Genome-wide identification of citrus histone acetyltransferase and deacetylase families and their expression in response to arbuscular mycorrhizal fungi and drought

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Abstract
Histone acetyltransferase (HAT) and histone deacetylase (HDAC) family members control histone acetylation levels to regulate gene expression and play significant roles in microbe interactions and abiotic stress responses. However, the HAT and HDAC family members in citrus have not yet been identified, and their responses to drought (abiotic stress) and arbuscular mycorrhizal fungi (AMF) colonization remain unknown. In the present study, HAT and HDAC family genes were characterized in the citrus genome. A total of 14 CsHATs and 11 CsHDACs were identified in the citrus genome, of which 8 CsHATs and 7 CsHDACs showed syntenic relationships with Arabidopsis HATs and HDACs, respectively. Furthermore, the 14 CsHATs were classified into GNAT-MYST, TAFII250, MYST, CBP, and one citrus-unique family. The 11 CsHDACs were classified into SIR2, HD2, and RPD3/HDA1. Gene expression analysis showed AMF colonization-induced drought induced the expression of OsHAT1, 2, 6, 7, 9, 11, 12, and 14, and repressed OsHDAC1, 2, 5, 6, 8, and 10. The regulation of HAT and HDAC expression was not always consistent with the cis-elements identified in their promoters. The present study offers useful insights into the citrus HAT and HDAC gene families and their expression in response to AMF colonization and drought stress.

1. Introduction
Protein lysine epsilon acetylation is a reversible and tightly regulated post-translational modification of proteins in plants, which regulates diverse cellular processes (Ma et al. 2013; Shen et al. 2015). Histone acetylation levels are controlled by two functional antagonistic enzymes, namely histone acetyltransferase (HAT) and histone deacetylase (HDAC) (Bannister and Kouzarides 2011). HATs and HDACs have been most extensively studied in Arabidopsis. HATs in Arabidopsis are encoded by 12 genes, and can be grouped into four classes, that is, general control nondepressible 5 (GCN5)-related N-acetyltransferase (GNAT), MOZ-YBF2/SA3-SA3/TP60 (MYST), the p300/CREB (cAMP-responsive element-binding protein)-binding protein (CBP) family, and TATA-binding protein associated factor 1 (TAF1). HDACs in Arabidopsis are encoded by 18 genes, and can be grouped into four types, that is, reduced potassium dependency 3 (RPD3) (Liu et al. 2012; Shen et al. 2015), histone deacetylase 1 (HDA1), silent information regulator 2 (SIR2), and plant-specific histone deacetylase 2 (HD2). The SIR2 family of HDAC, also called sirtuins, differs from other HDAC groups in catalyzing deacetylation via an NAD+-dependent reaction (Gregoretti et al. 2004; Ma et al. 2013).

It is well known that histone lysine acetylation plays a pivotal role in chromatin regulation and gene expression and can induce gene activation (Shahbazian and Grunstein 2007). Conversely, the removal of histone acetylation can lead to gene repression and silencing (To et al. 2011). In plants, changes in the acetylation level are associated with changes in gene expression under different stresses or symbiotic interactions. Arabidopsis RPD3-type histone deacetylase HDA9 is involved in modulating plant responses to salt and drought stresses (Hu et al. 2015; Zheng et al. 2016). HDA9 mutation can lead to the up-regulation of several genes involved in response to water-deprivation stress (Zheng et al. 2016). In plants such as rice, drought stress significantly increases the expression levels of four HATs, that is, OsHAC703, OsHAG703, OsHAF701, and OsHAM701, and the acetylation level at certain lysine sites of histones H3 (lysine 9, lysine 18, and lysine 27) and H4 (lysine 5) increase with increasing OsHAT expression. The significant increase in the transcript levels of OsHATs and the acetylation level of lysine residues of histones H3 and H4 suggest that OsHATs are involved in drought stress responses in rice (Fang et al. 2014). In terms of symbiotic interactions, plant-specific histone deacetylase encoding MtHDT2 is induced in mycorrhized roots of Medicago, especially in arbuscule-containing cells. In these cells, the MtHDT2 protein exhibits variable sub-nuclear localization patterns. MtHDT2 knockdown significantly reduces arbuscule formation in mycorrhized root segments and possibly affects the maintenance of arbuscular mycorrhizal (AM) symbiosis (Li 2017).

Drought stress is known to restrict vegetative growth and yield in citrus (Rodríguez-Gamir et al. 2011). Owing to their fewer root hairs and high AM dependency, citrus plants are strongly associated with mycorrhizal fungi, and it is well documented that arbuscular mycorrhizal fungi (AMF) can enhance drought tolerance in citrus plants (Wu et al. 2013). HAT and HDAC control histone acetylation levels
and are involved in plant stress responses and symbiotic interactions. However, HAT and HDAC have not yet been accurately identified in citrus (Xu et al. 2015), and their responses to drought stress and AMF colonization remain unclear. Thus, we attempted to identify the members of HAT and HDAC families in citrus by recently released software (TBtools) for gene family analysis (Chen et al. 2020). The exon–intron organization, motif compositions, gene duplications, chromosome distribution, phylogeny, and synteny, and cis-elements of citrus HATs and HDACs were further investigated. Global expression analysis was performed to identify the involvement of HAT and HDAC gene family members in drought stress and AMF interactions. This study provides valuable insights into the functional characterization of HAT and HDAC gene family members in citrus.

2. Materials and methods

2.1. HAT and HDAC sequence analyses and characteristics

To identify HAT and HDAC genes of citrus genome, the complete genome assembly of citrus (Citrus sinensis ‘Valencia’) was downloaded from the Citrus Genome Database (https://www.citrusgenomedb.org/organism/Citrus/sinensis; C. sinensis genome v2.0). The 12 HAT and 18 HDAC protein sequences of Arabidopsis were obtained from The Arabidopsis Information Resource (TAIR) (https://www.arabidopsis.org/). Citrus HATs and HDACs were searched using Arabidopsis query sequences with BLASTP. Some repeated sequences were manually deleted based on their E-values. The molecular weights (MW) and isoelectric points (pI) of candidate protein sequences were determined using ExPASy (https://web.expasy.org/compute_pi/) (Gasteiger et al. 2003). The presence of conserved HAT and HDAC domains was verified for all potential proteins using the NCBI Batch CD-Search program (https://www.ncbi.nlm.nih.gov/Structure/bwrgpsb/bwrgpsb.cgi).

2.2. Sequence alignment and phylogenetic analysis

To analyze phylogenetic relationship between HAT and HDAC of citrus and Arabidopsis, the full-length HAT and HDAC protein sequences of both Arabidopsis and citrus were aligned using Muscle (Edgar 2004) in MEGA version 7.0 with default parameters (Kumar et al. 2016), following which a neighbor-joining tree was generated with 1000 bootstrap test replications. Phylogenetic relationships of the HAT or HDAC gene family members of citrus were estimated from another phylogenetic tree based on the alignment of only the citrus proteins.

2.3. Conserved motif and gene structure analysis

A motif analysis was carried out using MEME (http://meme-suite.org/tools/meme) to identify conserved motifs, with the following optimized parameters: zero or one occurrence per sequence, a maximum number of 15 motifs, and an optimum motif width of 6–50 residues. The default settings were retained for other parameters. Gene structures corresponding to the HAT and HDAC protein sequences were determined by comparing the coding sequences with their genomic sequences using TBtools (Chen et al. 2020).

The Citrus Generic Feature Format (GFF) file was downloaded from the Citrus Genome Database and used to elucidate the structure of HAT and HDAC genes. In addition, citrus HAT and HDAC protein motifs, conserved domains, gene structures, and phylogenetic genes were visualized using TBtools (Chen et al. 2020).

2.4. Chromosomal distribution, gene duplication, and collinearity relationships

Chromosome locations of candidate citrus HAT and HDAC genes were analyzed based on the GFF information and then visualized using TBtools software (Chen et al. 2020). Gene duplication events of citrus HAT and HDAC proteins, and their collinearity relationships with Arabidopsis HAT and HDAC sequences were investigated using MCScanX (Wang et al. 2012). The results were then visualized using TBtools (Chen et al. 2020).

2.5. Expression analysis of HATs and HDACs under AMF colonization and drought stress

A completely randomized two-factor block experiment was performed, with watering regime (i.e. drought stress) and AMF colonization as treatments. In the water treatment, the samples were either well-watered or subjected to drought stress, whereas samples were grown with or without Funneliformis mosseae in the AMF treatment. Four treatments (well-watered without AMF (W_Non), well-watered with AMF (W_AMF), drought stress without AMF (D_Non), and drought stress with AMF (D_AMF)) were thus obtained. Treatments were performed with 3 biological replicates; each treatment had 10 pots and there were 3 seedlings per pot (30 seedlings per treatment), and each replicate consisted of 10 seedlings. The experimental protocol was adapted from Zhang et al. (2020). The seeds of citrus were germinated in autoclaved sand for four weeks. Subsequently, three seedlings with 4 leaf old were transplanted into 2.3 L pots containing 2.7 kg of an autoclaved (0.11 MPa for 2 h) mixture of soils and sands with the same volume ratio (maximum water holding capacity: 18.9 ± 0.09% (mean ± standard error)). For the AMF treatment, approximately 2000 spores of Funneliformis mosseae (Nicol. & Gerl.) were added into each pot at transplantation of seedlings. For non-AMF treatment, an equal amount of autoclaved inoculums was added. The treated seedlings were exposed to conditions of 900 µM m m⁻² s⁻¹ photon flux density, 28/20°C day/night temperature, and 68% relative humidity under greenhouse. After transplantation of the seedlings, the inoculated and un-inoculated seedlings were subjected to 75% of maximum water holding capacity of pot substrates for ten weeks. After that, the water status in half of the plants was changed to 50% of maximum water holding capacity of substrates (drought stress) for seven weeks, while the other plants were kept in well-watered condition (75% of maximum water holding capacity of pot substrates) for seven weeks and then harvest. The substrate water content was daily monitored through weighing, and lost water of pots was supplied.

Transcriptome data of seedling roots from the four treatments was analyzed as described by Shu et al. (2016). Twelve libraries of seedling roots were sequenced using the Illumina
HiSeq 2000 system, and then reads containing adapters, reads with more than 10% unknown nucleotides, and low-quality reads with more than 50% bases with a quality value ≤5 were removed to obtain clean sequences from the raw data. These clean sequences were then mapped to the genome of sweet orange *C. sinensis* (status: draft, version2-draft, version2) for annotation. The transcriptome data were uploaded to the NCBI Sequence Read Archive, with accession numbers SRR10413223, SRR10413224, SRR10413225, and SRR10413226. Gene expression was then analyzed from the transcriptome data. Transcript abundances of *HAT* and *HDAC* genes were calculated as fragments per kilobase of exon model per million mapped reads (FPKM), using the 'cuffdiff' package in Cufflinks version 2.2.1. The FPKM value of the W_Non treatment was calculated using the 2^−ΔΔCt method described by Livak and Schmittgen (2001), where β-actin was taken as the reference gene. The measured transcripts were normalized to the relative expression value in W_Non treatment. Significant differences between treatments were determined by Duncan’s multiple range tests at p = 0.05 with SAS (SAS Institute, Inc., Cary, NC, USA). Different letters indicate statistically significant differences.

### 2.6. cis-acting regulatory element analysis of citrus HAT and HDAC genes

To identify *cis*-elements in the *HAT* and *HDAC* genes, 2000 bp sequences upstream of the transcription start site of the candidate genes were extracted from the citrus genome. PlantCARE software (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) was used for searching the *cis*-acting regulatory elements (Romans et al. 1999), and the results were visualized using TBtools (Chen et al. 2020).

### 3. Results

#### 3.1. Identification and characteristics of HATs and HDACs in the citrus genome

Fourteen HATs and eleven HDACs were identified from the citrus genome after searching for *HAT* and *HDAC* domain sequences, respectively. The HATs and HDACs were named according to their positions on each chromosome. Considerable variation was observed in the protein length, MW, and pl of both HATs and HDACs. The HAT protein length ranged from 182 aa (CsHAT2) to 1943 aa (CsHAT14), pI ranged from 5.05 (CsHAT3) to 9.30 (CsHAT12), and MW ranged from 20.73 kDa (CsHAT3) to 218.78 kDa (CsHAT13). The HDAC protein length ranged from 359 aa (CsHDAC3) to 1442 aa (CsHDAC6), pI ranged from 5.1 (CsHDAC11) to 9.13 (CsHDAC1), and MW ranged from 40.48 kDa (CsHDAC3) to 162.00 kDa (CsHDAC6) (Table 1).

#### 3.2. Chromosomal distribution

Thirteen CsHATs were distributed across seven chromosomes, and one CsHAT was distributed on chromosome Unknown. Chromosomes 3 and 5 included the highest number of CsHATs (three genes), whereas no CsHAT genes were found on chromosomes 7 and 9. Eleven CsHDACs were distributed from chromosomes 1–8, with chromosomes 1, 4, and 5 containing two CsHDACs each, whereas no CsHDAC was located on chromosome 9. Some CsHAT (CsHAT5 and CsHAT12) and CsHDAC (CsHDAC3 and CsHDAC5) genes exhibited segmental duplication, and some CsHATs also exhibited tandem duplication (CsHAT10 and CsHAT19) (Figure 1).

#### 3.3. Synteny analysis of HATs and HDACs between *A. thaliana* and *C. sinensis*

To further investigate the genome synteny of HATs and HDACs, a comparative syntenic map of citrus was constructed in association with *A. thaliana*. Eight CsHATs, localized on chromosomes 1, 3, 4, 5, 6, and 8, showed syntenic relationships with *A*HATs. Four of these eight CsHATs presented more than one syntenic gene in *A. thaliana* (Figure 2). Seven CsHDACs, localized on chromosomes 1, 3, 4, 5, 7, and 8, showed syntenic relationships with *A*HDACs. No HDAC exhibited more than one syntenic gene in *A. thaliana* (Figure 2; Supplementary Table 2). The observed synteny suggests that these eight HATs and seven HDACs may have played an important role during evolution.

#### 3.4. Phylogenetic analysis of HATs and HDACs in *A. thaliana* and *C. sinensis*

Phylogenetic relationships of HATs and HDACs were analyzed based on the phylogenetic tree constructed using citrus and *Arabidopsis* protein sequences. As shown in Figure 3, HATs were clustered into five major groups; Group I was classified as the GNAT-MYST family with four members.

### Table 1. Basic information of histone acetyltransferase and histone deacetylase genes identified in the *C. sinensis*.

| Gene Name | Original ID | Length (aa) | pl | MW (kDa) |
|-----------|-------------|-------------|----|----------|
| CsHAT1    | Cs1g21740.2 | 379         | 8.9 | 52.94    |
| CsHAT2    | Cs2g01900.1 | 182         | 6.18 | 75.64    |
| CsHAT3    | Cs3g04970.2 | 585         | 5.05 | 62.41    |
| CsHAT4    | Cs3g18990.1 | 449         | 7.23 | 20.73    |
| CsHAT5    | Cs4g27350.1 | 687         | 8.41 | 43.25    |
| CsHAT6    | Cs4g10180.1 | 887         | 8.84 | 81.64    |
| CsHAT7    | Cs4g13370.1 | 466         | 5.9  | 64.52    |
| CsHAT8    | Cs5g06590.2 | 363         | 9.24 | 51.92    |
| CsHAT9    | Cs5g06780.1 | 564         | 8.63 | 98.42    |
| CsHAT10   | Cs5g30470.1 | 561         | 5.98 | 198.07   |
| CsHAT11   | Cs6g19010.1 | 1342        | 7.4  | 98.42    |
| CsHAT12   | Cs8g14601.1 | 741         | 9.13 | 41.20    |
| CsHAT13   | Cs8g21090.1 | 1768        | 8.36 | 218.78   |
| CsHAT14   | orang1:104242.1 | 1943  | 5.79 | 152.00   |
| CsHDAC1   | Cs1g08250.2 | 388         | 9.13 | 43.21    |
| CsHDAC2   | Cs1g09520.1 | 439         | 6.68 | 48.02    |
| CsHDAC3   | Cs2g19220.1 | 359         | 8.83 | 40.48    |
| CsHDAC4   | Cs3g07600.1 | 437         | 5.17 | 50.04    |
| CsHDAC5   | Cs4g08730.1 | 465         | 8.89 | 51.97    |
| CsHDAC6   | Cs4g18610.1 | 1442        | 9.26 | 162.00   |
| CsHDAC7   | Cs5g05970.1 | 465         | 5.31 | 52.21    |
| CsHDAC8   | Cs5g11820.1 | 646         | 6.05 | 71.63    |
| CsHDAC9   | Cs6g12720.3 | 386         | 5.22 | 41.63    |
| CsHDAC10  | Cs7g26360.2 | 617         | 5.92 | 68.31    |
| CsHDAC11  | Cs8g01020.1 | 499         | 5.1  | 56.45    |

Notes: MW: molecular weight; pl: isoelectric point.
Group II was classified as TAFII250 and included three members. Group III was comprised of four citrus HATs but no A Hat. Group IV was classified as the MYST family and consisted of three members. Group V was classified as the CBP family with nine members. HDACs were clustered into three major groups; Group I was classified as the SIR2 family with five members, Group II was classified as the HD2 family and included four members and Group III was classified as the RPD3/HDA1 family with twenty members (Figure 3).

3.5. Conserved domains and motifs of CsHATs and CsHDACs

Fifteen conserved motifs were identified in both CsHATs and CsHDACs. The motifs of the 14 CsHATs exhibited considerable variation. CsHAT3, 5, 6, 10, 12, and 14 had either or both motifs 1 and 4; CsHAT2, 4, 7, and 9 did not present any motifs; CsHAT1 and CsHAT8 included three motifs (motifs 2, 14, and 15); CsHAT11 and CsHAT13 exhibited 10 motifs (Figure 4). CsHDAC2, 8, 9, and 10 presented similar motifs, including motifs 1, 2, 6, 7, and 12; CsHDAC4, 7, and 11 exhibited the same motif constitution and possessed the highest number of motifs; CsHDAC3 and CsHDAC5 possessed similar motifs, including motifs 6, 9, 10, 11, and 14 (Figure 4). HATs and HDACs with similar motif compositions were clustered in the same group. All motifs have been detailed in Supplementary Figure 1. TAFs_like (CsHAT14), TBP-binding superfamily (CsHAT14), Hat N (CsHAT7), HAT KAT11 superfamily (CsHAT11 and 13), and PHD_HAC_like (CsHAT11 and 13) domains were identified in the conserved domain analysis of CsHATs. Domains of the HDAC class (CsHDAC2, 6, 7, and 9) and
3.6. CSHAT and CsHDAC expression profiles in citrus seedlings

Expression analysis showed that all CSHATs and CsHDACs were expressed in the roots; however, they exhibited differential expression patterns. The expression of CSHAT1, 2, 6, 7, and 11 was induced by AMF colonization under both well-watered and drought conditions. However, the expression of CSHAT3, 4, 5, 8, 9, 10, and 12 was repressed by AMF. Drought treatment significantly promoted the expression of CSHAT6, 13, and 14, and downregulated CSHAT5 and CSHAT8, under both AMF and non AMF conditions. Furthermore, AMF colonization and drought stress had a synergistic effect, evident from the weakened repression of CSHAT3, 9, 10, and 12 and enhancement of CSHAT1, 6, 10, and 11 exposed to AMF during drought stress (Figure 5; Supplementary Table 3). The expression of CsHDAC1, 2, 5, 6, 8, and 10 was repressed and CsHDAC4 and CsHDAC7 were induced by AMF under both well-watered and drought conditions. Drought treatment significantly promoted CsHDAC1, 7, and 10 expression under both AMF and non AMF conditions. Furthermore, AMF colonization and drought stress exerted a synergistic effect, evident from the weakened repression of CsHDAC1, 8, 9, 10, and 11 and the enhancement of CsHDAC7 expression on exposure to AMF during drought stress (Figure 5; Supplementary Table 3). Collectively, the present study provides valuable insights into the citrus HATs and HDACs and their responses to AMF and drought stress.

The expression of 10 selected CSHATs and CsHDACs was analyzed using qRT-PCR to confirm the transcriptome data. qRT-PCR showed the expression of CSHAT2, 6, and CsHDAC4 was induced by AMF or/drought stress, and the expression of CSHAT4, 8, CsHDAC5, and 8 was repressed by AMF or/drought stress (Figure 6). The expression pattern identified by qRT-PCR was in accordance with that in transcriptome data. This study offers valuable depiction of the CSHATs and CsHDACs and the regulation of their expression during AMF colonization and/or drought.

3.7. Analysis of cis-elements in the CSHAT and CsHDAC promoters

Several cis-elements, for example, ‘anaerobic induction’ and ‘hormone responsive’, were identified in the upstream regulatory regions (promoters) of CSHATs and CsHDACs. Cis-elements belonging to ‘defense and stress responsive’ and ‘drought inducibility’ were responsible for their responses to mycorrhizal colonization and drought stress. ‘Drought inducibility’ cis-elements were noted in the promoters of six CSHATs (CSHAT2, 3, 7, 8, 9, and 12), and ‘defense and stress responsive’ cis-elements were identified in the promoters of four CsHDACs (CsHDAC3, 5, 10, and 13). ‘Drought inducibility’ cis-elements were also identified in the promoters of six CsHDACs (CsHDAC2, 3, 7, 9, and 10), and ‘defense and stress responsive’ cis-elements were observed in the promoters of four CsHDACs (CsHDAC1, 3, 4, 8, and 11). Two ‘drought inducibility’ cis-elements were identified in CsHDAC3 and CsHDAC7, indicating that these two CsHDACs were more affected than others by drought stress. In addition, the promoters of CsHDAC3 and CsDAC3 included both ‘drought inducibility’ and ‘defense and stress responsive’ cis-elements, suggesting that these two genes might be regulated by both drought conditions and microbial interactions (Figure 7).
4. Discussion

4.1. Number variation of HATs and HDACs in different species

To date, HATs and HDACs have been identified in several plant species, including *Arabidopsis*, rice, and grape (Pandey et al. 2002; Hu et al. 2009; Aquea et al. 2010; Fang et al. 2014). Numbers of HAT and HDAC proteins vary among different plant species. The *Arabidopsis* genome contains 12 HATs and 18 HDACs (Pandey et al. 2002), whereas the rice genome contains 8 OsHATs and 19 OsHDACs (Hu et al. 2009; Fang et al. 2014), and the grape genome contains 7 and 13 genes coding putative HATs and HDACs (Aquea et al. 2010). In this study, 14 HATs and 11 HDACs were identified in the citrus genome. It is known that segmental (or whole-genome duplications) and tandem duplications are the two major drivers of gene family expansion in plants (Hématy and Höfte 2008). CsHAT genes exhibited one segmental and one tandem duplication, and CsHDAC genes also presented one segmental duplication (Figure 1), suggesting that the expansion of HAT and HDAC families occurred a few times during evolution. In addition, 8 of the 14 CsHATs and 7 of the 11 CsHDACs showed syntenic relationships with *AtHATs* and *AtHDACs*, respectively. In conjunction

Figure 4. Phylogenetic tree of deduced CsHAT (a) and CsHDAC (b) proteins associated with the motif compositions and exon–intron composition of CsHATs and CsHDACs. The phylogenetic trees were constructed using the maximum likelihood method implemented in MEGA 7.0 (left in the figure). The reliability of the predicted tree was tested using bootstrapping with 1000 replications. The motif composition related to each CsHAT and CsHDAC protein is displayed in the middle of the figure. Motifs 1–15 are displayed in different colored boxes and have been detailed in Supplementary Figure 1. The exon–intron structure and domains of CsHATs and CsHDACs are displayed on the right in the figure. Green boxes indicate untranslated 5’ and 3’ regions, yellow boxes indicate CDS, and black lines indicate introns. The length of the protein can be estimated from the scale provided at the bottom.

Figure 5. Expression profiles of CsHATs and CsHDACs in response to AMF colonization and drought stress. The color scale represents the log2 expression values, and red and blue colors indicate higher or lower transcript abundances compared with the control (non AMF plants under well-watered condition), respectively. W_Non means treatment of well-watered without AMF, W_AMF means treatment of well-watered with AMF, D_Non means treatment of drought stress without AMF, and D_AMF means treatment of drought stress with AMF.
with gene duplication and synteny analysis, these results confirmed that the citrus HATs and HDACs were relatively conserved.

4.2. Phylogenetic characteristics of HAT and HDAC genes

A phylogenetic tree was generated based on the protein sequence alignment of HATs and HDACs. Members of the same group exhibited similar protein sequence length, motif composition, and gene structure, reflecting a close relationship. Thus, homologous genes in the same branch might have similar functions in plant-microbe interactions and abiotic stress. CsHAT10 and AtHAG1 both included the ‘COG5076’ domain, and CsHAT14 and AtHAF2 included the ‘TAFs_like’ domain. AtHAG1 or named as ATGNC5 shows HAT activity (Stockinger et al. 2001) and is known to acetylate H3K14 (Earley et al. 2007) and contribute to cold-regulated gene expression (Vlachonasios et al. 2003; Pavangadkar et al. 2010). AtHAF2 regulates the expression of several cold-regulated genes belonging to the TAFII250 family (GroupII) (Pavangadkar et al. 2010). Thus, CsHAT10 and CsHAT14, with similar domains, might also respond to cold stress. Group III was composed of four CsHATs with the ‘Bromodomain’ domain; however, the biological functions of these members require further analysis. Group V included the highest number of CsHATs, along with AtHAC1, 2, 4, 5, and 12, which were classified into the CBP family. Several domains have been identified in this family, including ‘HAT_K11’ and ‘PHD_HAC like’. AtHAC members in this group possess broad-specificity HAT activities and likely act together to acetylate histone H3 lysine 9 (Earley et al. 2007). Among the AtHACs, AtHAC1 interacts with a tomato heat stress transcription factor, HsB1, in vitro and in vivo, which acts as a transcriptional coactivator for a heat-shock-inducible gene (Bharti et al. 2004). CsHAT13 clustered together with AtHAC1, suggesting that it might respond to heat stress in citrus.

CsHDAC1 and AtSRT2, with ‘SIR’ domains, were clustered in the same branch of the SIR2 family. Arabidopsis histone deacetylase AtSRT2 has been shown to negatively affect plant basal defenses against pathogens, by suppressing salicylic acid biosynthesis (Wang et al. 2010). Resistance to PstDC3000 was increased in AtSRT2 knockout plants, whereas it was suppressed in AtSRT2-overexpressing plants. CsHDACs with the ‘HDAC’ domains were classified into the RPD3/HDA1 family in Group III (Figure 3); AtHDA6, CsHDAC7, AtHDA19, and CsHDAC11, with the ‘HDAC’ domains, were clustered into the same branch. AtHDA6 and AtHDA19 are known to be involved in plant-microbe interactions and abiotic stress. Transcription of AtHDA19 is induced by pathogen-related hormones (jasmonic acid and ethylene) and the fungal pathogen Alternaria brassicicola, and overexpression of AtHDA19 enhances Arabidopsis resistance to A. brassicicola (Zhou et al. 2005). Thus, CsHDAC1, CsHDAC7, and CsHDAC11 might play a role in plant defense.

4.3. Responses of citrus HAT and HDAC genes to AMF colonization and drought

Citrus HAT and HDAC genes exhibited different expression patterns under AMF colonization and drought, indicating their differential function in response to microbe-interaction and abiotic stress. Drought stress significantly promoted CsHAT6 and CsHAT14 expression, even though their promoters did not contain the ‘drought inducibility’ cis-elements. The expression of CsHAT2, 7, 9, and 12, containing the ‘drought inducibility’ cis-element, were induced by drought stress. Of these, CsHAT9 and CsHAT12 were repressed by AMF under drought conditions, which might be due to improved seedling water conditions and feedback-mediated downregulation of CsHAT9 and 12 expressions by AMF. This expression pattern is comparable to that of polyamine synthetase and sucrose metabolism, which are induced by drought and downregulated by AMF (Rodríguez-Gamir et al. 2011; Wu et al. 2017; Zhang et al. 2020). However, the induction of CsHAT2 and CsHAT12 was enhanced under AMF conditions, suggesting a synergistic effect of AMF and drought on the two CsHATs. AMF induced the expression of CsHAT1, 2, 6, 7, and 11, and repressed CsHAT3, 4, 5, 8, 9, 10, and 12. In comparison with the W_non treatment, all CsHATs with ‘defense and stress responsive’ cis-elements in the promoter (CsHAT3, 5, 10, and 13) were suppressed in the W_AMF treatment. AMF are symbiotic, and the interaction of AMF with the host varies among plant species (Borowicz 2001); thus, the response of ‘defense and stress responsive’ cis-element to AMF highlights a difference in the regulation pattern of different plants.
As expected, the expression of CsHDAC7 and CsHDAC10, with ‘drought inducibility’ cis-elements, was induced by drought, and the expression of CsHDAC1 and CsHDAC8, with ‘defense and stress responsive’ cis-elements, was repressed by AMF. In addition, AMF induced CsHDAC7 and repressed the expression of CsHDAC2, 5, 6, 8, and 10, even though they did not contain ‘drought inducibility’ or ‘defense and stress responsive’ cis-elements, respectively. The expression of CsHDAC7, with two ‘drought inducibility’ cis-elements, was induced by drought under both AMF and non AMF conditions, whereas the expression of CsHDAC3, with two ‘drought inducibility’ cis-elements and one ‘defense and stress responsive’ cis-element, was repressed in the D_Non treatment and induced in the D_AMF treatment. This suggests that CsHDAC3 was more likely regulated by ‘defense and stress responsive’ cis-elements. The regulation of CsHAT and CsHDAC expression was not consistent with the cis-elements present in their promoters. AMF or drought induced the expression of CsHAT1, 2, 6, 7, 9, 11, 12, and 14 and repressed CsHDAC1, 2, 5, 6, 8, and 10, regulating the histone acetylation levels in response to microbe interaction and stress. Overall, the present study provides valuable insights into the CsHATs and CsHDACs and their responses to AMF and drought stress. It would provide basic information for further gene function analysis.

**Author contributions**

Bo Shu and Chun Luo conceived the experiments and drafted the manuscript. Bo Shu, Fei Zhang, and Chun Luo conducted the experiments. Chun Luo, Yachao Xie, Dejian Zhang, and Chunyan Liu analyzed the data. Qiangsheng Wu and Chun Luo critically reviewed the manuscript.

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