fig. 3A) and infection ability (Fig. 3B) of *B. cinerea*. Further, RNA-Seq analysis showed that a total of 11 genes related to cell-wall degradation were downregulated (Fig. 4A), including xyloglucanases, endoglucanases, and exoglucanases (Fig. 4A). Among these, BcXygA (NCBI BC1G_04913) is a xyloglucanase with a GH12 (glycosyl hydrolase family 12) domain (pfam01670). Another xyloglucanase (BcXyg1) with a GH12 domain was determined to be involved in plant necrosis (Zhu et al. 2017). Furthermore, Xyn11A, a xylanase with a GH11 domain, is necessary for plant cell death caused by *B. cinerea* (Brito et al. 2006; García et al. 2017; Noda et al. 2010). Collectively, these observations reveal that *BcSAS2* is a global regulator of these enzymes in *B. cinerea*.

To avoid infection by pathogens, plants have evolved two types of sophisticated immune systems, pathogen-associated molecular pattern-triggered innate immunity and effector-triggered innate immunity. After the invasion of pathogens, the plant immune response is activated, which leads to a series of reactions, including the accumulation of reactive oxygen species (ROS), stomatal closure, ethylene production, and callose deposition (Cheval et al. 2013; Kaku et al. 2006; Wada et al. 2019). H_2O_2 , a ROS produced by plants, is toxic to pathogens.

We found that disrupting the *BcSAS2* gene can both significantly reduce tolerance to H_2O_2 (Fig. 3C and D) and the pathogenicity of *B. cinerea* (Fig. 3A and B). Consistent with our observations, the deletion of the *BcYAK1* gene in *B. cinerea* also led to increased sensitivity to H_2O_2 and decreased pathogenicity of *B. cinerea* (Yang et al. 2018a). Furthermore, many proteins were found to be associated with oxidative sensitivity and pathogenicity in *B. cinerea*, such as BcSkn7 (a response regulator) (Yang et al. 2015), BcPtpA and BcPtpB (two protein tyrosine phosphatases) (Yang et al. 2013), Brrg-1 (a putative response regulator) (Yan et al. 2011), and BcCat (Fig. 6). All these observations support the concept that increased H_2O_2 sensitivity is associated with decreased *B. cinerea* pathogenicity to plants.

In addition to cell-wall degradation and oxidative stress-response genes, other genes are known to be involved in pathogenicity of *B. cinerea* (Amselem et al. 2011; Hahn et al. 2014). For example, BcSpl1 (a cerato-platanin protein) (Frías et al. 2011), BcVel1 (Schumacher et al. 2012), BcAtrB (an ABC transporter) (Stefanato et al. 2009), BcPP2Ac (a phosphoprotein phosphatase), SPT3 (a transcription factor) (Giesbert et al. 2012), and CRZ1 (a calcineurin-responsive zinc finger transcription factor) (Schumacher et al. 2008) are evidently crucial for virulence of *B. cinerea*. Among this group, only the CRZ1 gene appeared to be affected by BcSas2 (it was downregulated in the *BcSAS2* deletion mutant). Taken together, these observations suggest that BcSas2 affected pathogenicity mainly by regulating plant cell-wall degradation and resistance to oxidative pressure.

In summary, the HAT BcSas2 of *B. cinerea* is located in the nucleus and is closely related to the acetylation histone of H4K16. BcSas2 plays an important role in virulence and the response to oxidative stress by regulating at least 13 genes involved in plant cell-wall degradation. The promoter regions of these pathogenicity-related genes were shown to directly interact with BcSas2 and H4K16ac, suggesting an important transcriptional regulatory role of BcSas2 and H4K16ac.

MATERIALS AND METHODS

Strains, media, and culture.

Wild-type strain B05.10 of *B. cinerea* isolated from *Vitis vinifera* was used for transformation experiments in this study (Yang et al. 2018a). Strains Δ BcSas2.1, Δ BcXygA.3, and Δ BcCat.5 were made by marker exchange of B05.10 as described (Yang et al. 2018a). Strains Δ BcSas2-C, Δ BcXygA-C, and Δ BcCat-C were the gene complements of Δ BcSas2.1, Δ BcXygA.3, and Δ BcCat.5, respectively. All strains were grown in YPD medium (1% yeast extract, 2% peptone, 2% glucose) and on PDA plates (20% potato, 2% dextrose, and 1.5% agar) supplemented with 0.01% hygromycin B or 0.01% sulfate geneticin G418. The number of conidia produced by wild type and mutant and complemented strains were counted after 10 days of incubation on PDA plates. The sclerotium formation of these strains was also investigated after 5 weeks of incubation on PDA plates in darkness. The sensitivity of different strains to H_2O_2 was investigated after 3 days of incubation on PDA plates supplemented with 0.2% H_2O_2 (Yan et al. 2010).

General genetic techniques.

An Ezup-Column-fungi genomic DNA purification kit (Sangon Biotech) was used to extract genomic DNA from different strains. A SimpliAmp thermal cycler (ABI) was used to perform PCR amplification with Q5 DNA polymerase (New England Biolabs) and primers (Supplementary Table S4). A DNA gene extraction kit (TsingKe) was used to purify DNA

fragments. Plasmids were extracted from *Escherichia coli* DH5 α using a Plasmid Miniprep kit (TsingKe).

Construction of gene-deletion cassettes, gene-complementation vectors, and transformation.

To construct the gene-deletion vectors, two flanking sequences of *BcSAS2* (NCBI BC1G_05395), *BcCAT* (BC1G_12146) and *BcXYGA* (BC1G_04913), were amplified from *B. cinerea* B05.10 genomic DNA by PCR, using the primers listed in Supplementary Table S4. The hygromycin B phosphotransferase (*HPH*) gene was amplified from the pBS-HPH1 vector (Yang et al. 2018a). The two flanking sequences and the *HPH* gene were fused by splice overlap PCR, and the resulting fragments (*Promoter::HPH::Terminator*) were ligated into the pMD19-T vector for sequencing. The deletion cassettes (*Promoter::HPH::Terminator*) were transformed into *B. cinerea* B05.10, using protoplast transformation methods, as previously described (Gronover et al. 2001). Positive transformants of $\Delta BcSas2.1$, $\Delta BcXygA.3$, and $\Delta BcCat.5$ were selected on PDA plates with 0.01% hygromycin B, and the gene deletions were verified by PCR using primers listed in Supplementary Table S4. The genes *BcSAS2*, *BcCAT*, and *BcXYGA*, including their native promoters and terminators were amplified from *B. cinerea* B05.10 genomic DNA using the primers shown in Supplementary Table S4 and were cloned into plasmid pBS-neo (Yang et al. 2018a). These were subsequently transformed into $\Delta BcSas2.1$, $\Delta BcCat.5$, and $\Delta BcXygA.3$, respectively, using standard procedures as described (Gronover et al. 2001; Yang et al. 2018a).

Assays of pathogenicity, onion epidermis penetration, and sensitivity to H₂O₂.

Pathogenicity assays of different strains on tomato leaves were measured as described previously (Yang et al. 2013). In brief, the strains of *B. cinerea* were incubated on PDA plates at 25°C for 4 days. Then, 5-mm-diameter plugs from the plates were transferred to tomato leaves (3 weeks old) and were incubated at 25°C for 3 days. Finally, the lesion diameters caused by different strains were measured. Onion epidermis penetration by different strains was performed using the procedures described previously (Viaud et al. 2006; Yang et al. 2018a). To measure strain sensitivity to H₂O₂, different strains of *B. cinerea* were incubated on PDA plates supplemented with 0.2% H₂O₂ at 25°C for 3 days. Mycelial growth was then measured.

RNA extraction, library construction, and sequencing.

After growth in YPD medium at 25°C for 24 h, the mycelia of different *B. cinerea* strains were harvested by centrifugation at 9,000 $\times$ g and 4°C for 15 min. Total RNA was extracted as described previously (Wang et al. 2018). RNA purity was detected using a K5500 spectrophotometer (Kaiao). A 2100-RNA Nano 6000 assay kit (Agilent) was employed to confirm the RNA concentration and integrity. mRNA was enriched using oligo (dT) magnetic beads and was fragmented into short segments. First-strand cDNA was synthesized using a 6-base random primer, and the second strand was subsequently synthesized using DNA polymerase I, dNTPs, and RNaseH. After purification by a QIAQuick PCR kit (Qiagen), the cDNA fragments were adenylated at the 3' ends and were ligated to sequencing adaptors. To enrich the cDNA fragments, PCR was further performed, and the generated sequencing library was sequenced on an Illumina platform (Wang et al. 2018).

Bioinformatics analysis of the RNA-Seq data.

To obtain high-quality reads, the adaptor sequences and low-quality sequences in the raw data were discarded. The obtained clean reads were mapped against the database of *B. cinerea*

using TopHat (v2.0.12) (Trapnell et al. 2009), and HTSeq (v 0.6.1) was employed to calculate the numbers of mapped genes (Anders et al. 2015). The reads per kilobase per million mapped reads value was used to quantitatively estimate gene expression values (Trapnell et al. 2010). The differentially expressed genes between the treatment group and the reference group were analyzed by DESeq, and a *P* value less than 0.05 in DESeq was selected as significant (Wang et al. 2010).

Fluorescent real-time qPCR.

RNA was extracted from different strains of *B. cinerea* according to a previously described method (Yang et al. 2018a). A PrimeScript RT reagent kit (Takara) was used to synthesize cDNA. The primers used in fluorescent real-time qPCR are listed in Supplementary Table S4, and the $2^{-\Delta\Delta C_t}$ method was employed to calculate the relative gene expression levels (Livak and Schmittgen 2001).

Western blot analysis.

The mycelia of different strains of *B. cinerea* were disrupted, and the nuclear proteins were purified using a Nuclear protein extraction kit (Solarbio). The obtained proteins were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis using a 12% gel and subsequently electrotransferred onto a polyvinylidene difluoride membrane (Wang et al. 2019). After blocking the membrane with 5% skim milk powder, the membrane was incubated with antihistone H4 antibody (1: 5,000 dilution [Abcam]), antiacetyl-histone H4 antibody (1: 4,000 dilution [Sigma-Aldrich]), and antihistone H4 (acetyl K16) antibody (1:4,000 dilution [Abcam]). Finally, an ECL immunoblotting-detection kit (ThermoFisher) was employed to visualize the signals.

ChIP-qPCR analyses.

ChIP was performed according to previously described methods (Liu et al. 2019; Luo et al. 2012; Zhang et al. 2014). In brief, after the mycelia of different strains had developed, mycelia were cross-linked with formaldehyde buffer (10 mM Tris-HCl, pH 8.0, 0.4 M sucrose, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride [PMSF], and 1% formaldehyde) at 25°C for 24 h, and the crosslinking was stopped with 125 mM glycine (pH 7.5) at 25°C and 180 rpm (HZQ-F100, Suzhou Peiying Equipment Manufacturing Co., Ltd.) for 5 min. After centrifugation, the supernatant was discarded. The obtained mycelia were then frozen and ground in liquid nitrogen. The resulting powder was suspended in lysis buffer (250 mM HEPES, pH 7.5, 0.15 M NaCl, 1% Triton X-100 [vol/vol], 0.1% sodium deoxycholate (wt/vol), 0.01 M dithiothreitol, 1 mM EDTA, and 100 mM PMSF), followed by DNA fragmentation using a sonicator (Scientz-650E). After centrifugation at 4°C and 16,200 $\times$ g for 10 min, the obtained sonicated lysates were diluted in buffer (1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl, pH 8.0, and 167 mM NaCl), and the chromatin concentration was determined using a biospectrometer (Eppendorf). Immunoprecipitation was performed using anti-GFP antibody (Abcam) or antihistone H4K16ac antibody (Abcam) together with protein A agarose beads (GE Healthcare) overnight at 4°C. After separation, the beads were washed sequentially with low-salt wash buffer, high-salt wash buffer, LiCl wash buffer, and Tris-EDTA buffer (Liu et al. 2019). The DNA bound to the beads was eluted at 65°C for 2 h, using elution buffer containing 1% SDS, 0.1 M NaHCO₃, and proteinase K. The eluted DNA was purified by a Spin dna purification kit (Sangon Biotech). The enriched DNA in ChIP was quantified by fluorescent real-time qPCR with the primers shown in Supplementary Table S4. The relative enrichment value was calculated by dividing the immunoprecipitation DNA by the input DNA (Liu et al. 2019).

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LITERATURE CITED

- Allfrey, V. G., Faulkner, R., and Mirsky, A. E. 1964. Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. *Proc. Natl. Acad. Sci. U.S.A.* 51:786-794.
- Amselem, J., Cuomo, C. A., van Kan, J. A., Viaud, M., Benito, E. P., Couloux, A., Coutinho, P. M., de Vries, R. P., Dyer, P. S., Fillinger, S., Fournier, E., Gout, L., Hahn, M., Kohn, L., Lapalu, N., Plummer, K. M., Pradier, J. M., Quévillon, E., Sharon, A., Simon, A., ten Have, A., Tudzynski, B., Tudzynski, P., Wincker, P., Andrew, M., Anthouard, V., Beever, R. E., Beffa, R., Benoit, I., Bouzid, O., Brault, B., Chen, Z., Choquer, M., Collémar, J., Cotton, P., Danchin, E. G., Da Silva, C., Gautier, A., Giraud, C., Giraud, T., Gonzalez, C., Grossetete, S., Güldener, U., Henrissat, B., Howlett, B. J., Kodira, C., Kretschmer, M., Lappartient, A., Leroch, M., Levis, C., Mauceli, E., Neuvéglise, C., Oeser, B., Pearson, M., Poulain, J., Poussereau, N., Quesneville, H., Rasche, C., Schumacher, J., Séguens, B., Sexton, A., Silva, E., Sirven, C., Soanes, D. M., Talbot, N. J., Templeton, M., Yandava, C., Yarden, O., Zeng, Q., Rollins, J. A., Lebrun, M. H., and Dickman, M. 2011. Genomic analysis of the necrotrophic fungal pathogens *Sclerotinia sclerotiorum* and *Botrytis cinerea*. *PLoS Genet.* 7:e1002230.
- Anders, S., Pyl, P. T., and Huber, W. 2015. HTSeq—A Python framework to work with high-throughput sequencing data. *Bioinformatics* 31: 166-169.
- Brito, N., Espino, J. J., and González, C. 2006. The endo-beta-1,4-xylanase xyn11A is required for virulence in *Botrytis cinerea*. *Mol. Plant-Microbe Interact.* 19:25-32.
- Cantarel, B. L., Coutinho, P. M., Rancurel, C., Bernard, T., Lombard, V., and Henrissat, B. 2009. The Carbohydrate-Active EnZymes database (CAZy): An expert resource for glycogenomics. *Nucleic Acids Res.* 37 (Database):D233-D238.
- Cheval, C., Aldon, D., Galaud, J. P., and Ranty, B. 2013. Calcium/calmodulin-mediated regulation of plant immunity. *Biochim. Biophys. Acta* 1833:1766-1771.
- Choudhary, C., Kumar, C., Gnäd, F., Nielsen, M. L., Rehman, M., Walther, T. C., Olsen, J. V., and Mann, M. 2009. Lysine acetylation targets protein complexes and co-regulates major cellular functions. *Science* 325: 834-840.
- Corona, D. F., Clapier, C. R., Becker, P. B., and Tamkun, J. W. 2002. Modulation of ISWI function by site-specific histone acetylation. *EMBO Rep.* 3:242-247.
- Deng, X., Berletch, J. B., Ma, W., Nguyen, D. K., Hiatt, J. B., Noble, W. S., Shendure, J., and Distech, C. M. 2013. Mammalian X upregulation is associated with enhanced transcription initiation, RNA half-life, and MOF-mediated H4K16 acetylation. *Dev. Cell* 25:55-68.
- Frías, M., González, C., and Brito, N. 2011. BcSpl1, a cerato-platanin family protein, contributes to *Botrytis cinerea* virulence and elicits the hypersensitive response in the host. *New Phytol.* 192:483-495.
- Füllgrabe, J., Lynch-Day, M. A., Heldring, N., Li, W., Struijk, R. B., Ma, Q., Hermanson, O., Rosenfeld, M. G., Klionsky, D. J., and Joseph, B. 2013. The histone H4 lysine 16 acetyltransferase hMOF regulates the outcome of autophagy. *Nature* 500:468-471.
- García, N., González, M. A., González, C., and Brito, N. 2017. Simultaneous silencing of xylanase genes in *Botrytis cinerea*. *Front. Plant Sci.* 8:2174.
- Giesbert, S., Schumacher, J., Kupas, V., Espino, J., Segmüller, N., Haeuser-Hahn, I., Schreier, P. H., and Tudzynski, P. 2012. Identification of pathogenesis-associated genes by T-DNA-mediated insertional mutagenesis in *Botrytis cinerea*: A type 2A phosphoprotein phosphatase and an SPT3 transcription factor have significant impact on virulence. *Mol. Plant-Microbe Interact.* 25:481-495.
- Gronover, C. S., Kasulke, D., Tudzynski, P., and Tudzynski, B. 2001. The role of G protein alpha subunits in the infection process of the gray mold fungus *Botrytis cinerea*. *Mol. Plant-Microbe Interact.* 14:1293-1302.
- Grunstein, M. 1997. Histone acetylation in chromatin structure and transcription. *Nature* 389:349-352.
- Hahn, M., Viaud, M., and Van Kan, J. A. L. 2014. The genome of *Botrytis cinerea*, a ubiquitous broad host range necrotroph. Pages 19-44 in: *Genomics of Plant-Associated Fungi and Oomycetes: Dicot Pathogens*, R. A. Dean, A. Lichens-Park, and C. Kole, eds. Springer Verlag, Berlin.
- Heise, F., Chung, H. R., Weber, J. M., Xu, Z., Klein-Hitpass, L., Steinmetz, L. M., Vingron, M., and Ehrenhofer-Murray, A. E. 2012. Genome-wide H4 K16 acetylation by SAS-I is deposited independently of transcription and histone exchange. *Nucleic Acids Res.* 40:65-74.
- Horikoshi, N., Kumar, P., Sharma, G. G., Chen, M., Hunt, C. R., Westover, K., Chowdhury, S., and Pandita, T. K. 2013. Genome-wide distribution of histone H4 lysine 16 acetylation sites and their relationship to gene expression. *Genome Integr.* 4:3.
- Kaku, H., Nishizawa, Y., Ishii-Minami, N., Akimoto-Tomiya, C., Dohmae, N., Takio, K., Minami, E., and Shibuya, N. 2006. Plant cells recognize chitin fragments for defense signaling through a plasma membrane receptor. *Proc. Natl. Acad. Sci. U.S.A.* 103:11086-11091.
- Kouzarides, T. 2007. Chromatin modifications and their function. *Cell* 128: 693-705.
- Kubicek, C. P., Starr, T. L., and Glass, N. L. 2014. Plant cell wall-degrading enzymes and their secretion in plant-pathogenic fungi. *Annu. Rev. Phytopathol.* 52:427-451.
- Kuo, M. H., and Allis, C. D. 1998. Roles of histone acetyltransferases and deacetylases in gene regulation. *BioEssays* 20:615-626.
- Lee, K. K., and Workman, J. L. 2007. Histone acetyltransferase complexes: One size doesn't fit all. *Nat. Rev. Mol. Cell Biol.* 8:284-295.
- Lee, Y., Min, K., Son, H., Park, A. R., Kim, J. C., Choi, G. J., and Lee, Y. W. 2014. ELP3 is involved in sexual and asexual development, virulence, and the oxidative stress response in *Fusarium graminearum*. *Mol. Plant-Microbe Interact.* 27:1344-1355.
- Liu, Z., Jian, Y., Chen, Y., Kistler, H. C., He, P., Ma, Z., and Yin, Y. 2019. A phosphorylated transcription factor regulates sterol biosynthesis in *Fusarium graminearum*. *Nat. Commun.* 10:1228.
- Livak, K. J., and Schmittgen, T. D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2^{-ΔΔCT} method. *Methods* 25:402-408.
- Luo, M., Yu, C. W., Chen, F. F., Zhao, L., Tian, G., Liu, X., Cui, Y., Yang, J. Y., and Wu, K. 2012. Histone deacetylase HDA6 is functionally associated with AS1 in repression of *KNOX* genes in *Arabidopsis*. *PLoS Genet.* 8:e1003114.
- Lv, B., Yang, Q., Li, D., Liang, W., and Song, L. 2016. Proteome-wide analysis of lysine acetylation in the plant pathogen *Botrytis cinerea*. *Sci. Rep.* 6:29313.
- Meijsing, S. H., and Ehrenhofer-Murray, A. E. 2001. The silencing complex SAS-I links histone acetylation to the assembly of repressed chromatin by CAF-I and Asf1 in *Saccharomyces cerevisiae*. *Genes Dev.* 15: 3169-3182.
- Noda, J., Brito, N., and González, C. 2010. The *Botrytis cinerea* xylanase Xyn11A contributes to virulence with its necrotizing activity, not with its catalytic activity. *BMC Plant Biol.* 10:38.
- Phillips, D. M. 1963. The presence of acetyl groups of histones. *Biochem. J.* 87:258-263.
- Sapountzi, V., and Côté, J. 2011. MYST-family histone acetyltransferases: Beyond chromatin. *Cell. Mol. Life Sci.* 68:1147-1156.
- Schumacher, J., de Larrinoa, I. F., and Tudzynski, B. 2008. Calcineurin-responsive zinc finger transcription factor CRZ1 of *Botrytis cinerea* is required for growth, development, and full virulence on bean plants. *Eukaryot. Cell* 7:584-601.
- Schumacher, J., Pradier, J. M., Simon, A., Traeger, S., Moraga, J., Collado, I. G., Viaud, M., and Tudzynski, B. 2012. Natural variation in the VELVET gene bev11 affects virulence and light-dependent differentiation in *Botrytis cinerea*. *PLoS One* 7:e47840.
- Stefanato, F. L., Abou-Mansour, E., Buchala, A., Kretschmer, M., Mosbach, A., Hahn, M., Bochet, C. G., Métraux, J. P., and Schoonbeek, H. J. 2009. The ABC transporter BcatB from *Botrytis cinerea* exports camalexin and is a virulence factor on *Arabidopsis thaliana*. *Plant J.* 58: 499-510.
- Su, J., Wang, F., Cai, Y., and Jin, J. 2016. The functional analysis of histone acetyltransferase MOF in tumorigenesis. *Int. J. Mol. Sci.* 17:99.
- Suka, N., Luo, K., and Grunstein, M. 2002. Sir2p and Sas2p opposingly regulate acetylation of yeast histone H4 lysine16 and spreading of heterochromatin. *Nat. Genet.* 32:378-383.
- Sutton, A., Shia, W. J., Band, D., Kaufman, P. D., Osada, S., Workman, J. L., and Sternglanz, R. 2003. Sas4 and Sas5 are required for the histone acetyltransferase activity of Sas2 in the SAS complex. *J. Biol. Chem.* 278:16887-16892.
- Trapnell, C., Pachter, L., and Salzberg, S. L. 2009. TopHat: Discovering splice junctions with RNA-Seq. *Bioinformatics* 25:1105-1111.
- Trapnell, C., Williams, B. A., Pertea, G., Mortazavi, A., Kwan, G., van Baren, M. J., Salzberg, S. L., Wold, B. J., and Pachter, L. 2010. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. *Nat. Biotechnol.* 28:511-515.
- Viaud, M., Fillinger, S., Liu, W., Polepalli, J. S., Le Pêcheur, P., Kunduru, A. R., Leroux, P., and Legendre, L. 2006. A class III histidine kinase acts

- as a novel virulence factor in *Botrytis cinerea*. Mol. Plant-Microbe Interact. 19:1042-1050.
- Wada, S., Cui, S., and Yoshida, S. 2019. Reactive oxygen species (ROS) generation is indispensable for haustorium formation of the root parasitic plant striga hermonthica. Front. Plant Sci. 10:328.
- Wang, G., Li, D., Miao, Z., Zhang, S., Liang, W., and Liu, L. 2018. Comparative transcriptome analysis reveals multiple functions for Mhy1p in lipid biosynthesis in the oleaginous yeast *Yarrowia lipolytica*. Biochim. Biophys. Acta Mol. Cell Biol. Lipids 1863:81-90.
- Wang, G., Xu, L., Yu, H., Gao, J., and Guo, L. 2019. Systematic analysis of the lysine succinylome in the model medicinal mushroom *Ganoderma lucidum*. BMC Genomics 20:585.
- Wang, L., Feng, Z., Wang, X., Wang, X., and Zhang, X. 2010. DEGseq: An R package for identifying differentially expressed genes from RNA-seq data. Bioinformatics 26:136-138.
- Williamson, B., Tudzynski, B., Tudzynski, P., and van Kan, J. A. 2007. *Botrytis cinerea*: The cause of grey mould disease. Mol. Plant Pathol. 8:561-580.
- Wolffe, A. P., and Pruss, D. 1996. Targeting chromatin disruption: Transcription regulators that acetylate histones. Cell 84:817-819.
- Yan, L., Yang, Q., Jiang, J., Michailides, T. J., and Ma, Z. 2011. Involvement of a putative response regulator Brrg-1 in the regulation of sporulation, sensitivity to fungicides, and osmotic stress in *Botrytis cinerea*. Appl. Microbiol. Biotechnol. 90:215-226.
- Yan, L., Yang, Q., Sundin, G. W., Li, H., and Ma, Z. 2010. The mitogen-activated protein kinase kinase BOS5 is involved in regulating vegetative differentiation and virulence in *Botrytis cinerea*. Fungal Genet. Biol. 47: 753-760.
- Yang, Q., Yin, D., Yin, Y., Cao, Y., and Ma, Z. 2015. The response regulator BcSkn7 is required for vegetative differentiation and adaptation to oxidative and osmotic stresses in *Botrytis cinerea*. Mol. Plant Pathol. 16: 276-287.
- Yang, Q., Yu, F., Yin, Y., and Ma, Z. 2013. Involvement of protein tyrosine phosphatases BcPtpA and BcPtpB in regulation of vegetative development, virulence and multi-stress tolerance in *Botrytis cinerea*. PLoS One 8:e61307.
- Yang, Q., Zhang, J., Hu, J., Wang, X., Lv, B., and Liang, W. 2018a. Involvement of BcYak1 in the regulation of vegetative differentiation and adaptation to oxidative stress of *Botrytis cinerea*. Front. Microbiol. 9:281.
- Yang, X. J., and Seto, E. 2008. Lysine acetylation: Codified crosstalk with other posttranslational modifications. Mol. Cell 31:449-461.
- Yang, Y., Yang, X., Dong, Y., and Qiu, D. 2018b. The *Botrytis cinerea* xylanase BcXyl1 modulates plant immunity. Front. Microbiol. 9:2535.
- Zhang, Q., Lei, X., and Lu, H. 2014. Alterations of epigenetic signatures in hepatocyte nuclear factor 4 α deficient mouse liver determined by improved ChIP-qPCR and (h)MeDIP-qPCR assays. PLoS One 9:e84925.
- Zhou, S., Yang, Q., Yin, C., Liu, L., and Liang, W. 2016. Systematic analysis of the lysine acetylome in *Fusarium graminearum*. BMC Genomics 17:1019.
- Zhu, W., Ronen, M., Gur, Y., Minz-Dub, A., Masrati, G., Ben-Tal, N., Savidor, A., Sharon, I., Eizner, E., Valerius, O., Braus, G. H., Bowler, K., Bar-Peled, M., and Sharon, A. 2017. BcXYG1, a secreted xyloglucanase from *Botrytis cinerea*, triggers both cell death and plant immune responses. Plant Physiol. 175:438-456.