Histone Acetyltransferases and Deacetylases Are Required for Virulence, Conidiation, DNA Damage Repair, and Multiple Stresses Resistance of *Alternaria alternata*

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Histone acetylation, which is critical for transcriptional regulation and various biological processes in eukaryotes, is a reversible dynamic process regulated by HATs and HDACs. This study determined the function of 6 histone acetyltransferases (HATs) (*Gcn5*, *RTT109*, *Elp3*, *Sas3*, *Sas2*, *Nat3*) and 6 histone deacetylases (HDACs) (*Hos2*, *Rpd3*, *Hda1*, *Hos3*, *Hst2*, *Sir2*) in the phytopathogenic fungus *Alternaria alternata* by analyzing targeted gene deletion mutants. Our data provide evidence that HATs and HDACs are both required for mycelium growth, cell development and pathogenicity as many gene deletion mutants (ΔGcn5, ΔRTT109, ΔElp3, ΔSas3, ΔNat3, ΔHos2, and ΔRpd3) displayed reduced growth, conidiation or virulence at varying degrees. In addition, HATs and HDACs are involved in the resistance to multiple stresses such as oxidative stress (*Sas3*, *Gcn5*, *Elp3*, *RTT109*, *Hos2*), osmotic stress (*Sas3*, *Gcn5*, *RTT109*, *Hos2*), cell wall-targeting agents (*Sas3*, *Gcn5*, *Hos2*), and fungicide (*Gcn5*, *Hos2*). ΔGcn5, ΔSas3, and ΔHos2 displayed severe growth defects on sole carbon source medium suggesting a vital role of HATs and HDACs in carbon source utilization. More SNPs were generated in ΔGcn5 in comparison to wild-type when they were exposed to ultraviolet ray. Moreover, ΔRTT109, ΔGcn5, and ΔHos2 showed severe defects in resistance to DNA-damaging agents, indicating the critical role of HATs and HDACs in DNA damage repair. These phenotypes correlated well with the differentially expressed genes in ΔGcn5 and ΔHos2 that are essential for carbon sources metabolism, DNA damage repair, ROS detoxification, and asexual development. Furthermore, *Gcn5* is required for the acetylation of H3K4. Overall, our study provides genetic evidence to define the central role of HATs and HDACs in the pathological and biological functions of *A. alternata*.

Keywords: histone acetylation, transcriptional regulation, DNA damage repair, pathogenicity, *Alternaria alternata*

INTRODUCTION

The fundamental structural subunit of the chromatin is the nucleosome, which consists of approximately 200 bp of DNA wrapped around an octamer of core histone proteins H2A, H2B, H3, and H4 (Kornberg and Lorch, 1999). The reversible acetylation of specific lysine residues in N-terminal tails of core histones is maintained by histone acetyltransferases (HATs) and histone deacetylases (HDACs) and has been found to play a pivotal role in chromatin-regulated DNA events, such as DNA replication, damage repair, recombination, and gene expression in eukaryotes (Lee and Workman, 2007; Yang and Seto, 2008). HATs are classified into five families, including MYST (Ybf2/Sas3, Sas2, MOZ, Tip60), GNAT (Gcn5-related N-acetyltransferases), p300/CBP, nuclear receptor cofactors, and basal transcription factors (Jeon et al., 2014). HDACs are grouped into four groups based on phylogenetic analysis and sequence homology, including Class I and II (Rpd3, Hda1), Class III (Sir2 or Sir2-like protein) and Class IV (HDAC11) (Gregoretti et al., 2004; Jeon et al., 2014).

In yeast, many HATs or HDACs have been found to be involved in transcriptional regulation and many cellular functions. Gcn5 belongs to the GNAT family and acts as a core subunit of several histone acetyltransferase (HAT) complexes, such as ADA, SAGA, or SALSA (Lee and Young, 2000). Gcn5 acetylates N-terminal lysine (K) residues of histones H2B (K11/16) or H3 (K14/18/23/27/36), and the loss of its function leads to defects in gene transcription, sexual differentiation and multiple stresses resistance (Helmlinger et al., 2008; Johnsson et al., 2009; Nugent et al., 2010). H3K9/27/56 can be acetylated by RTT109, a HAT of p300/CBP family (Fillingham et al., 2008; Das et al., 2009; Burgess et al., 2010; Da Rosa et al., 2010). In yeast, H3K56ac regulates expression homeostasis and resistance to DNA-damaging agents (Masumoto et al., 2005; Ozdemir et al., 2005; Han et al., 2007; Voichek et al., 2016). Both Sas3 and Esa1 belong to MYST family, which function as the subunits of Nucleosome Acetyltransferase of histone H3 (NuA3) and H4 (NuA4) complexes, respectively (Lee and Workman, 2007). Studies revealed that Esa1 is essential for cell cycle progression in yeast (Clarke et al., 1999). In *S. cerevisiae*, the transcription of FLO1 was significantly reduced in the absence of both Sas3 and Ada2 (Church et al., 2017). Rpd3 is a founding member of class I and comprises an N-terminal deacetylase domain (Vidal and Gaber, 1991). In yeast, Rpd3 functions as a catalytic subunit in sin3 complexes and has been linked to many biological functions, such as transcriptional regulation, DNA repair, replication, ROS resistance, or azole resistance (Vogelauer et al., 2002; Jazayeri et al., 2004; Baker et al., 2013; Li et al., 2015; Bosio et al., 2017). Hos2 and Hda1 belong to class II, and both of them also contain an N-terminal deacetylase domain (Yang and Seto, 2008). In yeast, Hda1 and Rpd3L contribute to the repair of replication-born DSBs (double strand breaks) by facilitating cohesin loading thus preventing genome instability (Ortega et al., 2019). Hos2 acts as a key subunit of Set3 complex which deacetylates H4 and H3 by counteracting Esa1 (Pijnappel et al., 2001; Torres-Machorro et al., 2015). The yeast Hos2 is involved in various biological functions, such as these involved in cell integrity pathway, cell resistance

to multiple stresses, DNA damage repair, and expression of growth-related genes (Pijnappel et al., 2001; Wirén et al., 2005; Cohen et al., 2008; Kim et al., 2012; Tkach et al., 2012). The best-characterized member in Class III is the NAD-dependent histone deacetylase (HDAC) Sir2. Yeast Sir2 acts as anti-aging gene required for cellular lifespan regulation by caloric restriction (Lin et al., 2000; Dang et al., 2009).

In filamentous fungi, HATs and HDACs have been found to be involved in growth, development, virulence, synthesis of secondary metabolites and multi-stress resistance. Studies in *Aspergillus nidulans* showed that GcnE (ortholog of Gcn5) controls asexual reproduction, and the biosynthesis of sterigmatocystin, penicillin and terrequinone A (Nützmann et al., 2011; Cánovas et al., 2014). Genetic deletion of *Gcn5* leads to severe defects in virulence, sexual or asexual development, and/or virulence in *Ustilago maydis*, *Beauveria bassiana*, and *Trichoderma reesei* (Xin et al., 2013; González-Prieto et al., 2014; Cai et al., 2018b). In *B. bassiana*, *RTT109* deletion mutant shows attenuated virulence, and increased sensitivity to the stress of oxidation and DNA-damaging agents (Cai et al., 2018c). Many other HATs also have been found to be involved in cell growth and pathogenicity in *M. oryzae* (Sas3), *F. graminearum* (Elp3), and so on (Lee et al., 2014; Dubey et al., 2019). HDAC Hos2 acts as a vital subunit of Tig1 complex crucial for conidiation and infectious growth in *M. oryzae*, and is required for pathogenesis and dimorphic switch in *U. maydis* (Ding et al., 2010; Elías-Villalobos et al., 2015). Although many HATs and HDACs have been identified to be critical for an array of biological processes in many fungi, the specific function of each gene is not completely the same among different fungi.

Alternaria alternata tangerine pathotype is a disastrous fungal pathogen which causes Citrus Brown Spot on young leaves, shoots and fruits of many citrus cultivars. Previous studies revealed that ROS detoxification-related genes such as *Ap1* and *Skn7* (Lin et al., 2009; Chen et al., 2012) or host-selective ACT-toxin biosynthesis genes such as *ACTT5* and *ACTT6* (Miyamoto et al., 2009), are critical for *A. alternata* to cause lesions on host tissues. Although numerous studies of HATs or HDACs indicate that many of these genes are involved in ROS detoxification, synthesis of secondary metabolites, and various biological functions in many pathogenic fungi, the roles of these genes in *A. alternata* have not been elucidated. In this study, we identified all the HATs and HDACs orthologs in *A. alternata* and clarified their roles in growth and development, carbon source utilization, multiple stresses resistance, DNA damage repair and virulence using genetic and biological analyses. We also performed a transcriptome analysis to explore the regulatory role of *Gcn5* and *Hos2* in this important citrus pathogen.

MATERIALS AND MERHODS

Fungal Strains and Culture Conditions

The wild-type Z7 strain of *A. alternata* (Fr.) Keissler used in the mutagenesis experiments was isolated from an infected citrus (*Citrus suavissima* Hort. Ex Tanaka) in Zhejiang, China (Huang et al., 2015; Wang et al., 2016). Fungal strains were cultured on

potato dextrose agar (PDA) at 26°C and conidia were collected after incubating on V8 medium for 8 days. Mycelium cultured in liquid potato dextrose broth (PDB) was collected by passing through cheesecloth and used for purification of DNA or RNA.

Targeted Gene Disruption and Genetic Complementation

Δ*Sas2*, Δ*Sas3*, Δ*Elp3*, Δ*Gcn5*, Δ*RTT109*, Δ*Nat3*, Δ*Rpd3*, Δ*Hos2*, Δ*Hos3*, Δ*Hda1*, Δ*Hst2,* and Δ*Sir2* strains were created by deleting *Sas2*, *Sas3*, *Elp3*, *Gcn5*, *RTT109*, *Nat3*, *Rpd3*, *Hos2*, *Hos3*, *Hda1*, *Hst2,* and *Sir2,* respectively, by integrating a bacterial *HYG* cassette under control of *TrpC* gene promoter and terminator in the genome of Z7 using a split marker approach mediated by protoplasts transformation as described previously (Chung et al., 2002; Ma et al., 2018). Fungal transformants were recovered from PDA containing 100 μg/ml hygromycin and examined by PCR with primers specific to its targeted gene. Oligonucleotide primers used in this study are listed in **Supplementary Table 1**.

Microscopy

Morphological observation of conidia and hyphae was carried using a Nikon microscope equipped with a LV100ND image system (Nikon, Japan).

Virulence Assays

Fungal virulence was assessed on detached Dancy (*Citrus reticulata* Blanco) leaves inoculated by placing a 5-mm dia. agar plug covered with fungal mycelium on each spot, and those inoculated leaves were kept in a plastic box at 26°C for 2–4 days for lesion development.

Phenotypic Experiments

All the phenotypes of mutants and wild-type were evaluated. To examine the vegetative growth under multiple stresses, mutants and wild-type were inoculated on PDA containing 10 mM H_2O_2, 1 mM cumyl hydroperoxide (CHP), 1 M sorbitol, 1 M KCl, 1 M NaCl, 250 mM $CaCl_2$, 1 mM $CuSO_4$, 0.01% sodium dodecyl sulfate (SDS), 0.2 mg/ml congo red (CR), 10 mg/L chlorothalonil, 2 mg/L Mancozeb, 3 mg/L Boscalid, 5 mM hydroxyurea (HU), 0.1% methyl methanesulfonate (MMS), or 5 μM camptothecin (CPT). To examine the carbon utilization ability, mutants and wild-type were inoculated on MM medium using 20 g/L glucose, 10 g/L sucrose, 10 g/L starch, or 10 g/L lactose as sole carbon source. All tests were repeated at least twice with three replicates of each treatment.

Western Blot Analysis

Wild-type, Δ*Gcn5* and Δ*Sas3* each with two replicates were cultured in PDB (120 rpm, 28°C) and were harvested after 36 h. The samples were ground and homogenized in 1 ml lysis buffer composed of 100 mM NaCl, 1% Triton X-100, 5 mM EDTA, 10 μl protease inhibitor cocktail (P8215, Sigma-Aldrich), 2 mM PMSF (P7626, Sigma-Aldrich), and 50 mM Tris-HCl, pH 7.5. The homogenates were centrifuged at 14,000 rpm, 20 min, 4°C. The Supernatants were collected as total protein. Antibody used in this study is H3K4ac (Abcam, ab176799).

Transcriptome Analysis

Wild-type, Δ*Gcn5* and Δ*Hos2* each with two replicates were cultured in PDB (120 rpm, 28°C) and were harvested after 36 h. RNA was extracted using Axygen RNA purification kit (Capital Scientific, Union City, CA, United States). The libraries were produced using an IlluminaTruSeq RNA Sample Preparation Kit, and they were sequenced on an Illumina HIseq 2500 platform, generating 150 bp paired-end reads. Trimmomatic Ver 0.36 (Bolger et al., 2014) was used to remove adaptors and low-quality reads. TopHat2 (Kim et al., 2013) was used to map sequences to the reference genome and the mapped reads on each gene were determined by HTSeq (Anders et al., 2015). Differential expression analysis was determined using DESEQ2 (Anders and Huber, 2012) based on the overall transcript counts after normalization. Transcripts with an adjusted FDR less than 0.01 and the absolute value of log2FC (log2 fold change) greater than 2 were considered to be differentially expressed genes (DEGs). DEGs were annotated by searching against the NCBI nr databases. GO and KEGG pathway were performed using clusterProfiler v3.6 (Yu et al., 2012). anti-SMASH 4.0 (Blin et al., 2017) was used to predict gene clusters associated with secondary metabolites. Protein domains were predicted in SMART database available at http://smart. embl-heidelberg.de/. Carbohydrate-active enzymes (CAZymes) were predicted using dbCAN meta server.[1] The transcriptomes raw data (SRS6969981, SRS6969980, SRS6969978, SRS6969979, SRS6969977, SRS6969976) have been deposited in NCBI's Sequence Read Archive.

Whole-Genome Resequencing Analysis

A paired-end library of each sample was constructed and sequenced by Hiseq2500, with insert sizes of approximately 350 bp. Trimmomatic was used to remove adaptors and low-quality reads. The high-quality resequencing reads was mapped to the *Alternaria alternata* reference genome with Bowtie2 (Langmead and Salzberg, 2012) using the default parameters. The aligned results were further filtered for unmapped reads and duplicated reads using SAMtools (Li et al., 2009) and Picard packages, respectively. SNP calling was carried out using Freebayes. Genomic variation analysis was performed using VCFtools (Danecek et al., 2011). IGV (Helga et al., 2013) was used to visualize reads on the genome. MapChart (Voorrips, 2002) was used to visualize SNPs on the genome. The raw data of whole-genome resequencing (SRS6971885, SRS6971887, SRS6971884, SRS6971886) have been deposited in NCBI's Sequence Read Archive.

Gene Expression Analyses

Quantitative Real-time PCR (qRT-PCR) was carried out on a 7300 Real Time PCR system (Applied Biosystems, Carlsbad, CA, United States) to validate the transcriptome data. RNA was extracted with an Axygen RNA purification kit (Capital Scientific) and the cDNA was synthesized from RNA using a PrimeScript RT regent kit (Takara, Shiga, Japan). Actin-coding gene (KP341672) was used as an internal control and the resulting

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[1]http://bcb.unl.edu/dbCAN2/blast.php

data were normalized using the comparative Cτ method as described previously (Sun et al., 2011).

Statistical Analysis

The statistical significance of treatments was determined by analysis of variance and means separated by Duncan's test ($P < 0.05$).

RESULTS

Identification and Genetic Deletion of Histone Acetyltransferases and Histone Deacetylases in *Alternaria alternata*

Seven HATs and six HDACs were identified combining the prediction results of dbHiMo[2] and hmm analysis, and the families to which these genes belong are also listed in **Table 1**. Nucleotide sequence length of corresponding predicted genes ranged from 654 to 3,450 bp, the number of introns contained varied from 0 to 8, and the length of proteins varied from 193 to 1,082 aa (**Figures 1A,B**). Functional domain prediction of amino acid sequences encoded by these genes revealed that all 13 proteins contain functional domains unique to HAT or HDAC, indicating these gene predictions are highly reliable. Mapping these HATs and HDACs to chromosomes revealed that all 13 genes are localized in the essential chromosomes, whereas no HATs or HDACs were identified in conditionally dispensable chromosomes (CDC) (**Supplementary Figure 1**). Phylogenetic analysis using the neighbor-joining method revealed that all HATs and HDACs predicted in *A. alternata* were highly similar to their corresponding genes in other fungi (**Figure 1C**).

To investigate the function of HATs and HDACs in *A. alternata*, mutants defective for *Gcn5* (ΔGcn5), *Elp3* (ΔElp3), *RTT109* (ΔRTT109), *Nat3* (ΔNat3), *Sas3* (ΔSas3), *Sas2* (ΔSas2), *Hos2* (ΔHos2), *Rpd3* (ΔRpd3), *Hos3* (ΔHos3), *Hda1* (ΔHda1), *Hst2* (ΔHst2), and *Sir2* (ΔSir2) were generated by the homologous recombination method. The mutants were verified

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[2]http://hme.riceblast.snu.ac.kr/

by PCR analysis (**Supplementary Figure 2**). However, no *Esa1* deletion mutants were obtained after multiple attempts.

Histone Acetyltransferases and Histone Deacetylases Are Required for Growth and Cell Development

In HATs disrupted mutants, vegetative growth rate of ΔGcn5, ΔElp3, ΔRTT109, ΔNat3, and ΔSas3 was reduced by 66, 44, 12, 71, and 41% compared to wild-type on potato dextrose agar (PDA), indicating that most HATs play a vital role on the growth of *A. alternata* (**Figure 2A** and **Supplementary Figure 3**). In addition, ΔGcn5 and ΔNat3 produced fewer aerial hyphae than wild-type on PDA. Microscopic examination revealed that the impacts of different HATs on the development of *A. alternata* varied greatly. The ΔGcn5 strain produced swollen and stubby hyphae and more hyphae branches, but failed to produce any spores (**Figures 2B,C** and **Supplementary Figure 4**). No spores were observed in ΔNat3 after 6 days of incubation, but a few spores appeared as incubation time increased. The hyphal tip of ΔNat3 was irregularly twisted and the hyphae were frequently branched. The conidiation of ΔElp3, ΔSas3, and ΔRTT109 decreased significantly, but the mycelium morphology remained the same as the wild-type level. Only ΔSas2 displayed wild-type levels of vegetative growth, hyphal development, and conidiation.

Unlike HATs, few HDACs were involved in vegetative growth and conidiation. Only ΔHos2 and ΔRpd3 grew significantly slower (with inhibition of 37 and 67%, respectively) than the wild-type (**Figure 2A**). Microscopic examination revealed that conidiation was inhibited significantly (>90%) in ΔHos2 and ΔRpd3. Similar to ΔGcn5, ΔRpd3 also produced stubby and swollen hyphae (**Figure 2B**). ΔHos3, ΔHda1, ΔHst2, and ΔSir2 displayed wild-type levels of vegetative growth and conidiation (**Figure 2C**).
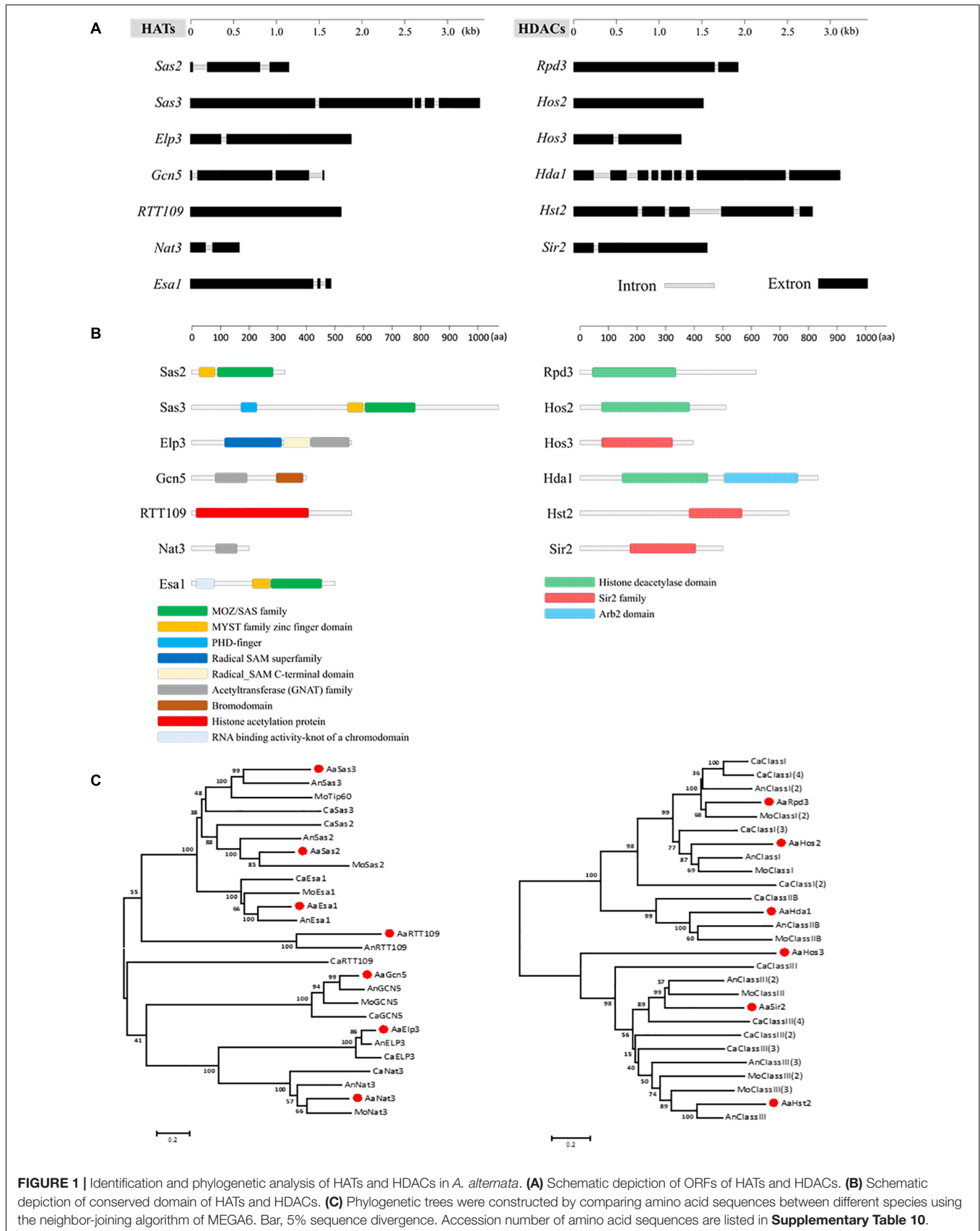
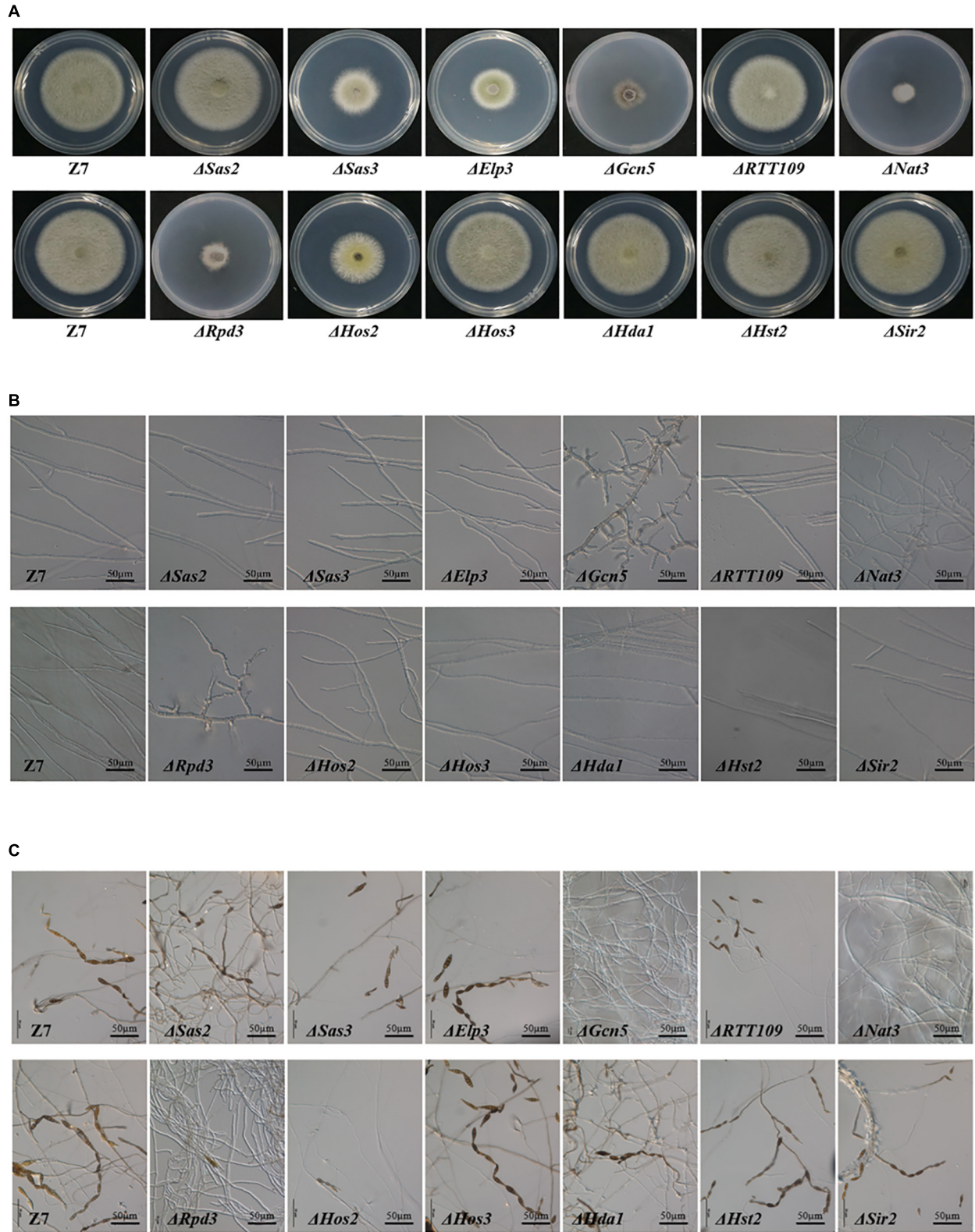### Histone Acetyltransferases and Histone Deacetylases Are Required for Fungal Virulence

To determine the effect of HATs and HDACs on the pathogenicity of *A. alternata*, virulence assays were performed on detached

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TABLE 1 | HATs and HDACs in *A. alternata*.

Gene	Accession no	Group	Length (aa)	Type	Deletion mutants	Mutants amount	Vegetative growth
Sas2	AALT_g11012	MYST	332	HATs	ΔSas2	5	Normal
Sas3	AALT_g515	MYST	1,082	HATs	ΔSas3	5	Inhibited
Elp3	AALT_g7656	GNATs	576	HATs	ΔElp3	7	Inhibited
Gcn5	AALT_g5778	GNATs	405	HATs	ΔGcn5	14	Inhibited
RTT109	AALT_g1972	P300/CBP	584	HATs	ΔRTT109	9	Inhibited
Nat3	AALT_g1183	GNATs	193	HATs	ΔNat3	2	Inhibited
Esa1	AALT_g11355	MYST	503	HATs	ΔEsa1	0	No information
Rpd3	AALT_g9756	ClassI	616	HDACs	ΔRpd3	6	Inhibited
Hos2	AALT_g8381	ClassI	504	HDACs	ΔHos2	15	Inhibited
Hos3	AALT_g1618	ClassII	397	HDACs	ΔHos3	6	Normal
Hda1	AALT_g4414	ClassII	844	HDACs	ΔHda1	4	Normal
Hst2	AALT_g8522	ClassIII	738	HDACs	ΔHst2	7	Normal
Sir2	AALT_g7248	ClassIII	495	HDACs	ΔSir2	21	Normal

FIGURE 1 | Identification and phylogenetic analysis of HATs and HDACs in *A. alternata*. **(A)** Schematic depiction of ORFs of HATs and HDACs. **(B)** Schematic depiction of conserved domain of HATs and HDACs. **(C)** Phylogenetic trees were constructed by comparing amino acid sequences between different species using the neighbor-joining algorithm of MEGA6. Bar, 5% sequence divergence. Accession number of amino acid sequences are listed in **Supplementary Table 10**.

FIGURE 2 | HATs and HDACs were required for growth and development. **(A)** Radial growth of the wild-type strain (Z7) and mutants. **(B)** Hyphae morphology of Z7 and mutants. **(C)** Conidiation of Z7 and mutants.

FIGURE 3 | HATs and HDACs were required for *A. alternata* pathogenicity. Pathogenicity was assayed on detached Dancy leaves by placing 5 mm agar plug covering fungal mycelium on the bottom of leaves (left, mutants; right, Z7). The leaves were incubated in a plastic box for lesion development and the necrotic lesions was observed at 2 days post inoculation (dpi). The percent changes of necrotic lesions appearing on the leaves calculated in relation to those induced by Z7 are also indicated.

Dancy leaves. For HATs deletion mutants, $\Delta Gcn5$, $\Delta Sas3$, and $\Delta Nat3$ failed to induce visible lesion on citrus leaves. $\Delta RTT109$ and $\Delta Elp3$ induced smaller necrotic lesions than those induced by the wild type 2 days post inoculation (dpi) (**Figure 3** and **Supplementary Figure 5**). Only $\Delta Sas2$ induced necrotic lesions at a rate and magnitude comparable to those for the wild-type on Dancy leaves 2 dpi. For HDACs deletion mutants, $\Delta Rpd3$ and $\Delta Hos2$ induced smaller necrotic lesions compared to those induced by the wild type at 2 dpi. $\Delta Hos3$, $\Delta Sir2$, and $\Delta Hda1$ produced necrotic lesions similar to those induced by Z7 (**Figure 3** and **Supplementary Figure 5**). Quantitative analysis revealed that the area of lesion induced by the $\Delta RTT109$, $\Delta Elp3$, $\Delta Sas2$, $\Delta Hos2$, $\Delta Rpd3$, $\Delta Hda1$, $\Delta Hos3$, $\Delta Hst2$, and $\Delta Sir2$ were about 79, 32, 92, 18, 11, 97, 104, 96, and 103%, respectively, of those induced by Z7.

Histone Acetyltransferases and Histone Deacetylases Coordinate Carbon Sources Utilization

To examine whether HATs or HDACs were required for the utilization of carbon sources, mycelial radical growth assays were performed on MM medium supplemented with sucrose, starch, glucose, or lactose as sole carbon sources (**Figure 4**). In HATs disruption mutants, the vegetative growth inhibition rate of $\Delta Gcn5$ on all tested sole carbon source media for 4 days was 100%. At the same time, the growth inhibition ratio of $\Delta Sas3$ on the MM medium supplemented with glucose, sucrose, lactose, and starch was increased by 28, 41, 39, and 41%, compared to the wild type. In contrast, $\Delta Sas2$, $\Delta Elp3$, and $\Delta RTT109$ displayed wild-type sensitivity to all tested sole carbon source media. Most HDACs disruption mutants, including $\Delta Sir2$, $\Delta Hos3$, $\Delta Hda1$, and $\Delta Hst2$, exhibited wild-type sensitivity to all test medium. Only $\Delta Hos2$ showed slightly increased sensitivity on carbon sources of glucose and starch.

To prove further that the severe growth defects of $\Delta Gcn5$ on sole carbon source medium was not caused by its slow vegetative growth, the incubation time was extended to 10 days. Growth examination indicated that the growth inhibition ratio of $\Delta Gcn5$ on the medium with lactose as sole carbon source was still 100%, and only a few hyphae of $\Delta Gcn5$ appeared on carbon sources of

sucrose, starch, or glucose (the growth inhibition ratio is close to 100%), confirming that the *Gcn5* was required for the utilization of carbon sources (**Supplementary Figure 6**).

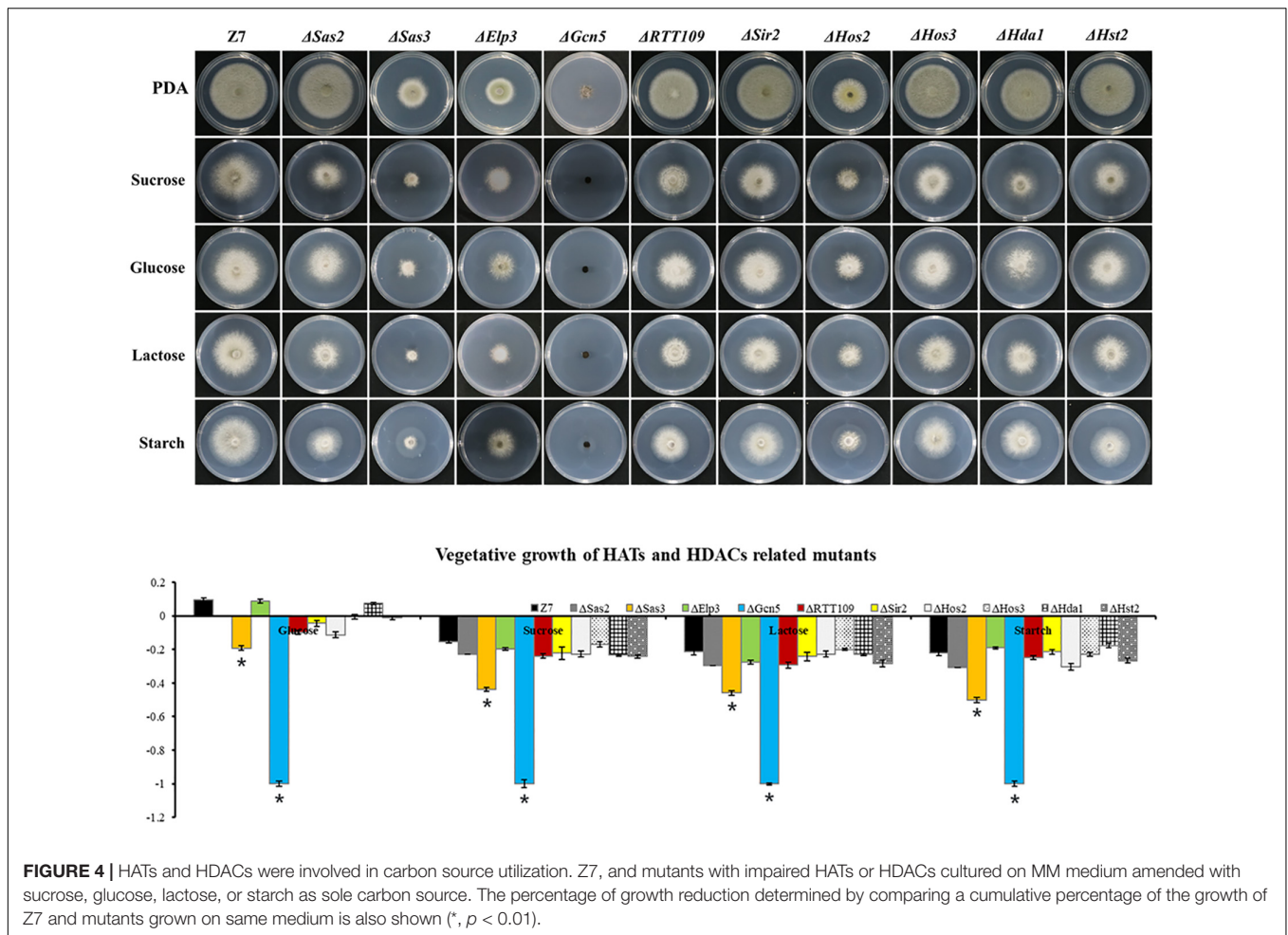## Histone Acetyltransferases and Histone Deacetylases Are Involved in Resistance to Multiple Stresses

In mutants defective for HATs, both $\Delta Elp3$ and $\Delta Gcn5$ were more sensitive to H_2O_2 and CHP (cumyl-H_2O_2), while $\Delta Sas3$ and $\Delta RTT109$ just showed increased sensitivity to H_2O_2, compared with wild-type. In addition, $\Delta Sas3$ showed elevated sensitivity to cell wall-targeting agents such as Congo red (CR) and sodium dodecyl sulfonate (SDS). At the same time, $\Delta Elp3$, $\Delta Gcn5$, and $\Delta RTT109$ just displayed increased sensitivity to CR. $\Delta Sas3$, $\Delta Gcn5$, and $\Delta RTT109$ displayed increased sensitivity to NaCl, KCl, and $CuSO_4$. Only $\Delta RTT109$ displayed increased sensitivity to $CaCl_2$. However, $\Delta Sas3$, $\Delta Elp3$, and $\Delta Gcn5$ displayed enhanced resistance to $CaCl_2$ compared to wild-type. Both $\Delta Sas3$ and $\Delta Elp3$ also showed increased tolerance to V8 medium compared to wild-type. Only $\Delta Sas2$ showed wild-type resistance to all test chemicals (**Figure 5**).

Unlike HATs which were widely involved in the adaption to various stresses, most of HDACs such as *Hos3*, *Hda1*, *Hst2*, and *Sir2* were not involved in the resistance to most tested chemicals. Sensitivity assays revealed that $\Delta Hos2$ showed increased sensitivity to H_2O_2, CHP, CR, SDS, NaCl, KCl, $CaCl_2$, and V8 medium, but not sorbitol and $CuSO_4$ (**Figure 6**).

Assays for fungicide sensitivity indicated that both $\Delta Gcn5$ and $\Delta Hos2$ displayed increased sensitivity to chlorothalonil, but not mancozeb and boscalid. All the other HATs or HDACs mutants displayed wild-type sensitivity to the test fungicides (**Supplementary Figure 7**).

Transcriptome Analysis Defines the Global Regulatory Role of Gcn5 and Hos2

The results described above indicated that both *Gcn5* and *Hos2* played vital roles in vegetative growth, development, multiple stresses resistance, carbon sources utilization, ROS detoxification, and virulence. To explore the transcriptional

FIGURE 4 | HATs and HDACs were involved in carbon source utilization. Z7, and mutants with impaired HATs or HDACs cultured on MM medium amended with sucrose, glucose, lactose, or starch as sole carbon source. The percentage of growth reduction determined by comparing a cumulative percentage of the growth of Z7 and mutants grown on same medium is also shown (*, *p* < 0.01).
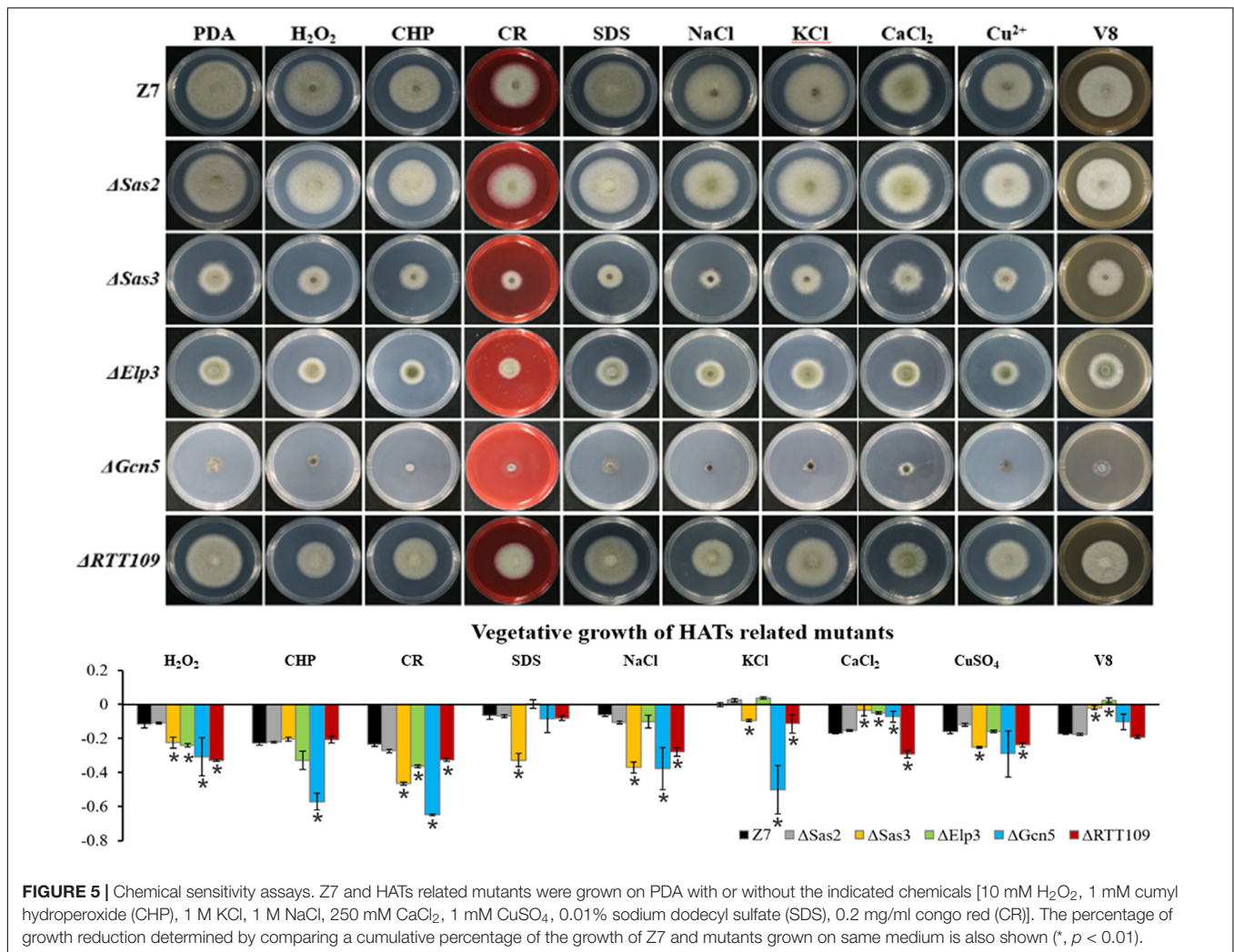
regulatory mechanism of *Gcn5* and *Hos2*, transcriptome analyses of *Gcn5* and *Hos2* compared to wild type were performed. Overall, 1,503 up-regulated and 1,051 down-regulated genes were identified in Δ*Gcn5* (**Supplementary Table 2**). In Δ*Hos2*, 469 up-regulated and 536 down-regulated genes were identified (**Supplementary Table 3**). *Gcn5* and *Hos2* were one of the genes with lowest transcription level in Δ*Gcn5* and Δ*Hos2*, respectively, indicating the genetic deletion of *Gcn5* and *Hos2* was reliable (**Supplementary Tables 2**, **3**). In addition, RT-qPCR results of 14 randomly selected differentially expressed genes (DEGs) was consistent with transcriptome data of Δ*Gcn5* and Δ*Hos2*, indicating that the transcriptome data were reliable. Although the DEGs in Δ*Gcn5* and Δ*Hos2* accounted for up to 20 and 8% of all genes in the genome, respectively, the transcription levels of the other 12 HATs and HDACs in Δ*Gcn5* or Δ*Hos2* did not change significantly, indicating that *Gcn5* and *Hos2* are not required for the transcription of the other HATs and HDACs.

COG (Clusters of Orthologous Groups of proteins) analysis of Δ*Gcn5* and Δ*Hos2* revealed that many DEGs were enriched in the metabolism category, such as Carbohydrate transport and metabolism (G), Amino acid transport and metabolism (E), Lipid transport and metabolism (I), and Inorganic ion

transport and metabolism (P). There are also many DEGs in Δ*Gcn5* or Δ*Hos2* enriched in Transcription (K) and Replication, recombination and repair (L) (**Supplementary Figures 8**, **9**). KEGG enrichment analysis revealed that the most affected metabolism-related pathways in Δ*Gcn5* or Δ*Hos2* were mainly related to carbon and nitrogen sources metabolism, such as starch and sucrose metabolism, galactose metabolism, Fructose and mannose metabolism, Methane metabolism, and Nitrogen metabolism. In addition, many DEGs in Δ*Gcn5* or Δ*Hos2* were enriched in genetic information processing, such as DNA damage repair, RNA transport, and tRNA biosynthesis (**Supplementary Figures 8**, **9**). Although many DEGs in Δ*Gcn5* and Δ*Hos2* were enriched in the same pathways, the amount and expression level of those genes varied greatly.

Gcn5 and *Hos2* are involved in regulating the transcription of many genes involved in carbon (sugar) source utilization. Analysis of DEGs in Glycolysis/Gluconeogensis (ko00010), Fructose and Mannose Metabolism (ko00051), Galactose Metabolism (ko00052), Starch and Sucrose metabolism (ko00500), Pentose Phosphate Pathway (ko00030), Citrate cycle (ko00020), and Pentose and Glucuronate interconversions (ko00040) in Δ*Gcn5* and Δ*Hos2* revealed that the number of

FIGURE 5 | Chemical sensitivity assays. Z7 and HATs related mutants were grown on PDA with or without the indicated chemicals [10 mM H_2O_2, 1 mM cumyl hydroperoxide (CHP), 1 M KCl, 1 M NaCl, 250 mM $CaCl_2$, 1 mM $CuSO_4$, 0.01% sodium dodecyl sulfate (SDS), 0.2 mg/ml congo red (CR)]. The percentage of growth reduction determined by comparing a cumulative percentage of the growth of Z7 and mutants grown on same medium is also shown (*, $p < 0.01$).
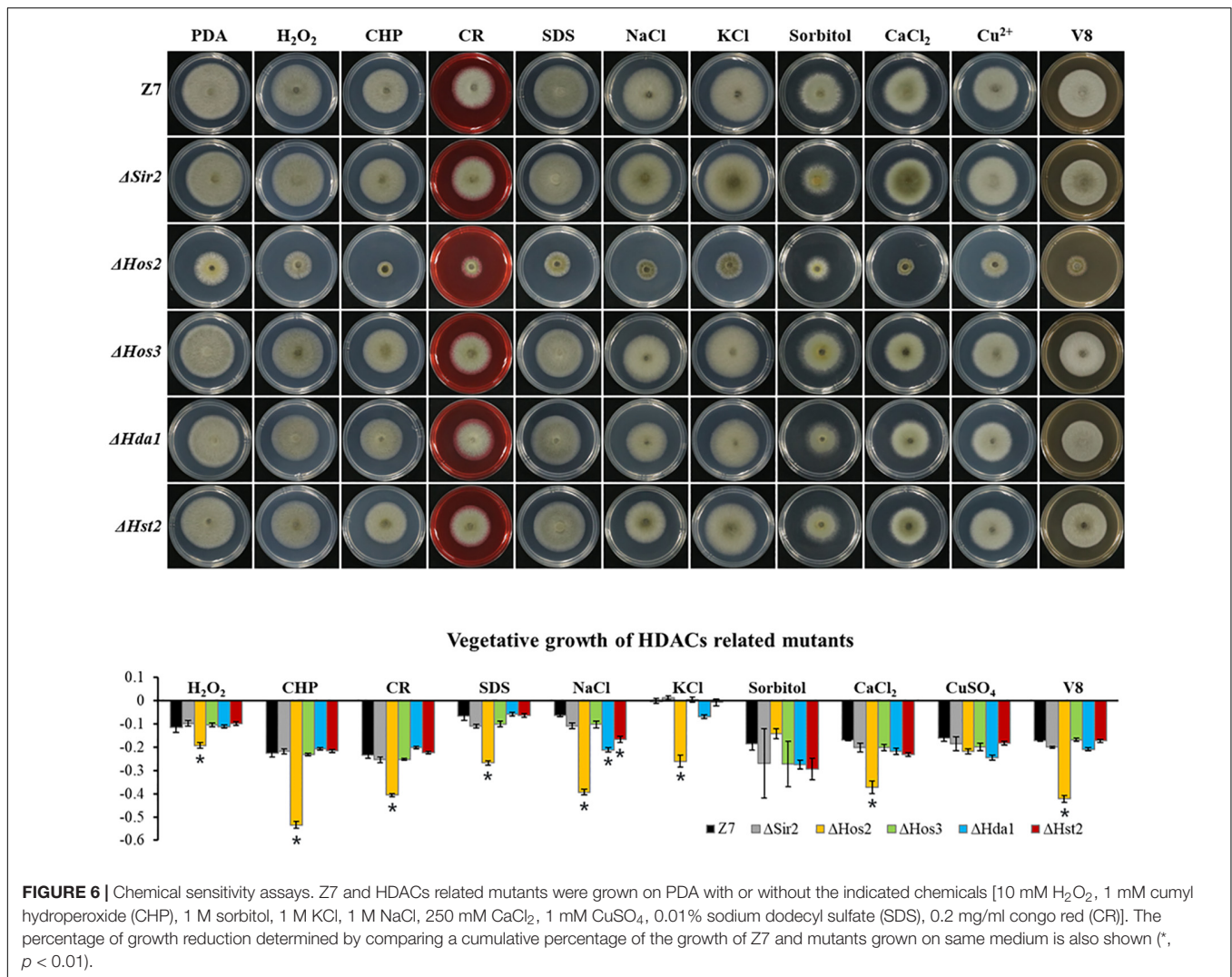
down-regulated genes ($\Delta Gcn5$, 22; $\Delta Hos2$, 15) was significantly greater than the up-regulated genes ($\Delta Gcn5$, 22; $\Delta Hos2$, 15) (**Figure 7**). In yeast, invertase and hexokinase are critical for the utilization of sucrose, lactose, glucose, or fructose (Carlson and Botstein, 1982). In *A. alternata*, AALT_g6039 encodes hexokinase, and AALT_g5797, AALT_g10082 and AALT_g2401 encode invertases. All four genes were significantly down-regulated in $\Delta Hos2$, but only AALT_g6039 and AALT_g2401 were significantly down-regulated in $\Delta Gcn5$.

$Gcn5$ and $Hos2$ affect the expression of ROS detoxification genes. In view of the increased sensitivity of $\Delta Gcn5$ and $\Delta Hos2$ to oxidative stress, we analyzed the transcription level of 96 ROS detoxification genes, including 7 catalases, 7 SOD superoxide dismutases, 42 glutathione system-related genes, 20 thioredoxin system-related genes, and 20 peroxidases (**Supplementary Table 4**). Although $\Delta Gcn5$ and $\Delta Hos2$ were more sensitive to oxidants, most of DEGs in these 96 ROS detoxification genes were up-regulated. In $\Delta Gcn5$, 21 DEGs were up-regulated and 10 DEGs were down-regulated (**Figure 7**). In $\Delta Hos2$, 9 DEGs were up-regulated and 4 DEGs were down-regulated.

$Gcn5$ and $Hos2$ mediate the expression of genes required for DNA damage repair. Base excision repair (BER), Nucleotide excision repair (NER), Mismatch repair (MMR), Homologous recombination (HR), and Non-homologous end-joining (NHEJ) play important roles in the repair of damaged DNA in organisms. Transcriptome analysis revealed that BER, NER, MMR, or HR-related genes including UDG (Uracil-DNA glycosylase, AALT_g5833), Polδ (DNA polymerase delta, AALT_g8966), and TFIIH (transcription factor b, AALT_g7297) were down-regulated in $\Delta Gcn5$ (**Supplementary Figure 10** and **Supplementary Tables 2**, **3**). In $\Delta Hos2$, BER and NER-related gene PARP [poly (ADP-ribose) polymerase, AALT_g9674], and NER related gene XPA (DNA-repair protein, AALT_g6731) were down-regulated (**Supplementary Table 3**). In addition, AALT_g76 (BER, endonuclease), AALT_g10858 (NER, RING-box protein 1) in $\Delta Gcn5$, and AALT_g15 (NHEJ, DNA ligase 4) in $\Delta Hos2$ were up-regulated (**Supplementary Table 3**).

$Gcn5$ and $Hos2$ regulate expression of genes involved in protein processing and degradation. In $\Delta Gcn5$, 7 DEGs enriched in "protein processing in endoplasmic reticulum (ko04141)" were up-regulated, including protein

FIGURE 6 | Chemical sensitivity assays. Z7 and HDACs related mutants were grown on PDA with or without the indicated chemicals [10 mM H_2O_2, 1 mM cumyl hydroperoxide (CHP), 1 M sorbitol, 1 M KCl, 1 M NaCl, 250 mM $CaCl_2$, 1 mM $CuSO_4$, 0.01% sodium dodecyl sulfate (SDS), 0.2 mg/ml congo red (CR)]. The percentage of growth reduction determined by comparing a cumulative percentage of the growth of Z7 and mutants grown on same medium is also shown (*, $p < 0.01$).

glycosyltransferase (AALT_g5706), Mannosyl-oligosaccharide glucosidase (AALT_g11241), Mannosyl-oligosaccharide alpha-1,2-mannosidase (AALT_g2989), HSP20 (AALT_g9536), UBC5 (AALT_g6888), UBC7 (AALT_g619), and RBX1 (AALT_g4961), which are involved in protein processing and degradation. Furthermore, eight up-regulated genes were enriched in "Ubiquitin mediated proteolysis (ko4120)" and "Regulation of Autophagy (ko04140)" (**Supplementary Figure 11** and **Supplementary Table 2**). Only 4 genes were down-regulated in the above three pathways. In $\Delta Hos2$, all four DEGs enriched in "protein processing in endoplasmic reticulum" (AALT_g173 and AALT_g6960), "Ubiquitin mediated proteolysis" (AALT_g6960), and "Regulation of Autophagy" were up-regulated (AALT_g11308) (**Supplementary Table 3**).

In addition, deletion of *Gcn5* or *Hos2* also affected the transcription of genes involved in Carbohydrate-active enzymes (CAZymes), secondary metabolite cluster, CYP450, and conidiation (**Supplementary Tables 5–8** and **Supplementary Figure 12**). The transcription of all ACT toxin synthesis-related genes did not change significantly in $\Delta Gcn5$ and $\Delta Hos2$

compared to wild-type. However, AALT_g11758 (CYP450), located in the ACT toxin gene cluster and required for pathogenicity but plays no role for ACT toxin synthesis (Wang et al., 2019), was significantly down-regulated in both $\Delta Gcn5$ (FC = −25) and $\Delta Hos2$ (FC = −6).

Histone Acetyltransferases and Histone Deacetylases Are Required for the Resistance to DNA-Damaging Agents

Previous studies showed that *Gcn5*, *Hos2*, and *RTT109* are required for DNA damage repair in *B. bassiana* and *S. cerevisiae* (Burgess et al., 2010; Cai et al., 2018a,c). Therefore, we tested whether *RTT109*, *Gcn5*, and *Hos2* were involved in the resistance to DNA-damaging agents such as camptothecin (CPT), hydroxyurea (HU) and methyl methanesulfonate (MMS) in *A. alternata*. Compared with wild-type, growth of $\Delta RTT109$ was significantly suppressed by 40 and 89% on PDA containing 5 µM CPT or 0.1% MMS, respectively (**Figure 8**). However, $\Delta RTT109$ displayed wild-type radial growth on PDA amended
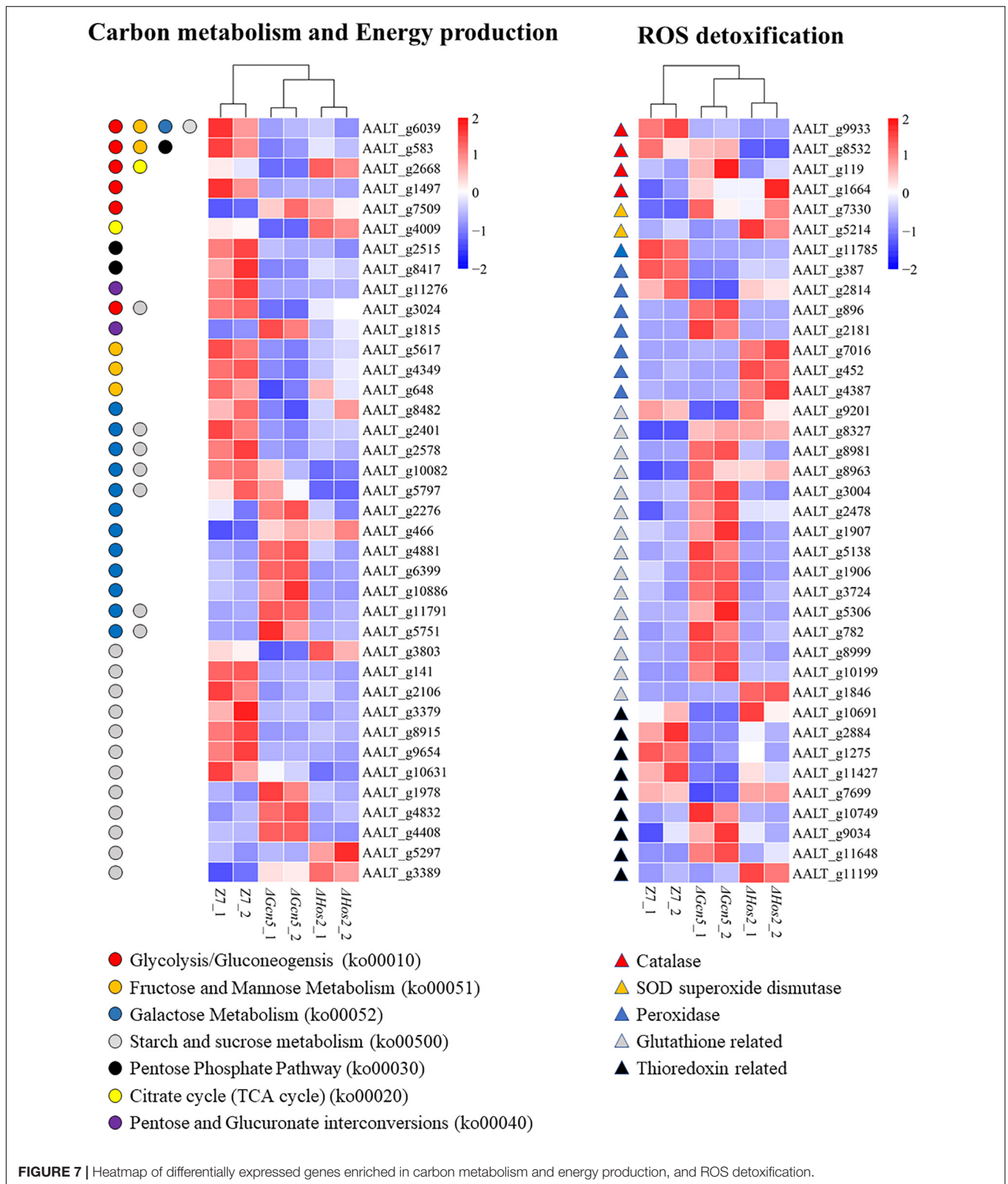
FIGURE 7 | Heatmap of differentially expressed genes enriched in carbon metabolism and energy production, and ROS detoxification.

with 5 mM HU. Unlike ΔRTT109, the growth of ΔHos2 on HU-added PDA was inhibited by 20% compared to wild-type. At the same time, ΔHos2 showed wild-type resistance

to 5 μM CPT or 0.1% MMS. In addition, ΔGcn5 displayed increased sensitivity to 5 mM HU and 0.1% MMS (the growth inhibition rate is 29 and 19%, respectively, compared
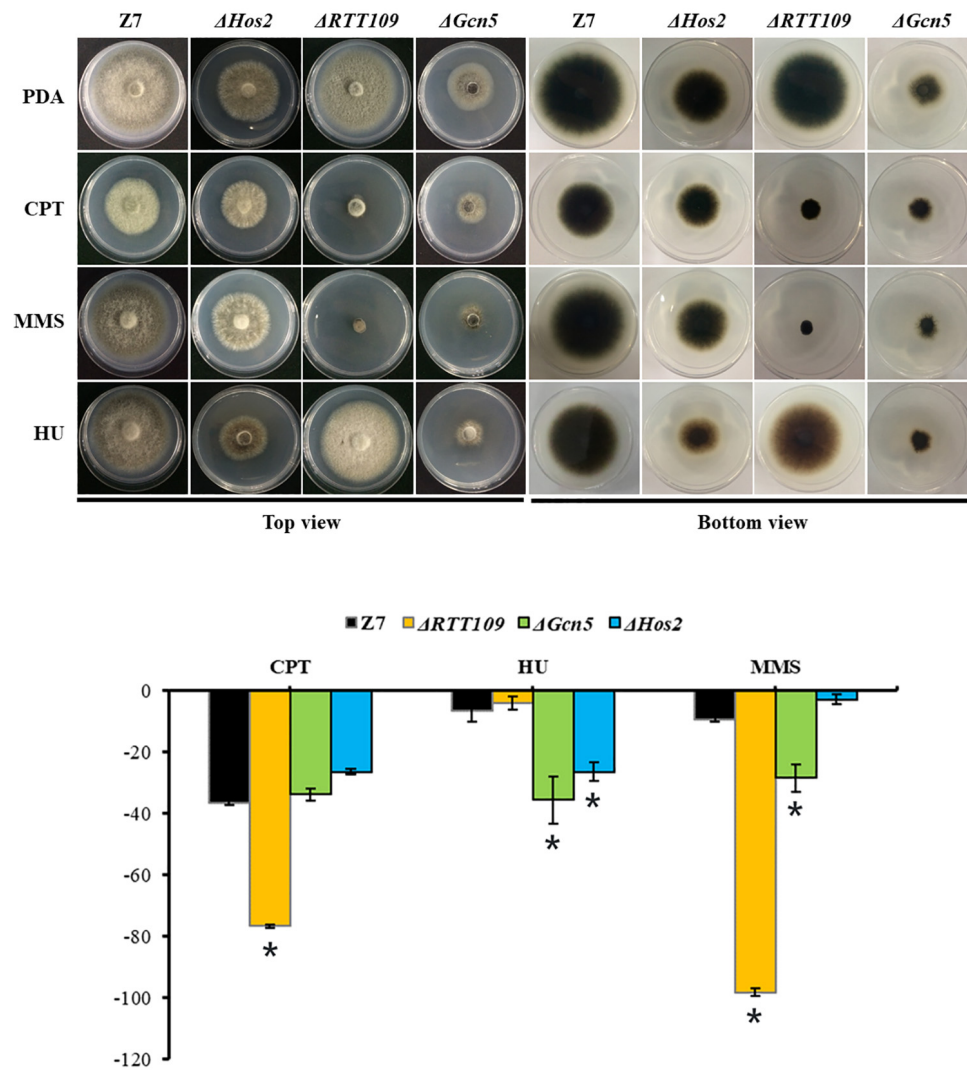
FIGURE 8 | HATs and HDACs were involved in the resistance to DNA-damaging agents. Z7, ΔHos2, ΔRTT109, and ΔGcn5 were grown on PDA with or without the indicated chemicals [5 mM hydroxyurea (HU), 0.1% methyl methanesulfonate (MMS), or 5 μM camptothecin (CPT)]. The percentage of growth reduction determined by comparing a cumulative percentage of the growth of Z7 and mutants grown on same medium is also shown (*, $p < 0.01$).

to wild-type) but displayed wild-type resistance to 5 μM CPT (**Figure 8**).

Gcn5 Is Required for DNA Damage Repair

In order to analyze the DNA damage repair ability of *Gcn5*, we performed genome resequencing using ultraviolet ray (UV) irradiated ΔGcn5 and wild-type. In the *Gcn5* coding region, no reads were detected in ΔGcn5, but many reads were found in wild-type, which proves once again that the knock-out experiment of *Gcn5* was successful (**Figure 9B**). In ΔGcn5, 949 unique SNPs were detected after UV irradiation. However, in UV irradiated wild-type, only 298 unique SNPs were found, which accounted for 31% of ΔGcn5, indicating that *Gcn5* is involved in the repair of DNA damage caused by UV (**Figure 9A**).

Histone Acetylation Targets of Gcn5

To test whether histone acetylation levels were altered in ΔSas3 and ΔGcn5, western blot was carried out using specific antibodies, directed against H3K4ac, while antibody against H3 was used as a loading control. As shown in **Figure 10**, the level of H3K4ac was significantly decreased ($P < 0.01$) in the ΔGcn5 compared to Z7. No reduction of the signal for H3K4ac was observed in ΔSas3.

DISCUSSION

Histone acetylation, which is important for multiple cellular processes, occurs in different organisms ranging from fungi to mammals, and the acetylation level manifested by HATs and HDACs dynamically changes over time during development and
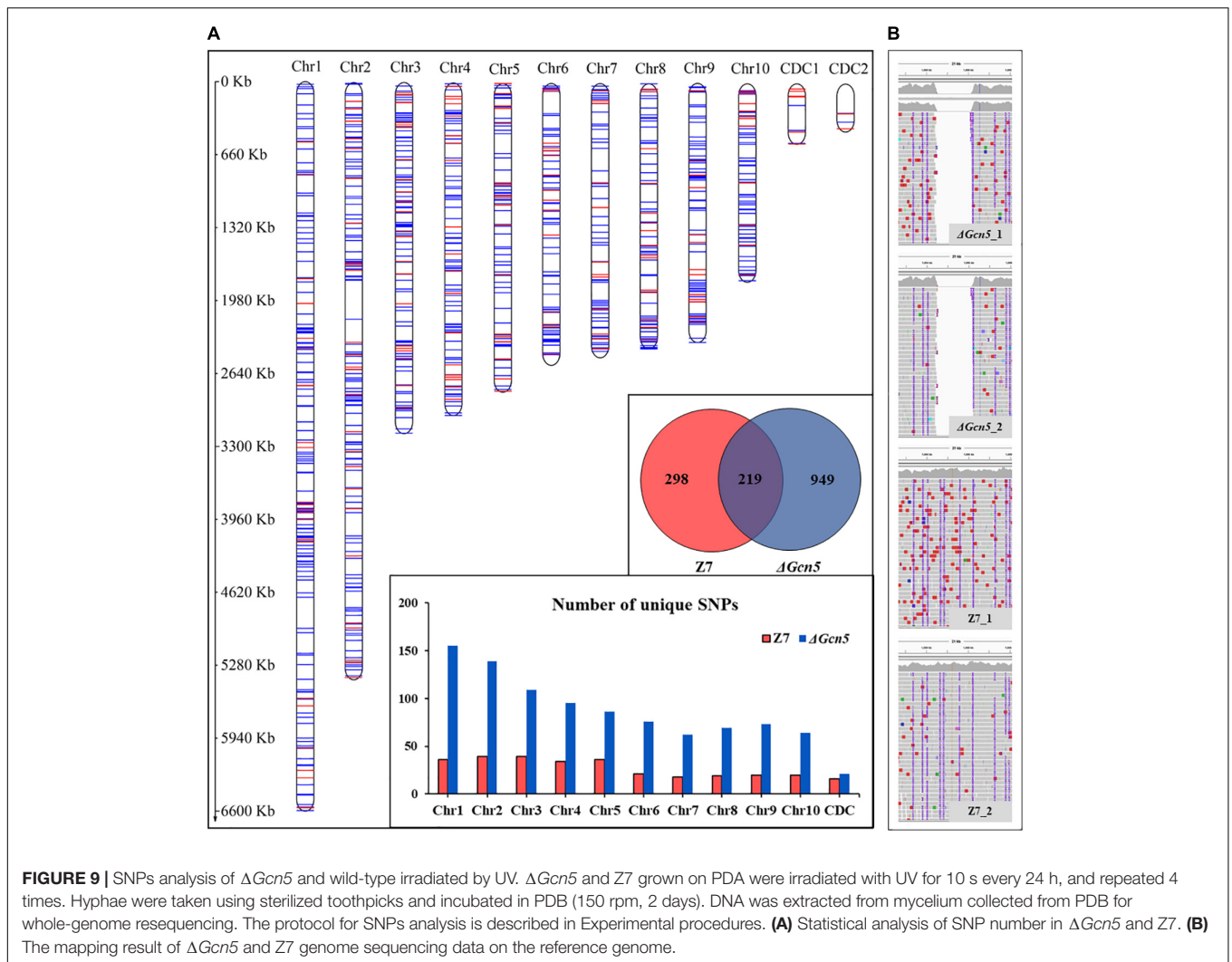
FIGURE 9 | SNPs analysis of Δ*Gcn5* and wild-type irradiated by UV. Δ*Gcn5* and Z7 grown on PDA were irradiated with UV for 10 s every 24 h, and repeated 4 times. Hyphae were taken using sterilized toothpicks and incubated in PDB (150 rpm, 2 days). DNA was extracted from mycelium collected from PDB for whole-genome resequencing. The protocol for SNPs analysis is described in Experimental procedures. **(A)** Statistical analysis of SNP number in Δ*Gcn5* and Z7. **(B)** The mapping result of Δ*Gcn5* and Z7 genome sequencing data on the reference genome.
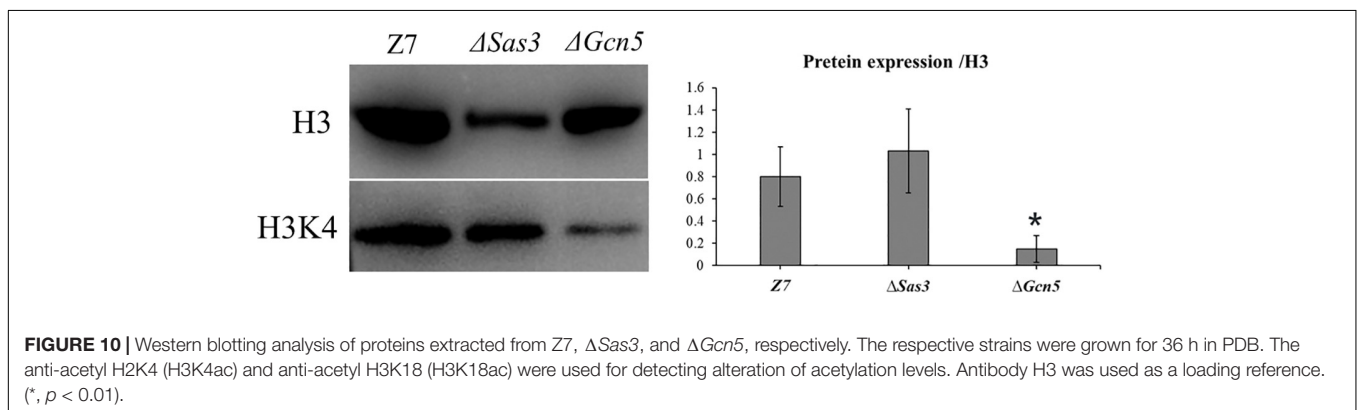
FIGURE 10 | Western blotting analysis of proteins extracted from Z7, Δ*Sas3*, and Δ*Gcn5*, respectively. The respective strains were grown for 36 h in PDB. The anti-acetyl H2K4 (H3K4ac) and anti-acetyl H3K18 (H3K18ac) were used for detecting alteration of acetylation levels. Antibody H3 was used as a loading reference. (*, $p < 0.01$).

differentiation (Lee and Workman, 2007; Kong et al., 2018). In plant pathogenic fungi, several HATs and HDACs have been found to be required for growth, virulence and adaptation to environmental stress (Maeda et al., 2017; Kong et al., 2018; Dubey et al., 2019). Although HATs and HDACs are conserved in cells, the functions of these genes are various in different species.

In this study, a total of 13 HATs and HDACs were identified in *A. alternata*. To systematically elucidate the functions of HATs and HDACs in *A. alternata*, we created 6 HATs and 6 HDACs deletion mutants. Our studies showed that HATs and HDACs were widely involved in biological processes of mycelial growth and development, conidial production, multiple stresses

resistance, carbon source utilization, DNA damage repair, and pathogenicity in *A. alternata*.

As shown in the present study, HATs and HDACs are involved in developmental processes because Δ*Gcn5*, Δ*Hos2*, Δ*Rpd3*, Δ*Nat3*, Δ*Sas3*, and Δ*RTT109* produce no or fewer conidia. In addition, Δ*Gcn5*, Δ*Rpd3*, and Δ*Nat2* produces more hyphal branches than wild-type. In some filamentous fungi like *N. crassa* and *A. nidulans*, the central development pathway relies upon the key activator genes *BrlA*, *AbaA*, and *WetA* (Etxebeste et al., 2010; Park and Yu, 2012). Six upstream developmental activators (*FlbA*, *FlbB*, *FlbC*, *FlbD*, *FlbE*, and *FluG*) are required for the activation of *BrlA* and the initiation of conidiation (Adams et al., 1988; Wieser et al., 1994). Our transcriptome data revealed that *FlbA*, *FlbC*, and *FlbD* were down-regulated in Δ*Gcn5* (**Supplementary Table 8**). Previous studies have revealed the conidiation of *A. alternata* is closely regulated by the calcium-mediated signaling pathway, Fus3 and Slt2 MAPK signaling pathway, as well as the cAMP dependent protein kinase A (PKA) (Lin et al., 2010; Wang et al., 2010; Yago et al., 2011; Tsai et al., 2013). Other factors including NADPH oxidase (Nox), thioredoxin reductase (Trr1), glutathione reductase (Glr1), nascent polypeptide associated complex α subunit (Nac1), and skn7 response regulator are also required for the developmental processes in *A. alternata* (Chen et al., 2012; Yang and Chung, 2013; Ma et al., 2018; Wang et al., 2020). Whether or not those regulators interact with HATs or HDACs remains to be determined. In addition to conidia formation, HATs and HDACs are required for vegetative growth. Δ*Gcn5*, Δ*Nat3*, Δ*Sas3*, Δ*RTT109*, Δ*Elp3*, Δ*Hos2*, and Δ*Rpd3* displayed growth reduction compared with wild-type, consistent with the findings in *B. bassiana* (*Gcn5*, *RTT109*, *Hos2*, and *Rpd3*), *F. graminearum* (*Gcn5*, *RTT109*, *Elp3*, and *Sas3*), and *S. cerevisiae* (*Nat3*) (Polevoda and Sherman, 2003; Lee et al., 2014; Cai et al., 2018a,b,c,d; Kong et al., 2018).

Experiments further demonstrated that Δ*Gcn5*, Δ*Sas3*, Δ*RTT109*, and Δ*Hos2* are required for cellular resistance to osmotic stress. In yeast, SAGA (Spt-Ada-Gcn5) is required for the transcription of Hog1-mediated genes under severe osmostress (Zapater et al., 2007). Chip-on-chip experiments revealed that acetylation at lysines 9 and 12 of histone H3 increases in osmostress up-regulated genes and decreases in repressed genes (Magraner-Pardo et al., 2014). The *Hog1* deletion mutant in *A. alternata* displayed reduced vegetative growth rate and increased sensitivity to KCl and NaCl, which is consistent with the phenotype of Δ*Gcn5*, Δ*Sas3*, Δ*RTT109*, and Δ*Hos2*, indicating that transcription of some genes regulated by *Hog1*, *Gcn5*, *Sas3*, and *Hos2* may be similar (Lin and Chung, 2010). Similar results were observed in *F. graminearum*, in which the *Hog1*, *Gcn5*, *Sas3*, *RTT109* are also involved in vegetative growth and resistance to osmotic stress (Nguyen et al., 2012; Kong et al., 2018). In addition, Δ*Sas3* and Δ*Hos2* are more sensitive to cell wall-disturbing compounds including CR and SDS, indicating both of these genes play a positive role in cell wall assembly.

Carbon source (sugar) metabolism, which is one of the most important biological processes in organisms, is involved in growth, development, and multiple stress resistance (Koch, 2004; Ene et al., 2012). This study found that *Sas3*, *Hos2*, and especially *Gcn5* displayed severe growth defects on sole carbon source medium, suggesting an involvement of these genes in carbon metabolism in *A. alternata*. In *C. albicans*, acetyl-CoA and acetate metabolism play a central role in growth on both glucose and non-glucose carbon sources (Carman et al., 2008). In *B. bassiana*, deletion of *Rpd3* and *Gcn5* resulted in growth defects on medium modified with different carbon sources, confirming the important role of histone acetylation in carbon source utilization (Cai et al., 2018b,d). In addition, most DEGs enriched in carbon source metabolism pathways, such as "Glycolysis/Gluconeogensis," Fructose and Mannose Metabolism," "Galactose Metabolism," "Starch and Sucrose Metabolism," etc., are down-regulated in Δ*Gcn5* and Δ*Hos2*, making it not surprising to see the reduced carbon source utilization ability in Δ*Gcn5* and Δ*Hos2*. In microorganisms, glucose metabolites would lead to carbon catabolite repression (CCR) and inhibit the utilization of other carbon sources (Celenza and Carlson, 1986; Kayikci and Nielsen, 2015). Sucrose non-fermenting 1 (SNF1) protein kinase complex is required for the utilization of non-fermentable carbon sources in fungi and enable them to adapt to the adversity of glucose deficiency (Alepuz et al., 1997). In *Fusarium virguliforme*, the growth of *FvSnf1* deletion mutants on medium amended with galactose as sole carbon source is completely inhibited (Islam et al., 2017). SNF1 complex-related genes (*AaSnf1*, *AaSip2*, and *AaSnf4*) were not significantly differentially expressed in Δ*Gcn5* and Δ*Hos2* compared to wild-type. However, the transcription of *SUC2* (AALT_g2401), which is regulated by *Snf1* and is essential for sucrose utilization in *S. cerevisiae* (Carlson et al., 1981; Oezcan et al., 1997), was down-regulated significantly in Δ*Gcn5* and Δ*Hos2*. Previous studies have shown that Snf1, which regulates Gcn5 occupancy and H3 acetylation at a specific sequence, works in concert with Gcn5 to regulate transcription (Lo et al., 2001; Abate et al., 2012). In addition, similar with *Gcn5* and *Hos2*, *Snf1* also plays an important role in the carbon source utilization in *A. alternata* (Wang et al., 2020). Therefore, we speculate that the interaction relationship between *Snf1* and these two genes may also exist.

Mutants lacking *Gcn5*, *Sas3*, *Elp3*, *RTT109*, or *Hos2* in *A. alternata* were defective in ROS detoxification at varying degrees, indicating all of these five genes are involved in resistance to oxidative stress. *RTT109*, *Gcn5*, and *Hos2* in *B. bassiana*, *Elp3* and *Sas3* in *F. graminearum* also play important roles in the resistance to oxidative stress (Lee et al., 2014; Cai et al., 2018a,b,c; Kong et al., 2018). However, *Gcn5* in *Aspergillus flavus* is not required for ROS resistance (Lan et al., 2016). In addition, *Hos2* plays a negative role in H_2O_2 resistance in *M. oryzae*, indicating that the function of specific genes in HATs and HDACs have been differentiated in fungi (Lee et al., 2019). In *A. alternata*, the ROS detoxification ability is critical for pathogenesis and survival (Chung, 2012; Ma et al., 2018; Gai et al., 2019). *A. alternata* strains lacking *Nox*, *Hog1*, *Yap1*, *Skn7*, *Gpx3*, or *Nac1* all exhibit increased cellular sensitivity to ROS and decreased pathogenicity on citrus leaves (Lin and Chung, 2010; Chen et al., 2012; Yang and Chung, 2012; Chung, 2014; Yang et al., 2016; Wang et al., 2020). Virulence assays revealed that the virulence of Δ*Gcn5* and Δ*Sas3* was completely lost, which is consistent with the findings in F. *graminearum* (Kong et al., 2018). Furthermore, the virulence of

ΔElp3, ΔRTT109, and ΔHos2 decreased significantly, supporting the requirement of ROS detoxification for successful infection by *A. alternata*. In addition, we observed that ΔNat3 and ΔRpd3 were significantly reduced in pathogenicity and vegetative growth rate. The severe growth defects of ΔNat3 and ΔRpd3 may contribute to its reduced virulence. Recent research shows that chlorothalonil causes a redox state change leading to oxidative stress generation in *Danio rerio* (Da Silva Barreto et al., 2020). Interestingly, sensitivity assays revealed that *A. alternata* strains with impaired *Gcn5* or *Hos2* displayed high sensitivity to chlorothalonil fungicide. In addition, transcriptome data of ΔGcn5 revealed that all DEGs in the sterol synthesis pathway were significantly down-regulated, including CYP51 homologous genes, which is closely related to DMI fungicide resistance. In *C. albicans*, the loss of *Hda1* and *Rpd3* both caused the pathogen to be significantly more sensitive to azole fungicides, indicating that histone acetylation levels are crucial for pathogens to resist DMI fungicides (Li et al., 2015). Transcriptome analysis also revealed that most DEGs involved in ROS detoxification were up-regulated in ΔGcn5 and ΔHos2 even without external oxidative stress, implying that the redox state in cells of these two mutants are changed. In eukaryotic cells, endoplasmic reticulum (ER) is essential for folding and trafficking of proteins that enter the secretory pathway, while the disorder of redox homeostasis of ER could result in protein misfolding (Wang and Kaufman, 2016). In ΔGcn5 and ΔHos2, many genes involved in "Protein processing in endoplasmic reticulum," "Ubiquitin mediated proteolysis," and "Regulation of autophagy" pathways are up-regulated, which mainly play roles in protein folding and degradation.

It is now clear that a subset of HATs and HDACs regulate the genome stability (Driscoll et al., 2007; Nagarajan et al., 2013). In *B. bassiana*, mutants lacking HATs (*Mst2* and *RTT109*) or HDACs (*Hos2*) displayed increased sensitivity to DNA-damaging stresses, indicating that all of these genes are involved in DNA damage repair (Cai et al., 2018a,c; Wang et al., 2018). In our study, many DEGs are enriched in DNA damage repair pathways. Moreover, ΔGcn5, ΔRTT109, and ΔHos2 displayed hypersensitivity to a variety of DNA-damaging agents such as MMS, HU, or CPT, all of which induce replication fork collapse or stalling (Interthal and Heyer, 2000; Boddy et al., 2001; Doe et al., 2002), suggesting *Gcn5*, *RTT109*, and *Hos2* play an important role in DNA damage repair in *A. alternata*. This speculation was evidenced by the result that more SNPs were generated in ΔGcn5 in comparison to wild-type when both of them were irradiated by UV.

In summary, our study revealed that HATs and HDACs are required for the vegetative growth, conidiation, pathogenicity, multiple stresses resistance, DNA damage repair, and carbon source utilization in *A. alternata*. In addition, *Gcn5* and *Hos2* play direct or indirect roles in the transcriptional regulation of genes involved in carbon source metabolism, DNA damage repair, ROS detoxification, protein processing and degrading, ergosterol synthesis, and secondary metabolite synthesis.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

HM, FG, and HL contributed to the conception of the study. HM, LL, YG, XYZ, YNC, XKZ, and YZC performed the experiments. HM, YG, and CJ performed the data analysis. HM wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2021.783633/full#supplementary-material

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